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Equilibrium, ^{1}H and ^{13}C NMR Spectroscopy, and X-ray Diffraction Studies on the Complexes Bi(DOTA)$^-$ and Bi(DO3A-Bu)

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Several Bi(III) complexes are used in medicine as drugs. Bi(DO3A-Bu) has recently been proposed as a nonionic contrast agent in X-ray imaging (H_3DO3A-Bu = 10-[2,3-dihydroxy-(1-hydroxymethyl)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid). The solution equilibria and NMR structure and dynamics of Bi(DO3A-Bu) and of the similar Bi(DOTA)$^-$ have been investigated (H_4DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid). The stability constants were determined with the study of the competition equilibria between Br$^-$ ions and the ligands DOTA or DO3A-Bu for the Bi(III) by spectrophotometry. The stability constants, obtained for Bi(DOTA)$^-$ and Bi(DO3A-Bu), are very high, log $K = 30.3$ and 26.8, respectively. Potentiometric titrations indicated the dissociation of one of the protons among the three alcoholic OH groups in Bi(DO3A-Bu). The dissociation constant is log $K =$ 7.53 (0.09) indicating that at physiological pH about 50% of the species possess –1 charge. It was shown by ^{1}H and ^{13}C NMR spectroscopy that the OH group attached to the middle carbon atom of the “butriol” side chain is coordinated to the Bi(III) and starts to deprotonate at pH > 5.5. The crystal structure of NaBi(DOTA)·H_2O shows an octacoordinated arrangement of the donor atoms around the Bi(III), with no water in the inner sphere. The crystals belong to the centrosymmetric space group *C*2/*c*. The temperature dependent ^{1}H and ^{13}C NMR spectra indicate that both Bi(DOTA)$^-$ and Bi(DO3A-Bu)$^-$ complexes are fluxional. For Bi(DOTA), the $\Delta(\delta\delta\delta\delta) \rightleftharpoons \Lambda(\lambda\lambda\lambda\lambda)$ fluxionality was identified, and on the basis of the activation parameters, a synchronous motion was suggested for the fluxional motion resulting in the change of ring conformation and of the helicity of the complex. The transition state is supposed to be more symmetrical than the initial state. The deprotonated Bi(DO3A-Bu) has a highly asymmetric NMR structure in solution, and its fluxional motion is slower than that of Bi(DOTA)$^-$.

Introduction

Various coordination compounds of Bi(III) have been used as drugs in medicine, but the complexation properties of Bi(III) have not attracted great interest.[1] This lack of interest was probably related to the difficulties involved in the study of the complexes of Bi(III) in solution, because of its strong tendency to hydrolyze. The Bi^{3+}(aq) ion is present only in strongly acidic solutions, and some Bi(OH)$^{2+}$ is formed even in 1.0 M $HClO_4$.[2] However, interest in Bi(III) has been increasing recently, and various complexes of Bi(III) have been successfully characterized.[1,3−7] Bi(III) compounds are important components of a number of remedies used for the treatment of gastrointestinal disorders.[1] The successful use of Bi(III)−citrate for the treatment of ulcers has initiated studies of the complexes of Bi^{3+} with citrate and other oxy acids, e.g., tartrate.[4−7]

Bi^{3+} is considered to be a soft metal ion, but it forms complexes of high stability with multidentate ligands containing O or O and N donor atoms. The stability constants (log K_{BiL}) of the Bi(III) complexes of polyaminopolycar-

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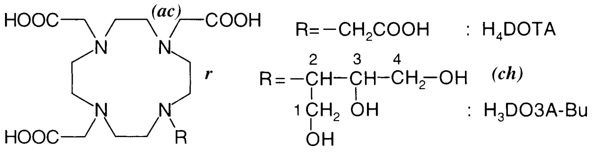
Scheme 1. Formula and Abbreviations Used for DOTA



boxylic acids are very high, e.g., the log K_{BiL} of $\mathrm{Bi(EDTA)^-}$ is 26.7.[8] Despite the strong propensity of Bi(III) compounds to hydrolyze, polyaminopolycarboxylate complexes are stable up to about pH 10.[8]

In recent years, Bi(III) complexes have been proposed for use as X-ray contrast agents in radiology.[9] The bismuth atom has a much larger mass attenuation when compared with iodine in the iodinated benzoic acid derivatives, which are currently used as X-ray contrast agents. Using Bi(III)-containing contrast agents, the energy of the X-rays used for the investigations could be significantly higher, so that the exposure to the patients to radiation would be lower.[9] Since the contrast agents are administered intravenously, Bi(III) complexes used for such aims must have high thermodynamic stabilities and kinetic inertness. Open-chain polyaminopolycarboxylic acids are known to form complexes of high stability with Bi(III), and it is to be expected that macrocyclic polyaminopolycarboxylates, such as DOTA ($\mathrm{H_4DOTA}$ = 1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid, Scheme 1), will behave similarly.

The complex Bi(DO3A-Bu), formed with the DOTA derivative ligand DO3A-butriol (Scheme 1), can be expected to have a high stability constant and high solubility, and on the basis of these properties, it is regarded as a potential nonionic X-ray contrast agent ($\mathrm{H_3DO3A}$-Bu = 10-[2,3-dihydroxy-(1-hydroxymethyl)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid).

However, the complexation equilibrium of Bi(DO3A-Bu) has not been studied. Similarly, there are no equilibrium data on the complex $\mathrm{Bi(DOTA)^-}$, although the DOTA complex formed with the α-emitting radioactive isotope ^{212}Bi (the daughter element of ^{212}Pb) was proposed to be used for the treatment of cancer (the complex ^{212}Pb(DOTA) is attached to monoclonal antibodies or proteins).[10,11]

The present article reports results characterizing the equilibrium properties and solution structures of $\mathrm{Bi(DOTA)^-}$ and Bi(DO3A-Bu), obtained by pH-potentiometry, spectrophotometry, and ^{1}H and ^{13}C NMR spectroscopy. The crystal structure of $\mathrm{Na[Bi(DOTA)]\cdot H_2O}$, determined by the X-ray diffraction method, is also reported.

Experimental Section

Chemicals, Stock Solutions, and Analysis. The chemicals used for the experiments were of analytical grade. $\mathrm{Bi(ClO_4)_3}$ solution was prepared by dissolving $\mathrm{Bi_2O_3}$ (99.9%, Fluka) in excess $\mathrm{HClO_4}$. The concentration of the $\mathrm{Bi(ClO_4)_3}$ solution was determined by complexometry with the use of standardized $\mathrm{Na_2H_2EDTA}$ solution and xylenol orange as indicator at about pH 1. DO3A-Butriol was produced by Schering AG (Berlin), while DOTA was kindly provided by Guerbet (Roissy, France). The concentrations of the ligand stock solutions were determined by pH-potentiometric titration in the absence and presence of a $\mathrm{Ca^{2+}}$ excess. The solutions of the complexes Na[Bi(DOTA)] and Bi(DO3A-Bu) were prepared by the reaction of equivalent amounts of $\mathrm{Bi(ClO_4)_3}$ and the ligand. To the vigorously mixed solution of $\mathrm{Na_4DOTA}$ or $\mathrm{Na_3DO3A}$-Bu was added $\mathrm{Bi(ClO_4)_3}$ solution dropwise. The pH of the solution was controlled, and it was set to 5−5.5.

Determination of Protonation and Stability Constants. The protonation constants of the ligands were determined by pH-potentiometric titration in 1.0 M NaBr and 1.0 M $\mathrm{Me_4NCl}$. Ligand samples (20 mL, 1.8×10^{-3} M) were titrated with NaOH or $\mathrm{Me_4NOH}$ solution. Titration vessels were thermostated at 25 °C. The titrated samples were stirred with a magnetic stirrer, and $\mathrm{N_2}$ gas was bubbled through the solution. Titrations were performed with a Radiometer PHM 85 pH-meter, an ABU 80 autoburet, and G202B glass and K401 calomel electrodes in the pH range 1.8−12.3.

Hydrogen ion concentrations were calculated from the measured pH values by a known method.[12] HCl samples (0.01 M) were titrated with NaOH or $\mathrm{Me_4NOH}$ in 1.0 M NaBr or $\mathrm{Me_4NCl}$, and the $[\mathrm{H^+}]$ versus pH data were used to calculate the hydrogen ion concentrations from the pH values obtained in the titrations of the ligands. The ionic product of water was also calculated from the 0.01 M HCl titration data. The pK_w values obtained in 1.0 M NaBr and $\mathrm{Me_4NCl}$ are 13.78 and 13.86, respectively.

Spectrophotometric measurements were carried out on the absorption band of $\mathrm{BiBr_6^{3-}}$ in the wavelength range 360−390 nm. Three series of samples were prepared, containing 3×10^{-5}, 5×10^{-5}, or 8×10^{-5} M $\mathrm{Bi(ClO_4)_3}$, 1.67×10^{-4}, 2.78×10^{-4}, or 4.45×10^{-4} M ligand, and 1.0 M NaBr. In each series, six samples were prepared with different pH values in the pH range 1.9−3.0. The samples were kept at 25 °C for about 4 weeks in order for the equilibrium to be attained. The molar absorptivity of $\mathrm{BiBr_6^{3-}}$ was determined in the concentration range 1×10^{-5} to 1×10^{-4} M $\mathrm{BiBr_6^{3-}}$. For the calculations of the equilibrium data, the absorbance values were measured at 10 wavelength values between 360 and 390 nm. The spectrophotometric measurements were made in 1.0 cm cells with a CARY 1E spectrophotometer. The protonation constants of the ligands and the stability constants of the complexes were calculated with the program PSEQUAD.[13]

NMR Measurements. The ^{1}H and ^{13}C NMR measurements were performed mainly on a Bruker AM 360 NMR spectrometer with a QNP 5 mm direct probe head. The chemical shifts are reported in ppm toward higher frequencies, with respect to TMS as an external standard (0 ppm for both cases).[14] The H−H COSY spectra were made with the standard Bruker program using a 45° mixing pulse. The ^{13}C NMR spectra were recorded in *J*-modulated decoupling or inverse gated decoupling mode, depending on the need for getting a quantitative spectrum. The H−C correlation spectrum was recorded on a Bruker DRX 500 spectrometer in inverse mode, using gradient pulses in the *z* direction with the usual Bruker HSQC pulse sequence. The spectra were analyzed with the Bruker WinNMR software package.

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Collection and Reduction of X-ray Diffraction Intensity Data. Microcrystals of NaBiDOTA·H_2O could easily be obtained from aqueous solutions. In order to increase their volumes, an aqueous solution close to saturation was pipetted into paraffin. Upon addition of a few seeding crystals, bigger crystals developed at the paraffin/water interface. Most of these crystals were twinned or had only moderate diffraction properties showing broad reflection profiles. For the analysis described here, a colorless platelet with dimensions $0.35 \times 0.35 \times 0.1$ mm^3 was mounted on a glass rod and used for data collection on a Siemens P4 diffractometer (sealed tube generator; Mo Kα radiation, $\lambda = 0.71073$ Å, graphite monochromator). Using an ω scan technique (scan width 1.2°), 3824 diffraction data were collected up to $2\theta_{\max} = 50°$. The intensities were corrected using a semiempirical absorption correction. The intensities of three standard reflections measured every 100 reflections indicated that the crystal was stable during the data collection.

The crystals are monoclinic with $a = 17.053(3)$ Å, $b = 6.587(2)$ Å, $c = 20.048(6)$ Å, and $\beta = 102.00(2)°$. The determination of the space group cannot be made without ambiguity as often observed for heavy atom structures. The structure was therefore solved and refined[15,16] in a number of space groups including *P*1, *P*2$_1$, *Cc*, and *C*2/*c*. Careful analysis of the E-statistics and the results of the structure refinement lead to the conclusion that the crystals belong to the centrosymmetric space group *C*2/*c*. In this space group, the asymmetric unit contains $^1/_2$ molecule of X, $^1/_2$ Na^+ ion, and 2 water molecules. Hydrogen atoms have been included into calculated positions and refined using a riding model. The full-matrix least-squares refinement converged at R1 = 3.7% (wR2(all data) = 9.7%) using anisotropic displacement parameters for all non-hydrogen atoms. The Na^+ ion is disordered over the crystallographic inversion center, and therefore, no special position constraint was used during refinement. The results of the X-ray analysis are shown in Tables 2 and 3. Figure 3a,b shows the molecular conformation of NaBiDOTA·H_2O together with the atomic numbering scheme used in this X-ray study.

Results and Discussion

Equilibrium Studies. Study of the complexation equilibria in the Bi(III)–aminopolycarboxylate ligand systems is difficult because of the high values of the stability constants and the formation of various Bi(III) hydroxo complexes. These difficulties can be overcome through the use of an appropriate competition reaction. If the stability constants of the complexes formed with the competing ligands are sufficiently high, the formation of hydroxo species in the equilibrium system is negligible. Our model calculations indicated that Br^- ion can successfully compete with the ligand DOTA and DO3A-Bu for Bi^{3+} in the pH range 1.8–3.2, even if the stability constant of the macrocyclic Bi(III) complex is log $K_{BiL} = 28.0$. This is a consequence of the relatively low values of the conditional stability constants of the Bi(III) complex formed with the macrocyclic ligand at lower pH values.

Table 1. Protonation Constants of the Ligands DOTA and DO3A-Bu and the Stability Constants of Their Complexes with Bi(III) and Some Endogenous Metals (25 °C)

	DOTA		DO3A-Bu	
species	1.0 M NaBr	1.0 M Me_4NCl[b]	1.0 M NaBr	1.0 M Me_4NCl[b]
HL	9.01 (0.05)	12.67 (0.02)	9.16 (0.01)	11.44 (0.02)
H_2L	9.08 (0.02)	9.76 (0.02)	9.15 (0.01)	9.04 (0.03)
H_3L	4.44 (0.04)	4.65 (0.02)	4.12 (0.01)	4.47 (0.03)
H_4L	3.74 (0.03)	4.16 (0.02)	2.79 (0.01)	3.41 (0.04)
H_5L	1.72 (0.05)	2.42 (0.02)	1.61 (0.02)	2.25 (0.04)
BiL	30.3 (0.02)		26.8 (0.02)	
Fe(III)L		29.4[a]		25.7
CuL		22.72[a]		20.8
ZnL		18.7[a]		18.7
CaL		16.37[a]		13.3

[a] Ref 33. [b] This work.

For successful competition reactions with DOTA and DO3A-Bu, the concentration of Br^- must be as high as about 0.5–1.0 M. In order to maintain a constant ionic strength, the equilibrium measurements were carried out in 1.0 M NaBr. At such a high Br^- concentration, the species $BiBr_6^{3-}$ predominates,[17] and the competition reactions studied are as follows:

$$BiBr_6^{3-} + H_nL \rightleftharpoons BiL + nH^+ + 6Br^- \qquad (1)$$

where $n = 3-5$ for both DOTA and DO3A-Bu.

For the determination of the stability constants, the protonation constants ($K_i^H = [H_iL]/[H_{i-1}L][H^+]$) of DOTA and DO3A-Bu were determined in 1.0 M NaBr. The protonation constants calculated from the data of three parallel titration curves are presented in Table 1. The log K_i^H values obtained in 1.0 M NaBr are much lower than those determined in 1.0 M Me_4NCl, indicating the formation of Na^+ complexes. On the basis of the differences in the protonation constants, the stability constants of $Na(DOTA)^{3-}$ and $Na(DO3A\text{-}Bu)^{2-}$ were calculated. The log K_{NaL} values ($K_{ML} = [ML]/[M][L]$) were found to be 4.65 (0.04) and 2.88 (0.05), respectively. (The standard deviations are presented in parentheses.) The determined log K_{NaL} values are quite close to those reported earlier for $Na(DOTA)^{3-}$ (4.38) and $Na(DO3A\text{-}Bu)^{2-}$ (2.32).[18]

The competition equilibria in the systems Bi^{3+}–Br^-–H_nL were studied by means of spectrophotometry. The absorption spectra of $BiBr_6^{3-}$ (6p ← 6s transitions) and $Bi(DOTA)^-$ or Bi(DO3A-Bu) differ considerably. The maximum of the absorption band of $BiBr_6^{3-}$ is at 372 nm, while those of $Bi(DOTA)^-$ and Bi(DO3A-Bu) are at 307 nm. The competition equilibria were studied via the absorption band of $BiBr_6^{3-}$. Spectra were recorded at three different Bi^{3+} concentrations and different pH values. Some characteristic absorption spectra obtained in the systems Bi^{3+}–Br^-–DOTA and Bi^{3+}–Br^-–DO3A-Bu are shown in Figure 1. The isosbestic points in the spectra indicate the presence of two absorbing species, $BiBr_6^{3-}$ and $Bi(DOTA)^-$ (A) or Bi(DO3A-Bu) (B). From the molar absorptivities and the stability product of $BiBr_6^{3+}$ reported by Fedorov et al. (log $\beta_6 = 7.94$),[17] the stability constants of $Bi(DOTA)^-$ and Bi(DO3A-Bu) were calculated, and the log K_{BiL} values are presented

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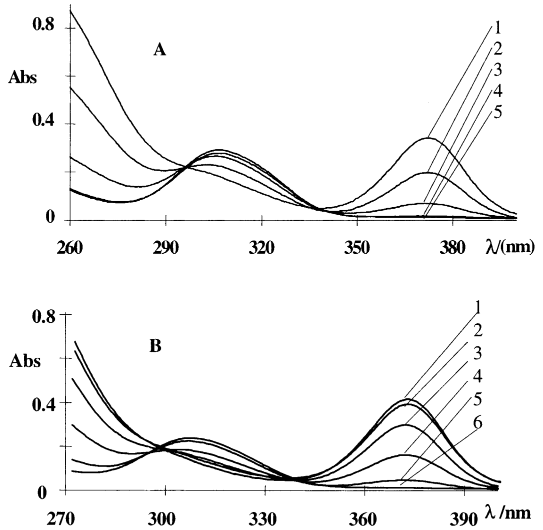

Figure 1. Absorption spectra of the system $Bi^{3+}-Br^{-}-$DOTA (A). $c_{Bi} = 3.18 \times 10^{-5}$M, $c_{DOTA} = 1.46 \times 10^{-4}$ M, pH = 1.92 (1); 2.12 (2); 2.32 (3); 2.53 (4); 2.71 (5); 1.0 M NaBr. Absorption spectra of the system $Bi^{3+}-Br^{-}$ −DO3A-Bu (B). $c_{Bi} = 3.1 \times 10^{-5}$ M, $c_{DO3A-Bu} = 1.67 \times 10^{-4}$ M, pH= 1.99 (1); 2.21 (2); 2.41 (3); 2.65 (4); 2.81 (5); 3.01 (6), 1.0 M NaBr.

in Table 1. The stability products of the complexes $BiBr_n^{(n-3)-}$ ($n = 1-6$) were determined by Fedorov et al. at different ionic strengths.[17] The background electrolytes were 1.0 M for $HClO_4$ and 0.5, 1.0, 2.0, 3.0, or 4.0 M for $LiClO_4$ and LiBr.[17] The stability product of $BiBr_6^{3-}$, log β_6, used for the calculations, was obtained by extrapolation to an ionic strength $I = 1.0$ M. The difference in the nature of the background electrolyte used in this work presumably does not result in any significant error in the log K_{BiL} values obtained because Fedorov et al. found that partial replacement of ClO_4^- for Br^- has practically no effect on the stability product of $BiBr_6^{3-}$.[17]

This statement seems to be reliable, since the log β_6 value obtained by Ahrland and Grenthe in 2.0 M ($HClO_4$ + $NaClO_4$ + NaBr)[19] is close to that obtained by Fedorov et al. in 1.0 M $HClO_4$ + 1.0 M ($LiClO_4$ + LiBr).[17] In Table 1, the stability constants of the complexes of Bi^{3+} are compared with those of some endogenous metal ions. It is seen that the stability constants of $Bi(DOTA)^-$ and Bi(DO3A-Bu) are in general significantly higher than the log K_{ML} values of the complexes formed with the other metal ions. The only exceptions are the complexes of Fe^{3+}, which are much more stable than the complexes of the dipositive ions Cu^{2+}, Zn^{2+}, or Ca^{2+}. In order to obtain information on the stability of the Bi^{3+} complexes in weakly basic solutions, the samples of $Bi(DOTA)^-$ and Bi(DO3A-Bu) were titrated with KOH in the pH range 3−11. The titration curves indicated no interaction between $Bi(DOTA)^-$ and OH^-. However, in the titration of Bi(DO3A-Bu), 1 equiv of KOH was consumed in the pH range 6−10, as may be seen in Figure 2. The consumption of base cannot be a result of the hydrolysis of Bi(III) because this was not observed in the titration of $Bi(DOTA)^-$.

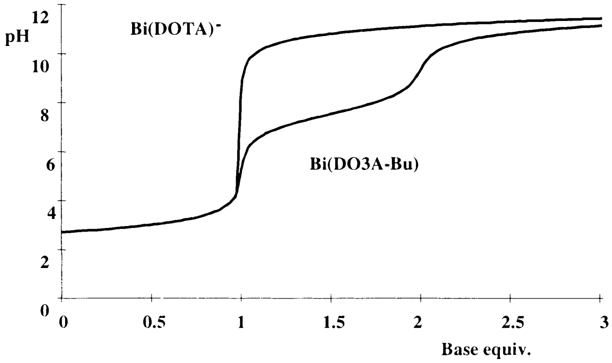

Figure 2. Titration curves of $Bi(DOTA)^-$ (1.95×10^{-3} M) and Bi(DO3A-Bu) (1.95×10^{-3} M). $c_{KOH} = 0.179$ M.

As regards Bi(DO3A-Bu), it can be assumed that its structure is similar to that of $Bi(DOTA)^-$ and that the Bi^{3+} occupies a position in the coordination cage determined by the four N and three carboxylate O atoms. It is very probable that one of the three alcoholic OH groups is also coordinated and at higher pH values the proton from the coordinated OH group can dissociate. On the assumption that the dissociation is as follows

$$\text{Bi(DO3A-Bu)} + H_2O \rightleftharpoons \text{Bi(DO3A-Bu)}^- + H_3O^+ \quad (2)$$

the dissociation constant K_d ($K_d = [BiL^-][H_3O^+]/[BiL]$) was calculated with the use of 86 titration data points. The dissociation constant obtained is $-\log K_d = 7.53$ (0.09). The dissociation of an alcoholic OH group was observed earlier for the lanthanide(III)−DO3A-Bu complexes, e.g., for Gd(DO3A-Bu), $-\log K_d = 9.45$.[18] In the case of Bi(DO3A-Bu), the dissociation of the coordinated alcoholic OH group occurs at lower pH than for the Ln^{3+} complexes, which probably means that the Bi^{3+}−alcoholic OH interaction is stronger than the Ln^{3+}−alcoholic OH interaction.

The high stability constants suggest that $Bi(DOTA)^-$ and Bi(DO3A-Bu) can be safely used in medical diagnostic investigations. However, it should be noted that Bi(DO3A-Bu) cannot be regarded as a nonionic diagnostic agent because at physiological pH about 50% of the complex is present as a mononegative anion as a result of the dissociation of an alcoholic OH group of the ligand. In view of the extremely high thermodynamic stabilities, $Bi(DOTA)^-$ and Bi(DO3A-Bu) are expected to be very inert. Kumar et al. found that the absorbance of $Bi(DOTA)^-$ did not change in 1.0 M $HClO_4$ even during 1 month, which indicates that any proton-assisted dissociation of the complex is extremely slow.[10]

Crystal Structure of $NaBi(DOTA)\cdot H_2O$. The fractional atomic coordinates and the isotropic temperature factors for the non-hydrogen atoms are listed in Table 2. All bond lengths and angles are in the range expected for this kind of structure.[20−22] The central Bi^{3+} is octacoordinated. The packing in the unit cell is stabilized by intermolecular

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Table 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients× for NaBi(DOTA)·H_2O[a]

	x	y	z	U(eq) × 10^3/Å^2
Bi(1)	0	560(1)	2500	24(1)
N(1)	−496(3)	−1605(9)	3360(2)	27(1)
C(2)	191(4)	−2491(12)	3844(3)	33(2)
C(3)	841(4)	−3264(12)	3498(3)	34(1)
N(4)	1146(3)	−1628(9)	3118(3)	29(1)
C(5)	1590(4)	−2481(12)	2624(3)	33(1)
C(12)	−1030(4)	−3264(11)	3017(3)	33(1)
C(13)	−945(4)	−238(12)	3729(4)	30(1)
C(14)	−509(4)	1754(12)	3939(3)	33(1)
O(14)	69(3)	2237(8)	3674(2)	36(1)
O(15)	−769(3)	2763(10)	4369(3)	57(2)
C(16)	1678(4)	−226(13)	3575(4)	35(2)
C(17)	1773(3)	1842(12)	3271(3)	34(2)
O(17)	1336(3)	2259(8)	2699(3)	39(1)
O(18)	2272(3)	2960(9)	3612(3)	46(1)
Na(1)	2510(3)	2912(9)	4813(3)	39(1)
O(1WA)	2911(5)	5958(13)	4762(4)	68(2)
O(2WA)	3799(3)	1315(11)	5053(3)	49(1)

[a] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

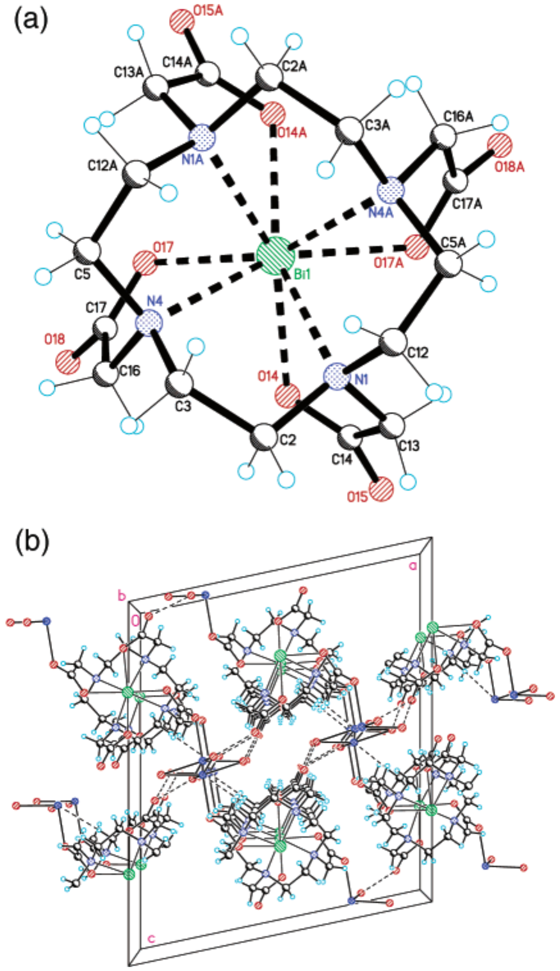

Figure 3. Crystal structure of NaBiDOTA·H_2O. (a) The numbering scheme is indicated. Bond distances and angles are given in the text and more in the Supporting Information. Packing of NaBiDOTA·H_2O and disorderd Na^+ ion along the crystallographic b axis (b).

Table 3. Selected Bond Lengths and Bond Angles for NaBi(DOTA)·H_2O[a]

bond	length/Å	bond	angle/deg
Bi(1)−N(1)	2.516(5)	N(1)−Bi(1)−N(4)	71.18(16)
Bi(1)−O(14)	2.580(5)	N(1)#−Bi(1)−N(4)	71.20(17)
Bi(1)−N(1)#	2.516(5)	N(1)#−Bi(1)−N(1)	110.9(2)
Bi(1)−O(14)#	2.580(5)	N(4)−Bi(1)−N(4)#	110.7(3)
Bi(1)−N(4)	2.535(5)	O(17)−Bi(1)−O(14)#	79.69(16)
Bi(1)−O(17)	2.495(4)	O(17)−Bi(1)−O(14)	78.17(16)
Bi(1)−N(4)#	2.535(5)	O(17)−Bi(1)−O(17)#	126.7(3)
Bi(1)−O(17)#1	2.495(4)	O(14)−Bi(1)−O(14)#	129.3(2)
		N(4)#−Bi(1)−O(14)#	85.03(17)
		N(4)#−Bi(1)−O(17)#	65.87(18)

[a] In Figure 3. Atoms denoted with letter A in Figure 3 (denoted in this table with #) are equivalent, generated by the following symmetry transformation: $-x, y, -z + {}^1/_2$.

hydrogen bonds involving carbonyl O atoms, solvent water and Na^+ ion. It should be noted that there are no direct interactions between the ligand molecules: all contacts are mediated via solvent molecules as Figure 3b shows. The Bi^{3+} ion is deeply located in the cage formed by the O and N atoms. The O14−Bi−O14A angle is 129.3°, while the N1−Bi−N1A angle is 110.9° (Table 3). If these are compared with the corresponding data for crystalline Lu(DOTA)$^-$, it can be seen that, despite its smaller ionic size, the Lu^{3+} is less deeply located in the cage.[21] The corresponding O−Lu^{3+}−O angles are between 141.7° and 143.3° while the N−Lu^{3+}−N angles are between 105.2° and 105.4°. The reason is that the softer Bi^{3+} bonds more strongly to the N donor atoms of the ring. Further, the lack of coordinated water in the Bi^{3+} complex lets the central ion sink deeper toward the ring. Accordingly, the mean Lu−N bond lengths are longer by more than 30 pm than the Lu−O bond lengths. The Bi−N and Bi−O bond lengths differ by less than 10 pm only as can be seen in Table 3, where selected bond distances and angles are shown.

This is in accordance with the higher thermodynamic stability (Table 1) and the differences in fluxional behavior (see later). The twisting angle between the squares determined by the four O and N atoms of the ligand is 39° for LuDOTA$^-$ while for Bi(DOTA)$^-$ it is only ca. 30° whereas 45° is considered to be ideal for the regular square antiprismatic arrangement. Other crystallographic parameters are listed in the Experimental Section and in the Supporting Information. The solution structure of the complex will be discussed in comparison with the crystal structure shown in Figure 3a.

^{1}H and ^{13}C NMR Studies. The proton spectrum of Bi(DOTA)$^-$ consists of broad lines at room temperature; these vary with a change of the temperature (Figure 4). Since the equilibrium studies indicate the presence of only one complex in solution, the broadening of the signals is probably a result of fluxional dynamic processes (vide infra), which are fairly common for DOTA complexes.[22−25] In order to acquire deeper insight into this behavior, we first assigned the ^{1}H NMR peaks on the basis of 2D COSY and HSQC at 274 K, where the coupling patterns are well visible. (Figure S1a,b, Supporting Information). We use the following notations for the carbon atoms: r denotes the ring carbons (and the protons on them), e.g., a proton of a ring CH_2 group is rH(a) or rH(b), ac denotes CH_2 carbons of the acetate

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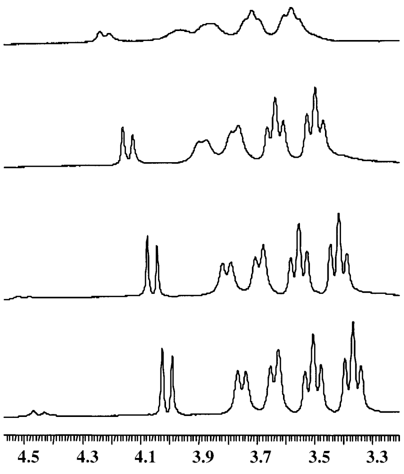
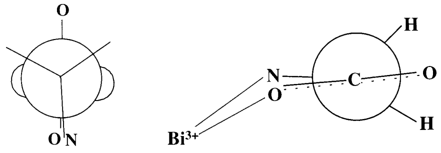


Figure 4. ^{1}H NMR spectra of 0.2 M BiDOTA solution at pH = 8.6 (20% D_2O) at different temperatures. From the bottom upward: 274, 280, 290, and 300 K. The water signal is suppressed by a binomial pulse sequence (Watergate, provided by Bruker Gmbh).

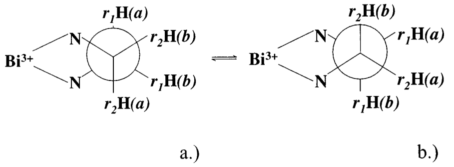
Scheme 2. Conformation of Acetate Arms in BiDOTA$^-$



groups, while ch denotes the carbons in the 2,3-dihydroxy-(1-hydroxymethyl)-propyl group, which of course is present only in the DO3A-Bu complex. (see Scheme 1).

The acetate protons of Bi-DOTA give an AB doublet with chemical shifts of 4.45 ppm for acH(a) (this sideband of the AB doublet is destroyed partially by the water suppression sequence in spectra of Figure 4) and 4.02 ppm for acH(b). The geminal coupling constant is 17.85 Hz, which is exceptionally large. There is a rule, supported by experimental findings and quantum mechanical calculations for $R-CH_2COO-$ fragments, which describes the variation in the $^2J_{HH}$ value with the dihedral angle of the R−C bond and the COO^- plane.[26-29] If this angle is about 90°, then $^2J_{HH}$ is about −12 Hz, while if it is 0° or 180°, then $^2J_{HH}$ is about −18 to −19 Hz. Therefore, we assume that the reason for this large geminal coupling is the coordination, which stabilizes a conformation with a dihedral angle of about 180°. The coordination mode is illustrated in Scheme 2, where the C−N bond is in an eclipsed position to the COO plane.[30] Indeed, it may be seen in the X-ray structure of the complex (Figure 3a) that, for example, the atoms N1A−C13A−C14A−O14A are almost in one plane.

Scheme 3. Conformation of BiDOTA$^-$ Ring



The ring exhibits four proton signals: r_1H(a) at 3.75 ppm (a doublet with $^2J_{HH}\{r_1H(b)\} = 14.2$ Hz), r_1H(b) at 3.36 ppm (a "pseudotriplet" with $^2J_{HH}\{r_1H(a)\} = 14.2$ Hz and $^3J_{HH}\{r_2H(b)\} = 14.0$ Hz; i.e., the geminal and the vicinal coupling constants are almost the same), r_2H(a) at 3.63 ppm (a doublet with $^2J_{HH}\{r_2H(b)\} = 13.6$ Hz), r_2H(b) at 3.53 ppm (a "pseudotriplet" with $^2J_{HH}\{r_2H(a)\} = 13.6$ Hz and $^3J_{HH}\{r_1(b)\} = 14.0$ Hz). The other vicinal couplings among the rest of the protons cannot be resolved either in the 1D or in the 2D spectra. From these coupling data, the conformations of the ring protons are axial (large $^3J_{HH}$) and equatorial (smaller $^3J_{HH}$ which cannot be detected even at 274 K),[28] similar to those of the Ln(III) complexes, as shown in Scheme 3.[20,23] Bi(DOTA)$^-$ has a simple ^{13}C NMR spectrum, with one signal at 179.1 ppm (CO), indicating the magnetic equivalence of the four coordinated carboxylate carbon atoms. Three CH_2 signals appear in the spectrum: one of them is due to the acetate arms (ac), at 58.8 ppm, while the other two to the ring carbon atoms, r_1 at 55.0 ppm and r_2 at 50.5 ppm. The probable reason is that the ring has a kind of symmetry that results in two different chemical environments for the CH_2 carbon(r) atoms while the acetate CH_2 carbon(ac) atoms are equivalent, similar to the carbonyls.

The analysis of NMR data indicates that the complex itself has C_{4v} symmetry in solution, which is very slightly distorted for the crystalline form (Figure 3, Tables 2 and 3). These findings are in accordance with the COSY and HSQC spectra (in Figure S1a,b in the Supporting Information). In contrast with the results obtained for most Ln(DOTA) complexes, no diastereomers were detected, which supports the observations of Aime et al. that the diameter of the ions is responsible for the diastereomerism.[25] The size of Bi^{3+} is very close to that of La^{3+}, for which only one diastereomer is dominant (vide infra). We have no direct evidence for the solution structure, but there is no reason to say that it is different from the X-ray structure, which is a distorted inverted antiprism similar to that of the Ln(III) complexes.

The dynamic pattern of the protons can be observed qualitatively via the 2D EXSY spectrum (Figure S2 in the Supporting Information) taken at 274 K. There are strong cross-peaks between protons r_1H(a) and r_2H(b) and between r_1H(b) and r_2H(a), which correspond to the motion of the protons originally in the axial position arriving at the equatorial position and vice versa, while the r_1 and r_2 carbon atoms virtually change their position (Scheme 3). With the notation introduced for ethylenediamine complexes, the reaction is $(\delta\delta\delta\delta) \rightleftharpoons (\lambda\lambda\lambda\lambda)$.[22,25] The existence of the weaker cross-peaks which connect the *geminal* protons (r_1H(a) to r_1H(b) or r_2H(a) to r_2H(b)), the *axial* protons (e.g., r_1H(a) to r_2H(a) in Scheme 3a), and the *equatorial* ones (e.g., r_1H(b) to r_2H(b) in Scheme 3a) cannot be understood as a result of this exchange process. Moreover, a detailed study of the molecular model indicates that these exchanges may require the rupture of all metal−ligand bonds which must be slow. The explanation of the appearance of these cross-peaks may

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be cross-relaxation resulting from closer and more distant neighborhood protons. The existence of the fluxionality $(\delta\delta\delta\delta) \rightleftharpoons (\lambda\lambda\lambda\lambda)$ already described and illustrated in Scheme 3 is supported by the temperature dependence of the 1D ^{1}H NMR spectra. At high temperature, the ring protons collapse to two signals, probably a still "exchange-broadened" pair of AB doublets, in which the geminal coupling could not be resolved even at this temperature (Figure S3). Further evidence is that the temperature dependence of the ^{1}H NMR spectra for the ring protons could be described quantitatively by taking into account only this exchange process with the activation parameters $\Delta H^{\ddagger} = 39.6$ kJ mol^{-1} and $\Delta S^{\ddagger} = -76.3$ J mol^{-1} K^{-1} (Figure S4a). The fluxionality of the ring in the Ln(III)−DOTA complexes can be described exclusively with this motion as well, which also supports our finding.[20−22,24,25] The AB doublets of the acetate arms broaden and collapse to one signal on elevation of the temperature (Figure S3). This process has activation parameters $\Delta H^{\ddagger} = 35.0$ kJ mol^{-1} and $\Delta S^{\ddagger} = -96$ J mol^{-1} K^{-1} (Figure S4b). This fluxional motion can be attributed to the change in the helicity of Bi(DOTA)$^-$, denoted by $\Lambda \rightleftharpoons \Delta$. This type of motion has also been detected in the case of Ln(III) complexes.[20−25]

The complex Bi(DOTA)$^-$ has one dominant diastereomer in the solution, which consists of an enantiomer pair denoted as $\Delta(\delta\delta\delta\delta)$ and $\Lambda(\lambda\lambda\lambda\lambda)$ according to the IUPAC standard, or m$_1$ and m$_2$ (this notation originates from the *minor* isomer of Ln^{3+}−DOTA complexes).[25] We came to this conclusion by investigating the X-ray picture that is shown in Figure 3a. This is not surprising because the size of Bi^{3+} is similar to that of La^{3+}. We calculated the ionic sizes as follows: Aime et al. published ionic sizes 101.9 and 107.5 pm for octacoordinated and nonacoordinated Y^{3+} ion, respectively, in the Y−DOTA$^-$ complex. Having known the Y^{3+}−O distances from the Cambridge Crystallographic Database, we calculated the "size" O donor atom and subtracted this value from the Bi−O distances in BiDOTA$^-$ complex. In this way, we obtained the size of the Bi^{3+} ion as 124−133 pm, and 122−125 pm was given for La^{3+} by doing the same calculation. On the basis of this similarity and in accordance with the observation of Aime et al. on La^{3+}, we can state that the concentration of the other enantiomer pair $\Delta(\lambda\lambda\lambda\lambda)$ (traditionally named *major*, M$_1$) and $\Lambda(\delta\delta\delta\delta)$ (M$_2$) is low; we could not even detect the presence of this enantiomer of BiDOTA$^-$.[25] Therefore, the exchange detected is as follows:

$$\Delta(\delta\delta\delta\delta) \rightleftharpoons \Lambda(\lambda\lambda\lambda\lambda)$$

The results support the suggestion of Desreux based on ^{13}C NMR measurements on the complex La(DOTA)$^-$ that the two types of motion, i.e., the motion of the ring and the acetate arms, proceed at the same rate in a synchronous way in Bi(DOTA)$^-$ as well.[23] The free energy of activation is the same as for the complex La(DOTA)$^-$, but the entropy of activation is a larger *negative* value for Bi(DOTA)$^-$. Generally, it is not an easy task to explain the activation parameters for fluxional systems, but some comment can be made. The entropy of activation characterizes the remaining hindrance of a certain process when there is no energetic barrier, which occurs at infinitely high temperature. For La^{3+}, it is close to zero,[23] $\Delta S^{\ddagger} = -4.6 \pm 3.3$, while for Yb^{3+} it is $\Delta S^{\ddagger} = 52 \pm 39$ J mol^{-1} K^{-1}.[24] These data were interpreted in such a way that in the transition state Yb(DOTA)$^-$ probably loses one water ligand resulting in increased disorder, while this is not the case for La(DOTA)$^-$.[23] The entropy of activation for Lu(DOTA)$^-$ is even more positive, 116 J mol^{-1} K^{-1}, which is not in contrast with the water free transition state concept, although Aime et al. did not analyze the reaction from this point of view in their paper.[21] Bi(DOTA)$^-$ has no water in the coordination sphere, and the transition state can be conceived as more symmetrical than either of the reactants or the products. This symmetric transition state can have a temporarily ordered water shell, explaining the high negative value of the entropy of activation.

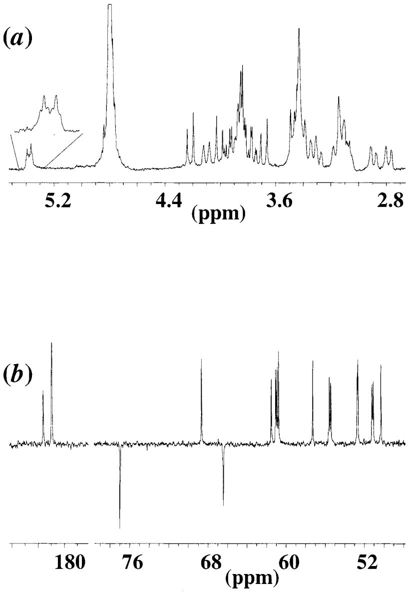

Figure 5. ^{1}H NMR spectrum (a) and ^{13}C (*J*-modulated) NMR spectrum (b) of 0.1 M Bi(DO3A-Bu) in D$_2$O at pH 8.73 at 298 K.

Bi(DO3A-Bu). As it is seen in Figure 5a,b, Bi(DO3A-Bu) exhibits complicated ^{1}H and ^{13}C NMR spectra. From these spectra, we can set out to find which of the three alcoholic OH groups of the "butriol" group is coordinated to the Bi^{3+}. With regard to the dissociation constant of Bi(DO3A-Bu), $-\log K_d = 7.53$, it is probable that at pH 8.73, where the spectrum (Figure 5a) was recorded, the coordinated alcoholic OH group of the ligand is predominantly deprotonated. In this respect, inspection of the ^{1}H NMR spectrum is very useful, because a proton signal is very strongly shifted to the lower fields, since it appears at 5.35 ppm (see insert in Figure 5a). A comparison of the integrated signal intensities shows that this signal is produced by one proton, which must be close to the coordinated alcoholic OH group because it is strongly deshielded.[31,32] The multiplet structure (a doublet of triplets by scalar coupling to one and two other protons) indicates that this

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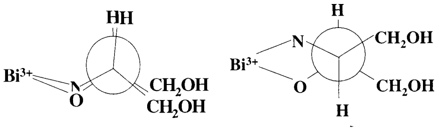
Scheme 4. Possible Conformations of BiDOTA-Butriol Side Chain



a SS or RR

b RS or SR

CH proton has a CH and a CH_2 group in its immediate neighborhood and is most probably attached to the ch3 carbon atom (Scheme 1), while the coordinated and deprotonated OH group also bonds to this carbon atom.

In order to find more evidence, we need to understand and interpret the ^1H and ^{13}C NMR spectra of Bi(DO3A-Bu). Therefore, we recorded 2D homonuclear and heteronuclear correlation spectra (see Figure S5a,b in Supporting Information). A comparison of the spectra, presented in Figures 5a and S5a, suggests that the proton signal at 5.35 ppm is part of the spin system and is coupled to three protons. These three protons form multiplets at 3.45, 3.76, and 3.85 ppm. However, it turns out from the HSQC spectrum (Figure S5b) that the proton at 5.35 ppm correlates to a CH carbon atom at 77 ppm (in the *J*-modulated ^{13}C NMR spectrum, the CH carbon signals appear in the negative phase). These findings indicate that the only possibility is that this proton is bonded to the ch3 carbon atom (Scheme 1), as stated already. Through the use of the correlation spectra, we could continue the assignment of the peaks of the "butriol" side chain. It follows from the HSQC spectrum (in Figure S5b) that the proton multiplets at 3.45, 3.76, and 3.85 ppm must belong to the protons which are on the ch2 carbon atom at 66.4 ppm and ch4 at 68.7 ppm.

Finally, the protons on the ch1 carbon atom appear as a multiplet at 3.78 ppm (see Figure S5a), and the corresponding carbon signal is at 60.8 ppm. The valuable vicinal (^3J) coupling constants for the butriol chain can be calculated from the spectrum, as follows: $^3J_{HH}[C(ch2)H-C(ch3)H] =$ 9.7 Hz, $^3J_{HH}[C(ch4)H_2-C(ch3)H] \cong 3$ Hz (from the triplet at 5.35 ppm), $^3J_{HH}[C(ch2)H-C(ch1)H_2] = 4.6$ Hz, and $^3J_{HH}[C(ch4)H_2-C(ch3)H] = 2.9$ Hz (from the group of peaks at 3.68 ppm). The lower coupling constants at around 3 Hz indicate that the attached protons are in the *gauche* position. The two CH protons with large coupling constants can be in the *trans* (a dihedral angle close to 180°) or in the *eclipsed* position (a dihedral angle near to 0°, see Scheme 4).

The ligand DO3A-Bu contains two asymmetric carbon atoms, ch2 and ch3. A simple molecular model clearly indicates that only the *eclipsed* position can give this large coupling constant $^3J_{HH}[C(ch2)H-C(ch3)H]$ if the ligand is in *S*,*S* or *R*,*R* configuration (see Scheme 4). However, if the ligand is in the racemic *R*,*S* or *S*,*R* configuration (enantiomers that are indistinguishable on NMR), then the *trans* position is possible, this latter being more favorable than the previous ones (Scheme 4a,b). Since we prepared the complex from the racemic ligand, conformation b is present, which corresponds to the *R*,*S* or *S*,*R* configuration of the butriol chain and *trans* position between ch2 and ch3 protons. The molecule can hold this *trans* position even in the probable $\Delta \rightleftharpoons \Lambda$ change in helicity.

From this point, the assignment cannot be continued in this way, because the chain is a separated spin system. Further investigation of the spectra allows the conclusion that the coordination of the "butriol" side chain results in complete asymmetry in the ligand structure, which, besides the complicated proton spectrum, leads to as many CH_2 carbon signals in the ^{13}C NMR spectrum as there are CH_2 carbon atoms in the molecule. In the Supporting Information, we describe the further assignments of the ^1H and ^{13}C NMR spectra of the Bi(DO3A-Bu) complex. From the relatively broad ^1H NMR peaks, we can conclude that the ring probably keeps the fluxional properties of the BiDOTA skeleton, causing this broadening. However, the lack of a single crystal X-ray structure prevented us to work out the details of it.

Concluding Remarks

The Bi(III) forms complexes of high stability with the cyclen derivative ligands DOTA and DO3A-Bu. In Bi(DO3A-Bu), the middle alcoholic OH group of the "butriol" deprotonates in the pH range 6−8. Therefore, at physiological pH about 50% of the Bi(DO3A-Bu) complex, which is a potential X-ray contrast agent, has negative charge.

The solid state structure of Bi(DOTA) is similar to that of the DOTA complexes of lanthanides, but no water molecule is coordinated in the inner sphere of Bi^{3+}. The absence of the water molecule in the inner coordination sphere of the Bi(DOTA) complex is probably due to the "lone" electron pair of the central atom and the shorter $Bi^{3+}-N$ bond distances which cause more compact coordination than in the case of the lanthanides.

The Bi(DOTA) complex shows fluxionality, both changes in ring conformation and acetate arm helicity in a synchronous way, probably via a symmetric transition state. The relatively large negative entropy of activation also supports the lack of the water molecule from the inner coordination sphere. Since Bi^{3+} is different in coordination properties from the lanthanides, the similarity in the dynamics of their DOTA complexes points to the fact that these dynamics look like the own feature of the ligand rather than the result of a special way of coordination.

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Supporting Information Available: Additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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