Inorg. Chem. **2003**, *42*, 4891−4897

Enantioselective Recognition between Chiral α-Hydroxy–Carboxylates and Macrocyclic Heptadentate Lanthanide(III) Chelates

Enzo Terreno,[†] Mauro Botta,[‡] Franco Fedeli,[§] Bruna Mondino,[†] Luciano Milone,[†] and Silvio Aime*[,†]

Dipartimento di Chimica IFM, Università degli Studi di Torino, Via P. Giuria 7, 10125, Torino, Italy, Dipartimento di Scienze e Tecnologie Avanzate, Università del Piemonte Orientale "Amedeo Avogadro", C.so Borsalino 54, 15100, Alessandria, Italy, and Laboratorio Integrato di Metodologie Avanzate, Bioindustry Park del Canavese, V. Ribes 5, 10010, Colleretto Giacosa (To), Italy

Received March 26, 2003

Three novel heptacoordinated Ln(III) complexes (Ln = Gd and Yb) have been synthesized and investigated by ^{1}H NMR spectroscopy. These complexes contain two stereogenic centers, one associated with a $\delta\delta\delta\delta$ or $\lambda\lambda\lambda\lambda$ conformation of the ethylenediamine moieties in the tetraazamacrocycle and the latter arises from the orientation (Δ or Λ) of the coordinating arms. Evidence has been gained for the occurrence of a fast exchange between all the possible conformers. Upon addition of several (*S*)-α-hydroxy–carboxylate substrates, the formation of stable ternary adducts has been obtained. Their ^{1}H NMR spectra are consistent with the presence of two diastereoisomers differing in the conformation adopted by the macrocyclic ligand wrapping the lanthanide(III) ion. The interaction leading to the formation of the ternary complexes is enantioselective depending on the hydrophilicity of the α-hydroxy–carboxylate.

Introduction

Chiral resolution by metal complexes is a topic of high relevance in several fields of chemistry, including catalysis and biochemistry.

As far as the latter topic is concerned, many attempts have been made for developing enantioselective probes able to recognize chiral substrates of biological interest. In principle, this aim may be pursued either by using enantiopure molecules able to resolve the chirality of the biological substrate or by exploiting the ability of the substrate itself to interact enantioselectively with a racemic mixture of the probe.

Metal complexes have often been considered for such applications, and paramagnetic lanthanide(III) chelates have been shown to be particularly favorable systems.[1,2] For instance, the ability of Ln(III) β-diketonates to act as chiral NMR shift reagents has been well-known for more than 30 years.[3] Moreover, lanthanide(III) complexes have been found to be useful in a number of biomedical applications, including their use as contrast agents and shift reagents in magnetic resonance-based techniques (MR imaging and spectroscopy),[4,5] as probes in nuclear medicine applications,[6,7] and as luminescent sensors in optical methods.[8,9]

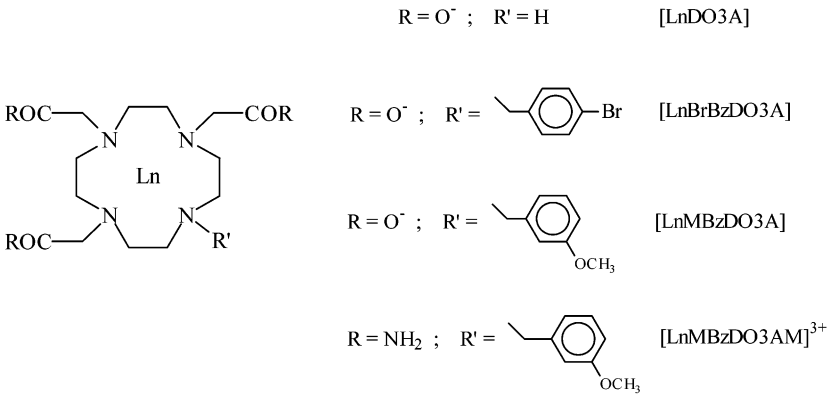
When paramagnetic Ln(III) complexes are used as NMR probes in experiments for chiral discrimination, most often the enantiopure form of the metal complex is used for resolving the enantiomeric pair of a given substrate.[10−15] Few

* To whom correspondence should be addressed. Telephone: 0039-011-6707520. Fax: 0039-011-6707855. E-mail: silvio.aime@unito.it.
[†] Università degli Studi di Torino.
[‡] Università del Piemonte Orientale "Amedeo Avogadro".
[§] Bioindustry Park del Canavese.

(1) Tsukube, H.; Shinoda, S. *Chem. Rev.* **2002**, *102*, 2389−2404.
(2) Parker, D.; Dickins, R. S.; Puschmann, H.; Crossland, C.; Howard, J. A. K. *Chem. Rev.* **2002**, *102*, 1977−2010.
(3) Whitesides, G. M.; Lewis, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 6979−6980.
(4) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.
(5) Balschi, J. A.; Clarke, K.; Stewart, L. C.; Bernard, M.; Ingwall, J. S. In *Methods in Biomedical Magnetic Resonance Imaging and Spectroscopy*; Young, I. R., Ed.; Chichester, U.K., 2000; Vol. 2, pp 871−879.
(6) Nayak, D.; Lahiri, S. *J. Radioanal. Nucl. Chem.* **1999**, *242*, 423−432.
(7) Shih, J.-L.; Brugger, R. M. *Med. Phys.* **1992**, *19*, 733−744.
(8) Horrocks, W. deW., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384−392.
(9) Parker, D. *Coord. Chem. Rev.* **2000**, *205*, 109−130.
(10) Wenzel, T. J.; Morin, C. A.; Brechting, A. A. *J. Org. Chem.* **1992**, *57*, 3594−3599.
(11) Rothchild, R.; Wyss, H. *Spectrosc. Lett.* **1994**, *27*, 225−246.

10.1021/ic034321y CCC: $25.00
Published on Web 07/10/2003

Chart 1. Ln(III) Complexes Investigated in This Work



examples are reported in which an enantiomer of the substrate is used for discriminating a racemic mixture of the metal complex.[16−18]

In principle, the recognition pathway between the two interacting partners can follow different routes, including the formation of ion-pairs, the setup of hydrophobic interactions, or the formation of ternary complexes.

Lanthanide ions usually exhibit coordination numbers (CN) ranging from 6 to 10, but in macrocyclic ligands based on the tetraazacyclododecane structure CN values are primarily restricted to 8−9.[19]

In the past decade, there has been a growing interest toward macrocyclic DO3A-like heptadentate Ln(III) complexes, mainly for their ability to interact with a wide array of anionic substrates.[20−25] In these chelates, when the Ln(III) ion is nona-coordinated (CN = 9), the interaction occurs through the replacement of the solvent molecule(s) coordinated to the metal ion by donor atoms of the substrate molecule. In such a case, adducts called ternary, mixed, or highly coordinated complexes may form. Of course, the coordinating ability of the "guest" molecule is dependent on both the affinity of its donor atoms toward the Ln(III) ion and, for bifunctional guests, on the stability of the additional chelate ring formed with the metal.

Recently, it has been reported that the Yb(III) complex of a cationic heptadentate triamide DO3A derivative can act as NMR chiral derivatizing agents toward lactate.[26] In fact, the enantiopure form of this chelate is able to interact quantitatively with the enantiomers of lactate, leading to two diastereisomers characterized by different ^{1}H NMR spectra. It is noteworthy that the authors did not observe any enantioselectivity in this interaction when mandelate, i.e., a more sterically demanding substrate, was used.

In this work, we report the observation that the interaction between the racemic mixture of the heptadentate DO3A-like Yb(III) complexes shown in Chart 1 and several enantiopure α-hydroxy−carboxylates is enantioselective. Moreover, we found that the enantioselectivity is related to the hydrophilicity of the interacting anionic substrate.

Experimental Section

Synthesis of the Ligands. TAZA and DO3A (TAZA = 1,4,7,10-tetraazacyclododecane; DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) were kindly provided by Bracco Imaging SpA (Milano, Italy).

MBzDO3A ligand (MBzDO3A = 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) was synthesized as follows: TAZA (0.25 mol) was N-alkylated with 3-(methoxyphenyl)methyl bromide (0.05 mol; 1:1 solvent acetonitrile/water; room temperature; overnight); the acetonitrile is then removed by slow evaporation, and the remaining aqueous solution was extracted with ethyl acetate. Then, the organic fractions were evaporated, giving a pale yellow oil (ca. 10 g) containing both the mono- and bis-alkylated TAZA derivatives. This crude product (7.9 g) was suspended in water, and bromoacetic acid (0.1 mol) was added dropwise. Then, the pH of the solution was brought up to 10 by adding 10 N NaOH. After heating (50−60 °C) for 6 h, the solution was filtered and acidified with 10% HCl up to pH 2.5. The final product was purified by liquid chromatography. A first eluition was carried out by using Amberlite XAD 1600 (eluent: gradient of methanol up to 40% in water) in order to separate the product from the bis-alkylated TAZA derivatives. Then, a second eluition was performed by using Duolite C20 MB (eluent: 2.5 M

(12) Jiang, J. L.; Li, A. H.; Jang, S. Y.; Chang, L.; Melman, N.; Moro, S.; Ji, X. D.; Lobkovsky, E. B.; Clardy, J. C.; Jacobson, K. A. *J. Med. Chem.* **1999**, *42*, 3055−3065.
(13) Inamoto, A.; Ogasawara, K.; Omata, K.; Kabuto, K.; Sasaki Y. *Org. Lett.* **2000**, *2*, 3543−3545.
(14) Watanabe, M.; Hasegawa, T.; Miyake, H.; Kojima, Y. *Chem. Lett.* **2001**, *1*, 4−5.
(15) Di Bari, L.; Lelli, M.; Pintacuda, G.; Salvadori, P. *Chirality* **2002**, *14*, 265−273.
(16) Aime, S.; Botta, M.; Parker, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **1995**, 2259−2266.
(17) Aime, S.; Botta, M.; Geninatti Crich, S.; Terreno, E.; Anelli, P. L.; Uggeri, F. *Chem. Eur. J.* **1999**, *5*, 1261−1266.
(18) Corsi, D. M.; van Bekkum, H.; Peters, J. A. *Inorg. Chem.* **2000**, *39*, 4802−4808.
(19) Aspinall, H. C. *Chem. Rev.* **2002**, *102*, 1807−1850.
(20) VanderElst, L.; Vanhaverbeke, Y.; Goudemant, J. F.; Muller, R. N. *Magn. Res. Med.* **1994**, *31*, 437−444.
(21) Bruce, J. I.; Dickins, R. S.; Govenlock, L. J.; Gunnlaugsson, T.; Lopinski, S.; Lowe, M. P.; Parker, D.; Peacock, R. D.; Perry, J. J. B.; Aime, S.; Botta, M. *J. Am. Chem. Soc.* **2000**, *122*, 9674−9684.
(22) Lowe, M. P.; Parker, D.; Reany, O.; Aime, S.; Botta, M.; Castellano, G.; Gianolio, E.; Pagliarin, R. *J. Am. Chem. Soc.* **2001**, *123*, 7601−7609.
(23) Aime, S.; Botta, M.; Mainero, V.; Terreno, E. *Magn. Res. Med.* **2002**, *47*, 10−13.
(24) Aime, S.; Delli Castelli, D.; Fedeli, F.; Terreno, E. *J. Am. Chem. Soc.* **2002**, *124*, 9364−9365.
(25) Faulkner, S.; Burton-Pye, B. P.; Khan, T.; Martin, L. R.; Wray, S. D.; Skabara, P. J. *Chem. Commun.* **2002**, 1668−1669.
(26) Dickins, R. S.; Love, C. S.; Puschmann, H. *Chem. Commun.* **2001**, *22*, 2308−2309.

NH_4OH) in order to separate the MBzDO3A ligand from salts and from the excess of bromoacetic acid. A 5.31 g amount of MBzDO3A (yield, 60%) was obtained.

^{1}H NMR (D_2O, *t*-BuOH as reference): δ 7.42 (t, 1H), 7.2 (d, 1H), 7.1 (s, 1H), 7.03 (d, 1H), 3.8 (s, 3H), 3−3.7 (m, 24H), 1.3 (s, 3H).

^{13}C NMR (D_2O, *t*-BuOH as reference): δ 177.0 173.2, 160.7, 137.5, 132.4, 124.8, 117.9, 115.9, 58.9, 57.2, 57.0, 53.4, 52.3, 51.8, 51.5.

MS (MALDI-TOF). Calcd for $C_{22}H_{34}N_4O_7$: 466.5 amu (atomic mass unit). Found: 467.4 (MH^+).

MBzDO3AM ligand (MBzDO3AM = 10-[(3-methoxyphenyl)-methyl]-1,4,7-tris[(aminocarbonyl)methyl]-1,4,7,10-tetraazacyclododecane) was synthesized by starting from the crude product of the TAZA alkylation described above. This product (4 g) was dissolved in dry acetonitrile, and then bromoacetamide (0.044 mol) and K_2CO_3 (0.055 mol) were added under stirring at room temperature. After 6 h, the suspension was filtered and the solid product (containing K_2CO_3, KBr and the ligand) was suspended in water and refiltered to isolate the MBzDO3AM ligand (white solid, 3.5 g; yield, 55%).

^{1}H NMR (CD_3OD): δ 7.23 (t, 1H), 6.9 (d, 1H), 6.86 (s, 1H), 6.82 (d, 1H), 3.7 (s, 3H), 3.55 (s, 2H), 3.1 (s, 2H), 3.0 (s, 4H), 2.7 (broad, 22H).

^{13}C NMR (CD_3OD): δ 176.7 , 176.3, 160.7, 140.8, 129.9, 122.3, 115.8, 113.0, 60.5, 58.6, 55.2, 54.7, 54.2, 53.5.

MS (MALDI-TOF). Calcd for $C_{22}H_{37}N_7O_4$: 463.6 amu (atomic mass unit). Found: 464.3 (MH^+).

BrBzDO3A ligand was synthesized and characterized according to the published procedure.[27]

Synthesis of the Ln(III) Complexes. The Ln(III) complexes (Ln = Gd and Yb) were synthesized in water at room temperature by adding equimolar amounts of $LnCl_3$ and one of the above-mentioned ligands. The pH was monitored during the synthesis in order to keep it in the 6−8 range. The final concentration of the metal chelates was checked through the Evans' method.[28]

NMR Measurements. All the NMR spectra were recorded on a Bruker Avance 300 spectrometer (Karlsruhe, Germany) operating at 7.05 T (proton Larmor frequency of 300 MHz).

The water proton longitudinal relaxation rates were measured with a Stelar Spinmaster spectrometer (Mede (Pv), Italy) operating at 0.47 T (proton Larmor frequency of 20 MHz). T_1 values were obtained by using the standard inversion−recovery method. The reproducibility of the T_1 data was ±1%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper−constantan thermocouple (uncertainty of ±0.1 °C).

The determination of the binding affinity between the Gd(III) chelates and the α-hydroxy−carboxylates investigated in this work has been carried out by using the PRE (proton relaxation enhancement) methodology whose experimental and theoretical aspects are reported in the literature.[29]

Results and Discussion

Figure 1 reports the ^{1}H NMR spectra of [Yb(MBzDO3AM)]$^{3+}$ (7.05 T, pH 6.5) recorded in the temperature range 278−298 K. As expected for a paramagnetic Yb(III) chelate, the proton resonances spread out over a wide range of chemical shifts (from ca. −80 to +130 ppm at the lowest temperature).

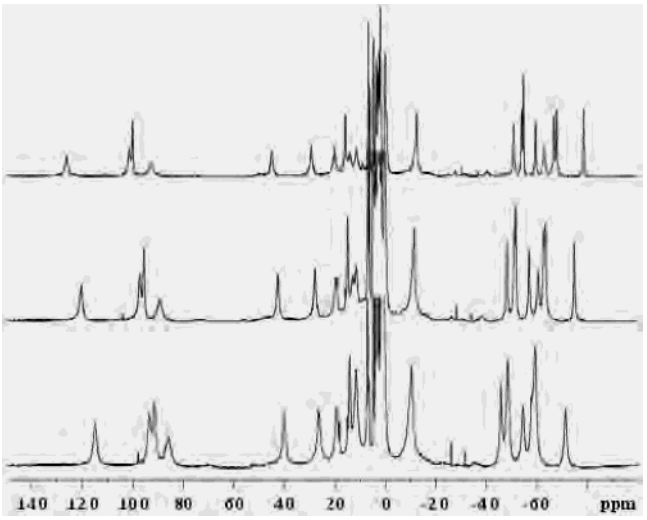


Figure 1. ^{1}H NMR spectra of an aqueous solution of [Yb(MBzDO3AM)]$^{3+}$ at different temperatures: from top to bottom 278, 288, and 298 K (7.05 T; pH 6.5).

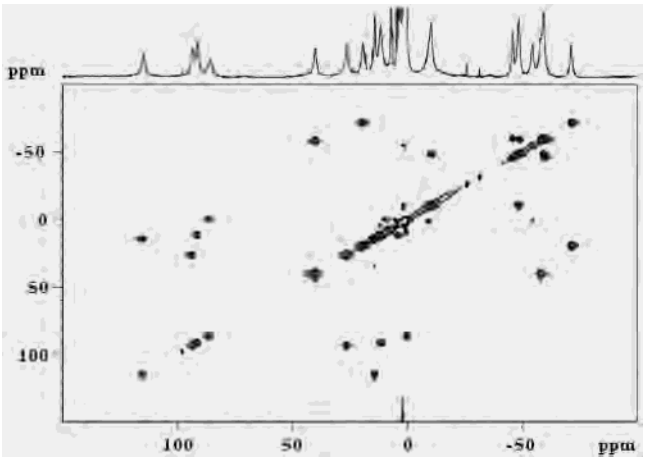


Figure 2. 2D-EXSY spectrum of an aqueous solution of [Yb(MBzDO3AM)]$^{3+}$ at 298 K (7.05 T; pH 6.5; mixing time, 10 ms).

The number of resonances observed in the spectra is consistent with the presence of a single predominant species in solution. However, the observation of a slight broadening of the resonances upon increasing the temperature is an indication of the occurrence of an exchange process. Further support for this suggestion has been gained by a 2D-EXSY experiment performed at 298 K (Figure 2). The cross-peak pattern, correlating pairs of exchanging protons, displays a close similarity with that observed for the main isomer in the related DOTA complex.[30]

The MBzDO3AM ligand does not possess a chiral center, but it is well-established that in macrocyclic ligands the metal complexation can yield two stereogenic centers: one generated from the two possible conformations of the four ethylenediamine groups of the macrocycle ($\delta\delta\delta\delta$ or $\lambda\lambda\lambda\lambda$) and the other arising from the two possible orientations of

(27) Aime, S.; Gianolio, E.; Terreno, E.; Giovenzana, G. B.; Pagliarin, R.; Sisti, M.; Palmisano, G.; Botta, M.; Lowe, M. P.; Parker, D. *J. Biol. Inorg. Chem.* **2000**, *5*, 488−497.

(28) Corsi, D. M.; Platas-Iglesias, C.; van Bekkum, H.; Peters, J. A. *Magn. Reson. Chem.* **2001**, *39*, 723−726.

(29) Aime, S.; Fasano, M.; Terreno, E.; Botta, M. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; Chichester, U.K., 2001; pp 203−209.

(30) Aime, S.; Botta, M.; Ermondi, G. *Inorg. Chem.* **1992**, *31*, 4291−4299.

the coordinating pendant arms (Δ or Λ). On this basis, it is expected that the [Yb(MBzDO3AM)]$^{3+}$ complex may be present in solution as two pairs of enantiomers ($\Lambda(\delta\delta\delta\delta)$/$\Delta(\lambda\lambda\lambda\lambda)$ and $\Delta(\delta\delta\delta\delta)$/$\Lambda(\lambda\lambda\lambda\lambda)$). On the basis of the analogy with DOTA-like systems, the first enantiomeric pair adopts a square-antiprismatic structure (SAP), whereas in the latter the complex displays a twisted-square-antisprimatic (TSAP) coordination geometry.[2] Either the ring inversion or the arm rotation causes the enantiomeric interconversion. The observation of only one predominant species in the ^{1}H NMR spectrum of [Yb(MBzDO3AM)]$^{3+}$ suggests that only one of the two enantiomeric pairs is present in aqueous solution. Thus, the isomerization process detected in the 2D-EXSY experiment is the result of the simultaneous occurrence of ring inversion and arm rotation. Despite the large number of papers dealing with the solution structure of octadentate macrocyclic Yb(III) complexes,[2,4] few data are available for heptadentate Yb(III) chelates.

Recently, Dickins et al. reported combined NMR and X-ray studies on a triamide derivative of Yb(III)−DO3A characterized by the presence of 1-methyl-1-phenyl−methyl groups on the three acetamide arms.[26,31] This complex crystallizes in the $\Lambda(\delta\delta\delta\delta)$ form and, very interestingly, this is also the only conformation found in solution. Likely, the presence of three bulky substituents on the acetamide arms considerably hinders any enantiomerization process. Conversely, in [Yb(MBzDO3AM)]$^{3+}$, the interconversion between the enantiomers occurs very rapidly and cross-peaks between axial and equatorial protons (ring inversion) and between acetamide protons (arm rotation) are clearly detected in the 2D-EXSY spectrum (Figure 2). The assignment of the proton resonances can be carried out on the basis of the previously reported shifts induced by the Yb(III) ion on the protons of DOTA-like ligands (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid). For instance, it has been demonstrated that in this class of compounds the most downfield-shifted resonances correspond to the four axial protons (ax1) which point downward with respect to the N4 square plane.[31] Usually, such resonances are very useful indicators of the solution structure adopted by the complex because their chemical shifts are very sensitive either to the coordination geometry (SAP or TSAP)[2] or to the polarizability of the donor atoms coordinating the Yb(III) ion.[32,33] The chemical shift values for the ax1 protons of [Yb(MBzDO3AM)]$^{3+}$ fall in a range (80−120 ppm at 298 K) very close to the values observed for [Yb(DOTA)]$^{-}$ [30] and for the tris(1-methyl-1-phenyl-methyl)amide derivative of DO3A in water.[31] Since it has been demonstrated that both these complexes in solution adopt the SAP conformation, it is reasonable to assume that the enantiomeric pair present in solution for [Yb(MBzDO3AM)]$^{3+}$ may have the $\Lambda(\delta\delta\delta\delta)$/$\Delta(\lambda\lambda\lambda\lambda)$ coordination geometry.

Chart 2. Schematic Representation of the Five-Membered Ring Formed by an Heptadentate Ln(III) Complex and α-Hydroxy−Carboxylates

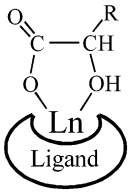


Table 1. Affinity Constants of (*S*)-Lactate with Heptadentate Gd(III) Complexes As Calculated from PRE Titrations at 298 K and pH 7

Gd(III) complex	K_A	Gd(III) complex	K_A
[Gd(DO3A)]	150 ± 10	[Gd(MBzDO3A)]	1500 ± 100
[Gd(BrBzDO3A)]	1500 ± 80	[Gd(MBzDO3AM)]$^{3+}$	8500 ± 450

Closely similar spectra were obtained for the other two Yb(III) complexes investigated in this study. However, the proton resonances of [Yb(DO3A)] are already very broad at temperatures close to 273 K, thus indicating a faster enantiomerization process. Nevertheless, it is likely that the predominant solution structure adopted by all three heptacoordinated DO3A-like Yb(III) chelates corresponds to the SAP geometry ($\Lambda(\delta\delta\delta\delta)$/$\Delta(\lambda\lambda\lambda\lambda)$ enantiomeric pair).

Ternary Complexes. One of the most peculiar properties of such Ln(III) chelates is represented by their ability to form ternary adducts with a variety of anionic substrates. In this context, α-hydroxy−carboxylates represent a very interesting class of "guest" molecules, because they may form a very stable five-membered ring with the metal ion (Chart 2).[23]

The following α-hydroxy−carboxylates (with S configuration at the α-CH group) have been considered: lactate (R = $-CH_3$); mandelate (R = phenyl); tartrate (R = $-CH(OH)COO^-$); malate (R = $-CH_2COO^-$); gluconate (R = $-CH(OH)CH(OH)CH(OH)CH_2OH$); trifluorolactate (R = $-CF_3$).

The binding affinity of the heptadentate Ln(III) complexes toward some of these α-hydroxy−carboxylates have been measured by means of PRE measurements.[29] This well-established method, which requires the use of Gd(III) complexes, is based on the fact that the ^{1}H-water relaxation rate is significantly influenced by the hydration state of the Gd(III) ion. The formation of the ternary adduct between the complex and the anionic substrate reduces the number of metal-coordinated water molecules, thus providing a useful route for a quantitative analysis of the binding equilibrium.

As expected, the affinity constant (K_A) depends on the structure of both the interacting partners. Generally, for a given substrate the affinity followed the order [Gd(MBzDO3AM)]$^{3+}$ > [Gd(XBzDO3A)] (X = M or Br) > [Gd(DO3A)] (Table 1, Figure 3), thus indicating the relevant roles played either by the electric charge of the metal complex or by the presence of the benzylic substituent on the macrocyclic nitrogen. On the other hand, for a given metal complex the affinity follows the order tartrate > lactate > malate > trifluorolactate (Table 2, Figure 4). The higher affinity displayed by tartrate is likely due to the presence of two equivalent α-hydroxy moieties, whereas the low K_A value obtained for trifluorolactate may be ascribed to the

(31) Dickins, R. S.; Aime, S.; Batsanov, A. S.; Beeby, A.; Botta, M.; Bruce, J. I.; Howard, J. A. K.; Love, C. S.; Parker, D.; Peacock, R. D.; Puschmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 12697−12705.
(32) Di Bari, L.; Pintacuda, G.; Salvadori, P. *J. Am. Chem. Soc.* **2000**, *122*, 9257−9264.
(33) Dickins, R. S.; Parker, D.; Bruce, J. I.; Tozer, D. J. *J. Chem. Soc., Dalton Trans.* **2003**, 1264−1271.

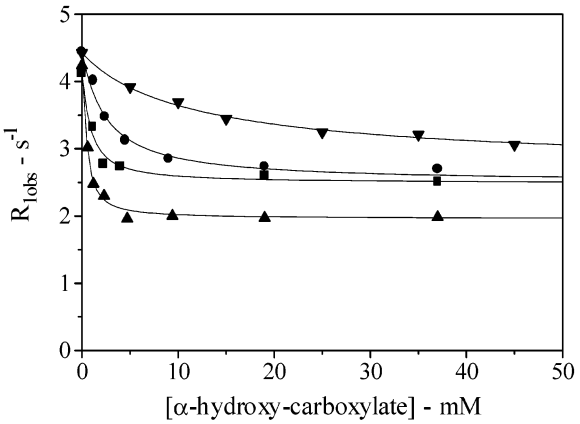


Figure 3. PRE titrations of [Gd(MBzDO3A)] with several α-hydroxy−carboxylates (S-form) {0.47 T (0.24 T for trifluorolactate); pH 7; 298 K; [Gd(MBzDO3A)], ca. 0.5 mM}: trifluorolactate (down triangle), malate (circle), lactate (square), tartrate (up triangle).

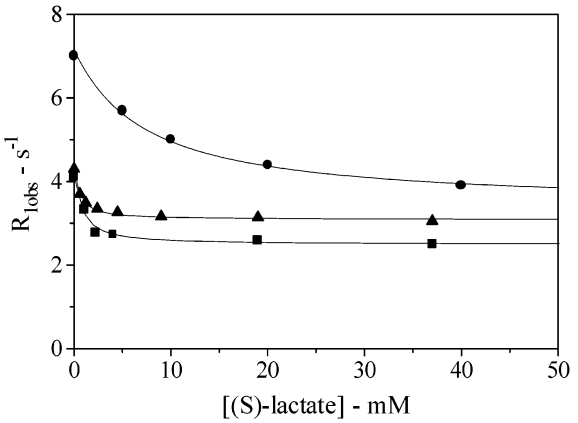


Figure 4. PRE titrations in the presence of (*S*)-lactate of the following {0.47 T (0.24 T for [Gd(DO3A)]); pH 7; 298 K; [Gd(MBzDO3A)] and [Gd(MBzDO3AM)]$^{3+}$, ca. 0.5 mM; [Gd(DO3A)], ca. 1 mM}: [Gd(MBzDO3A)] (square); [Gd(DO3A)] (circle); [Gd(MBzDO3AM)]$^{3+}$ (triangle).

Table 2. Affinity Constants of [Gd(MBzDO3A)] with α-Hydroxy−Carboxylates As Calculated from PRE Titrations at 298 K and pH 7

α-hydroxy−carboxylate	K_A	α-hydroxy−carboxylate	K_A
(*S*)-lactate	1500 ± 100	(*S*)-malate	500 ± 90
(*S*)-tartrate	3000 ± 320	(*S*)-trifluorolactate	90 ± 10

reduced electronic availability of the donor atoms of this substrate. The knowledge of the association constant between the two interacting molecules allows the preparation of solutions in which the metal complex is totally bound to a given anionic substrate.

In most cases, upon addition of the α-hydroxy−carboxylates to the solution of DO3A-like Ln(III) complexes, a new set of proton resonances appear in the ^{1}H NMR spectrum.[24,26] As an example, Figure 5 reports the low-field region of the ^{1}H NMR spectra of [Yb(BrBzDO3A)] upon addition of (*S*)-lactate.

The addition of small amounts of lactate leads to the appearance of a new set of signals that corresponds to the protons of the ternary adduct. Of course, in the presence of an excess of substrate only these resonances are detected in the spectrum. This finding is a clear indication that the

Figure 5. Low-field portion of the ^{1}H NMR spectra of an aqueous solution of [Yb(BrBzDO3A)] free (top) and upon addition of (*S*)-lactate with a molar ratio of 0.9:1 (middle) and 10:1 (bottom) (7.05 T; pH 6.5; 298 K).

exchange of the substrate coordinated to the Yb(III) ion is slow on the NMR time scale.[24]

Figure 6 reports the low-field portion of the ^{1}H NMR spectra of [Yb(MBzDO3AM)]$^{3+}$ free (top) and fully bound to the (*S*)-α-hydroxy−carboxylates investigated herein.

The formation of the ternary adduct is accompanied by two main changes in the NMR spectra, namely, (i) the resonances are much sharper and (ii) each signal is split into two peaks, whose ratio is dependent on the nature of the interacting substrate.

The sharpening of the NMR signals of the ternary adduct can be reasonably ascribed to the reduction of the dynamic motions of the metal complex. Actually, the $\Lambda(\delta\delta\delta\delta)/\Delta(\lambda\lambda\lambda\lambda)$ interconversion may still be observed also for the ternary adduct but at slightly higher temperature with respect to the free metal chelate.

The presence of two sets of signals for the ternary species can be accounted for either in terms of a different modality of chelation of the substrate to the metal ion or in terms of the formation of two diastereoisomers between the substrate and the enantiomeric pair of the metal complex. The former hypothesis may be excluded essentially on the basis of two pieces of experimental evidence: (i) the X-ray structures reported so far in the literature for ternary adducts formed by cationic Yb(III)−DO3A-like complexes and α-hydroxo−carboxylates indicate that the substrate adopts only one chelation mode with the carboxylic group in the equatorial position and the α-hydroxy group in the axial position above the Yb(III) ion;[26,31] (ii) the ^{13}C NMR spectrum recorded for the adduct formed by [Yb(MBzDO3AM)]$^{3+}$ and $^{13}C_3$-enriched (*S*)-lactate (Figure 7) indicates that the two signals for each carbon (at least for −CH and $-COO^-$) of the lactate bound to the Yb(III) complex have very close chemical shift values, thus confirming that their spatial position with respect to the metal center is almost identical.

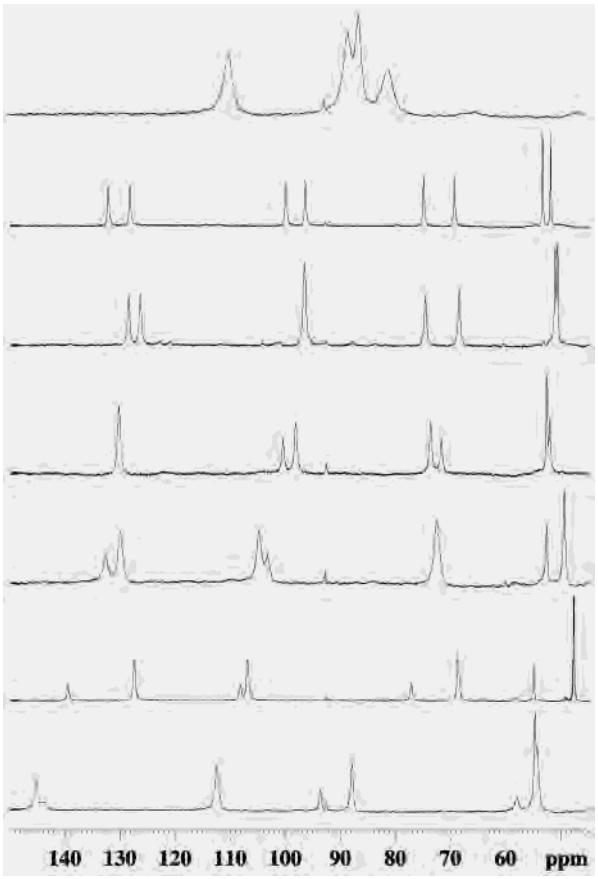


Figure 6. Low-field portion of the ^{1}H NMR spectra at 298 K and pH 6.5 of [Yb(MBzDO3AM)]$^{3+}$ free (top) and fully bound to the S-form of the following (from top to bottom): lactate, mandalate, malate, gluconate, tartrate, and trifluorolactate.

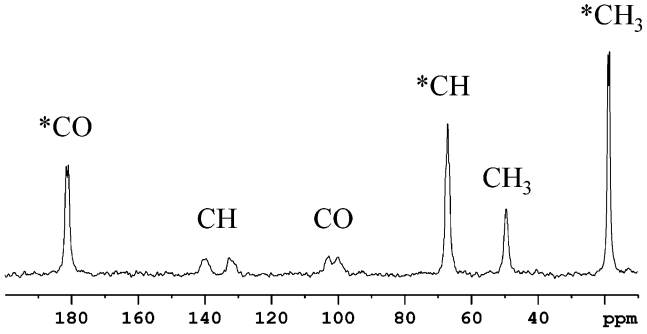


Figure 7. ^{13}C NMR spectrum (7.05 T; 298 K; pH 7) of a 50 mM solution of $^{13}C_3$-enriched (*S*)-lactate in the presence of 15 mM [Yb(MBzDO3AM)]$^{3+}$. Resonances of free lactate are labeled with an asterisk.

Indeed, the two sets of signals detected for the ternary adducts of Yb(MBzDO3AM)$^{3+}$ with α-hydroxy−carboxylates (see Figure 5) can be reasonably ascribed to the diastereoisomeric pair Δ(*λλλλ*)/(*S*)-substrate and Λ(*δδδδ*)/(*S*)-substrate. Therefore, the intensity ratio within each pair of protons of the two diastereoisomers provides a clear indication about the enantioselectivity of the interaction. This ratio is 1:1 for lactate and mandelate, 1.7:1 for malate, 2:1 for gluconate and citromalate, 2.7:1 for tartrate, and 3:1 for trifluoro-lactate. Interestingly, according to previously reported observations,[26] lactate and mandelate bind the two enantiomers of the Yb(MBzDO3AM)$^{3+}$ complex with similar

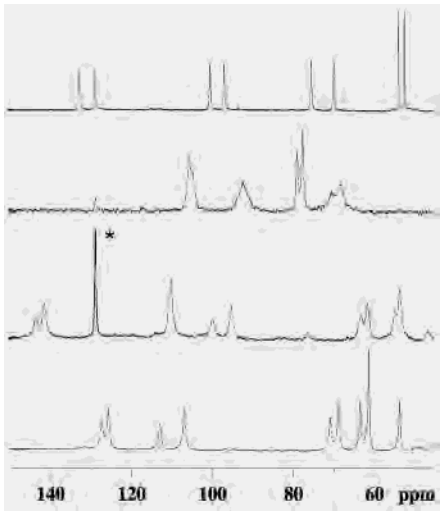


Figure 8. Low-field portion of the ^{1}H NMR spectra at 298 K and pH 6.5 of the ternary adduct with (*S*)-lactate of the following (from top to bottom): [Yb(MBzDO3AM)]$^{3+}$, [Yb(DO3A)], [Yb(MBzDO3A)], and [Yb(BrBzDO3A)]. The signal labeled with an asterisk refers to the presence of [Yb(DOTA)]$^-$ as impurity.

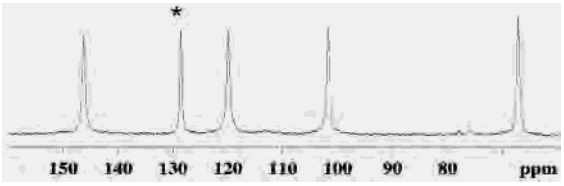


Figure 9. Low-field portion of the ^{1}H NMR spectrum at 298 K and pH 6.5 of the ternary adduct formed by (*S*)-tartrate and [Yb(MBzDO3A)]. The signal labeled with an asterisk refers to the presence of [Yb(DOTA)]$^-$ as impurity.

affinity, whereas the other α-hydroxy−carboxylates interact preferentially with a specific enantiomer. This finding suggests that the enantioselectivity cannot simply be driven by steric effects. Furthermore, it is worth noting that all the enantioselective α-hydroxy−carboxylates investigated herein possess hydrophilic moieties (−CF_3, −OH, and −COO^- groups) on the α-CH carbon. It is reasonable that such groups are responsible, likely through the formation of hydrogen bonds, for the stabilization of one diastereoisomer with respect to the other.

The enantioselectivity of the α-hydroxy−carboxylates toward heptadentate Ln(III)−DO3A-like complexes is also affected by the structure of the metal complex. In fact a signal intensity ratio of about 1.7:1 has been observed for the adduct with the (*S*)-lactates of [Yb(DO3A)], [Yb(MBzDO3A)], and [Yb(BrBzDO3A)] (see Chart 1), respectively (Figure 8).

Clearly, there is an enhanced enantioselectivity of α-hydroxy−carboxylates toward tricarboxylic vs triamidic Yb(III)−DO3A-like chelates. Further support arises from the observation that (*S*)-tartrate binds only one enantiomer of [Yb(MBzDO3A)] (Figure 9).

In summary, one may draw the conclusion that the stronger affinity of α-hydroxy−carboxylates to cationic complexes (e.g., [Yb(MBzDO3AM)]$^{3+}$) reduces its ability to discriminate between the two enantiomeric structures of the metal complex. Conversely, in the case of the neutral Yb(DO3A)-like complexes, the decreased electrostatic attraction implies

a more relevant role of the weaker interactions in pursuing the coordination of the substrate to the metal center in the macrocyclic complex. Likely, the setup of hydrogen bonding interaction becomes important for the determination of the observed enantioselectivity.

Acknowledgment. Financial support from MIUR and Bracco Imaging SpA (Milano, Italy) is gratefully acknowledged. This work was carried out under the framework of the COST-D18 action

IC034321Y