Coordination Isomers

Towards the Rational Design of Magnetic Resonance Imaging Contrast Agents: Isolation of the Two Coordination Isomers of Lanthanide DOTA-Type Complexes**

*Mark Woods, Zoltan Kovacs, Shanrong Zhang, and A. Dean Sherry**

The high spin state and long electronic relaxation time of the gadolinium(III) ion renders it an ideal basis for contrast media for use in magnetic resonance imaging (MRI). The through-space dipole–dipole interactions by which the unpaired f electrons of gadolinium catalyze the relaxation of proximate water-molecule protons are strongly distance dependent. For this reason, the protons of water molecules coordinated to gadolinium are the most effectively relaxed. Exchange of

[*] Prof. A. D. Sherry, Dr. M. Woods, Dr. Z. Kovacs
Department of Chemistry
University of Texas at Dallas
P.O. Box 830668, Richardson, Texas 75080 (USA)
Fax: (+1) 972-8832925
E-mail: sherry@utdallas.edu

Dr. Z. Kovacs
Macrocyclics Inc.
17815 Davenport Road, Suite 120
Dallas, Texas 75252 (USA)

Prof. A. D. Sherry, Dr. S. Zhang
Rogers Magnetic Resonance Center
Department of Radiology
University of Texas Southwestern Medical Center
5801 Forest Road, Dallas, Texas 75235 (USA)

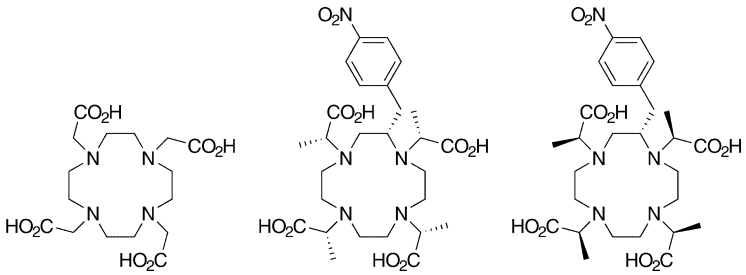
[**] M.W. thanks Craig Sheehan for the translation of an invaluable article (ref. [9]). The authors also gratefully acknowledge financial assistance from the National Institutes of Health (NIH) Biotechnology Research Program (Grant no. RR-02584), NIH Grant no. CA-84697, the Robert A. Welch Foundation (Grant no. AT-584), and the Texas Advanced Technology Program. DOTA = 1,4,7,10-tetraaza-1,4,7,10-tetrakis(carboxymethyl)cyclododecane.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200352234

these coordinated water molecules with the bulk solvent is essential for the efficiency or relaxivity of an effective contrast agent.[1] To maximize relaxivity, it is imperative that coordinated water molecules reside on the gadolinium ion just long enough to be relaxed; any longer and they needlessly occupy coordination sites that could be used for relaxing other water molecules. If the residence lifetime is too short, the relaxation process will be less effective. An optimal water residence lifetime (τ_M) of 30 ns has been calculated[1] by using the theory of Solomon, Bloembergen, and Morgan[2–6]

The toxicity of the aqua gadolinium ion necessitates that this paramagnetic agent must be administered in the form of a kinetically and thermodynamically robust chelate. Octadentate polyaminocarboxylate ligands such as DOTA (1,4,7,10-



tetraazacyclododecane-1,4,7,10-tetraacetic acid) have proven particularly effective for this purpose.[7] DOTA effectively sandwiches the central gadolinium ion between the four coplanar nitrogen atoms of the macrocycle and four coplanar oxygen atoms of the pendant arms. A water molecule occupying the apical position completes the coordination of the gadolinium ion. The macrocyclic ring in lanthanide–DOTA complexes adopts a [3333] conformation and the torsion angle between the square formed by the nitrogen atoms and a second square made up of coordinating oxygen atoms defines the coordination geometry of the complex. A large torsion angle of around 39° defines a capped square antiprismatic geometry (SAP), whereas a smaller torsion angle of around 25° defines a capped twisted square antiprismatic geometry (TSAP).[8]

These two coordination isomers are in dynamic equilibrium in solution and interconvert either by a flip in the macrocyclic ring conformation or by rotation of the oxygen pendant arms relative to the square defined by the nitrogen atoms.[9] Since the conformation of an ethylene bridge in a macrocycle may be defined as λ or δ (according to the sign of the torsion angle), the conformation of the macrocycle in DOTA may be defined as either (δδδδ) or (λλλλ). In addition, the orientation of the pendant arms (namely, the O-Gd-N torsion angle) may be defined as Δ or Λ. Thus, four stereoisomeric coordination geometries related as two enantiomeric pairs are possible: Δ(λλλλ) and Λ(δδδδ) (square antiprisms), and Δ(δδδδ) and Λ(λλλλ) (twisted square antiprisms). Sequential rotation of the arms and flipping of the ring interconvert the enantiomers.[9] Recent studies have suggested that the rate of water exchange in the two enantiomeric coordination isomers may differ by as much as two orders of magnitude.[10–13] The τ_M value measured for $[Gd(dota)]^-$ (244 ns) is a weighted average of the values for the coordination isomers present.[14] Although adequate for low-relaxivity agents, such a long average residence lifetime of water molecules is considered undesirable in the design of high-relaxivity contrast media.

Woods et al. recently demonstrated that an α substituent on an acetate arm of DOTA could "sterically lock" the conformation of that pendant arm in complexes.[12,15] The orientation of the arms is determined by the configuration at the α-carbon atom; the Λ orientation is generated by an *R* configuration, while an *S* configuration gives rise to a Δ orientation. We have shown that the conformation of the macrocycle may also be locked by substitution on the ring.[16] Although four methyl groups are necessary to "sterically lock" the macrocycle,[17,18] we found that the same effect can be achieved by using one nitrobenzyl substituent.[16] These observations suggest that a DOTA complex may be "locked" into one or other coordination geometry by suitably substituting both the macrocyclic ring and the pendant arms of the complex. The coordination isomer obtained may be determined by controlling the chirality at each center: if the configuration at the chiral centres in the macrocycle and in the pendant arms is the same, then a twisted square antiprism (TSAP) is obtained, whereas opposite configurations define a square antiprism (SAP).

With this in mind, two stereoisomers of the DOTA-like 2-(*p*-nitrobenzyl)-DOTMA (**1**; DOTMA = (1*R*,4*R*,7*R*,10*R*)-α,α′,α″,α‴-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)) were synthesized. The *p*-nitrobenzyl macrocycle was synthesized from L-*p*-nitrophenylalanine by known methods,[19] which led to an *S* configuration at the macrocyclic ring. By altering the configuration at the pendant arms (*RRRR* or *SSSS*), we envisioned that it should be possible to produce two ligands, each of which exclusively adopts one of the coordination geometries. *S*-2-(*p*-nitrobenzyl)-1,4,7,10-tetraazacyclododecane was alkylated by treatment with either D- or L-ethyl *O*-trifluoromethansulfonyl lactate in chloroform in the presence of Hünig's base, to afford the tetraethyl esters of the (*S-SSSS*) and (*S-RRRR*) isomers of 2-(*p*-nitrobenzyl)-DOTMA, respectively. After purification by column chromatography (SiO_2, 20% tetrahydrofuran in chloroform), the esters were hydrolyzed by treatment with lithium hydroxide in tetrahydrofuran and water, followed by acidification with hydrochloric acid. Purification of the two ligands at this stage proved impractical so they were used in this crude state to form the corresponding complexes upon addition of the appropriate lanthanide chloride (pH 5.5, 60 °C, 48 h). The complexes so obtained were purified by reverse-phase (C-18) semi-preparative HPLC.

Extended-sweep-width high-resolution ^1H NMR analysis of the two europium complexes $[Eu(S\text{-}RRRR\text{-}\mathbf{1})]^-$ and $[Eu(S\text{-}SSSS\text{-}\mathbf{1})]^-$ clearly reveals the presence of only one isomeric coordination geometry in each case (Figure 1). The shifts of the axial proton resonances (34–44 ppm) in the $[Eu(S\text{-}RRRR\text{-}\mathbf{1})]^-$ isomer are consistent with the expected SAP geometry, Δ(λλλλ), while those of the $[Eu(S\text{-}SSSS\text{-}\mathbf{1})]^-$ isomer, at 14–

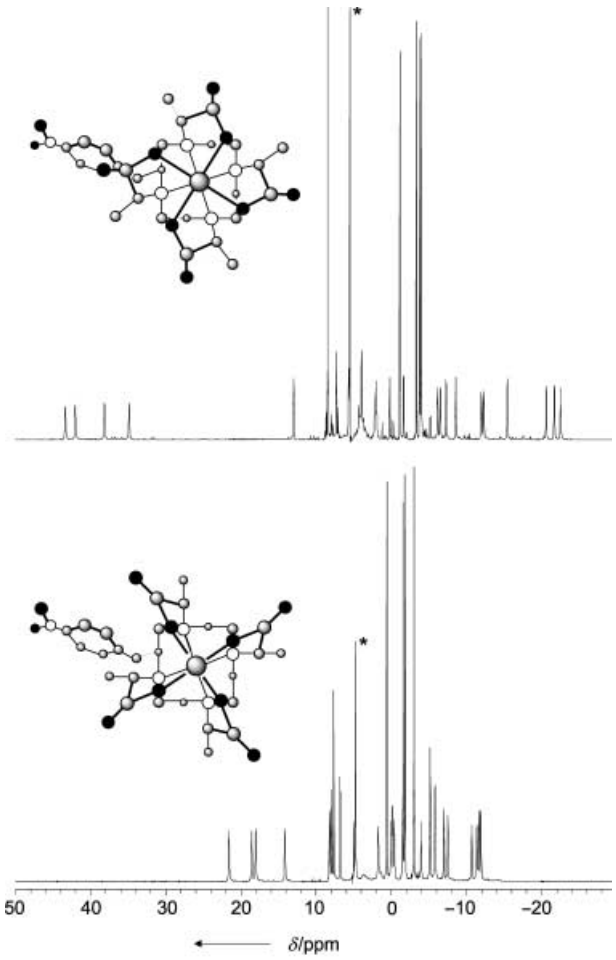


Figure 1. The extended-spectral-width high-resolution ^{1}H NMR spectra of the SAP [Eu(*S-RRRR*-**1**)]$^-$ (Δ(λλλλ); top) and TSAP [Eu(*S-SSSS*-**1**)]$^-$ (Δ(δδδδ); bottom) complexes, with schematic representations of the coordination geometries adopted by each isomer. The spectra were recorded at 270 MHz in D_2O at pH 5 and 25 °C (* denotes the HOD peak).

22 ppm, correspond to the more open TSAP geometry, Δ(δδδδ).[12] These results demonstrate that the coordination geometry of DOTA-like complexes may be controlled by the appropriate choice of substituent chirality at the ring and pendant arms. We next took advantage of this unique opportunity to examine some of the physical-chemical differences between the two coordination geometries.

Both isomers were expected to have one inner-sphere water molecule coordinating the central lanthanide ion. The quenching effect of proximate OH oscillators on the luminescence of europium may be used to establish the hydration state q of the europium ion.[20,21] This method is subject to errors arising from other closely diffusing OH or NH oscillators and often gives rise to non-integer hydration states. A revised method has recently been developed to account for the contributions of these diffusing oscillators.[22] The hydration states of [Eu(*S-RRRR*-**1**)]$^-$ and [Eu(*S-SSSS*-**1**)]$^-$ determined by this method show that one coordinated water molecule is present in each complex. The values of q given by the method of Horrocks and Sudnick[20,21] and the revised method of Beeby et al.[22] are given in Table 1. Regardless of the method employed, the hydration state of TSAP [Eu(*S-SSSS*-**1**)]$^-$ is around 0.2 water molecules smaller

Table 1: The hydration states, relaxivity, and water residence lifetimes of the europium/gadolinium complexes.

	Lanthanide Ion	[Ln(*S-RRRR*-**1**)]$^-$ Δ(λλλλ)	[Ln(*S-SSSS*-**1**)]$^-$ Δ(δδδδ)
q[a]	Eu	1.16	0.98
q'_{corr}[b]	Eu	1.00	0.81
r_1^{298}[c] [mM^{-1}s^{-1}]	Gd	4.96	5.20
r_1^{310}[d] [mM^{-1}s^{-1}]	Gd	3.84	3.84
τ_M^{298}[e] [ns]	Gd	120	15

[a] Hydration state measured by Horrock's method (q). [b] Hydration state measured by the revised method of Beeby (q'_{corr}) and co-workers. [c] Relaxivity at 25 °C. [d] Relaxivity at 37 °C (20 MHz). [e] Residence lifetimes measured by ^{17}O NMR spectroscopy at 67.8 MHz.

than that obtained for the SAP [Eu(*S-RRRR*-**1**)]$^-$ isomer. Given the strong distance dependence of the quenching effects of OH oscillators, this observation is consistent with a longer Eu^{3+}–water bond distance for [Eu(*S-SSSS*-**1**)]$^-$, with the twisted square antiprismatic geometry, than for the other isomer.[22]

The most important comparison, with respect to the design of high-relaxivity contrast agents, is that of the rate at which these water molecules exchange with the bulk solvent. The rate of water exchange is reflected in the transverse relaxation rate of ^{17}O nuclei in the bulk solvent. Thus, water exchange rates may be obtained by line-width analysis of the ^{17}O NMR spectrum of the solvent recorded as a function of temperature.[10] The two complexes [Gd(*S-RRRR*-**1**)]$^-$ and [Gd(*S-SSSS*-**1**)]$^-$ are characterized by extremely different transverse relaxation rate temperature profiles (Figure 2). The profile of [Gd(*S-RRRR*-**1**)]$^-$ (SAP), which rises to a maximum and then falls away with increasing temperature, is indicative of fairly slow exchange. In contrast, the profile of [Gd(*S-SSSS*-**1**)]$^-$ (TSAP) does not reach a maximum within a

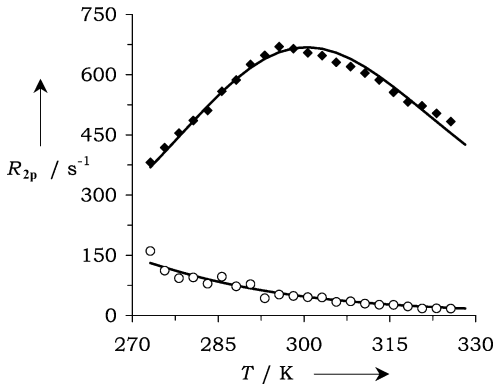


Figure 2. The profiles of the transverse relaxation rates (R_{2p}) of the SAP [Gd(*S-RRRR*-**1**)]$^-$ (◆) and the TSAP [Gd(*S-SSSS*-**1**)]$^-$ (○) complexes as a function of temperature, measured by ^{17}O NMR spectroscopy at 67.8 MHz. The differences in the shapes of the profiles are indicative of differences in water exchange rates.

measurable temperature range, which is indicative of much more rapid exchange. The values of τ_M obtained from fitting these data by using the procedures described by Merbach and co-workers[14,23] are consistent with this observation (Table 1). Indeed, the value obtained for the TSAP isomer [Gd(*S*-*SSSS*-**1**)]$^-$ is one order of magnitude smaller than that of the SAP isomer [Gd(*S*-*RRRR*-**1**)]$^-$. This difference is smaller than that observed by Dunand et al.[13] for the more slowly exchanging [Eu(dotam)]$^{3+}$) isomers (dotam = 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane). However, the two complexes [Gd(*S*-*RRRR*-**1**)]$^-$ and [Gd(*S*-*SSSS*-**1**)]$^-$ are related as individual coordination isomers of two separate diasteroisomeric systems and the relationship between these isomers therefore may not be the same as that between the [Eu(dotam)]$^{3+}$ isomers. Nonetheless, such a large difference in water exchange rates clearly renders the more quickly exchanging isomer preferable for use in the design of high-relaxivity MRI contrast agents.

The effect of these differing water exchange rates upon the relaxivities of the complexes [Gd(*S*-*RRRR*-**1**)]$^-$ and [Gd(*S*-*SSSS*-**1**)]$^-$ (20 MHz) is relatively small (Table 1). The water relaxivity of low-molecular-weight chelates at this frequency is strongly influenced by rotational reorientation of the complex (τ_R).[1] Since the two complexes are isomeric and are expected to exhibit identical τ_R values, the relaxivity will be limited to a similar extent in each case, regardless of water exchange rate. So, even though [Gd(*S*-*SSSS*-**1**)]$^-$ (TSAP) has a near-optimal water exchange rate, its relaxivity at 25 °C is only about 5 % higher than that of the more slowly exchanging [Gd(*S*-*RRRR*-**1**)]$^-$ (SAP). At 37 °C even that advantage has disappeared and both complexes exhibit the same relaxivity (Table 1). Indeed, the improvement in relaxivity over that of [Gd(dota)]$^-$ ($r_1 = 4.2\ \mathrm{mM^{-1}s^{-1}}$, 20 MHz, 25 °C)[24] is comparatively small. However, the benefits of an optimally exchanging system may only be evident upon slowing the rotation of the complex (longer τ_R). In practice, this is likely to be achieved by either covalent or noncovalent association of the chelate with a slowly rotating macromolecular structure. That experiment, however, was not within the scope of this paper.

In conclusion, we have demonstrated that the coordination geometry of a DOTA-type lanthanide complex may be controlled by careful consideration of the chirality when substituting both the macrocycle and the pendant arms. The bound water molecule lies further from the lanthanide ion in the twisted square antiprismatic geometry as compared to the square antiprism. This increased bond distance is reflected in more rapid exchange of the bound water molecule with the bulk solvent. The rate of water exchange observed for the twisted square antiprismatic geometry (15 ns) is close to the theoretical optimum value for the design of high-relaxivity contrast agents.

Received: June 26, 2003
Revised: September 16, 2003 [Z52234]

Keywords: coordination modes · lanthanides · macrocyclic ligands · MRI contrast agents

[1] S. Aime, M. Fasano, E. Terreno, *Chem. Soc. Rev.* **1998**, *27*, 19.
[2] N. Bloembergen, E. M. Purcell, R. V. Pound, *Phys. Rev.* **1948**, *73*, 679.
[3] I. Solomon, *Phys. Rev.* **1955**, *99*, 559.
[4] I. Solomon, N. Bloembergen, *J. Chem. Phys.* **1956**, *25*, 261.
[5] N. Bloembergen, *J. Chem. Phys.* **1957**, *27*, 572.
[6] N. Bloembergen, L. O. Morgan, *J. Chem. Phys.* **1961**, *34*, 842.
[7] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[8] M. Meyer, V. Dahaoui-Gindrey, C. Lecomte, R. Guilard, *Coord. Chem. Rev.* **1998**, *178–180*, 1313.
[9] S. Hoeft, K. Roth, *Chem. Ber.* **1993**, *126*, 869.
[10] S. Aime, A. Barge, M. Botta, D. Parker, A. S. De Sousa, *J. Am. Chem. Soc.* **1997**, *119*, 4767.
[11] S. Aime, A. Barge, J. I. Bruce, M. Botta, J. A. K. Howard, J. M. Moloney, D. Parker, A. S. De Sousa, M. Woods, *J. Am. Chem. Soc.* **1999**, *121*, 5762.
[12] M. Woods, S. Aime, M. Botta, J. A. K. Howard, J. M. Moloney, M. Navet, D. Parker, M. Port, O. Rousseaux, *J. Am. Chem. Soc.* **2000**, *122*, 9781.
[13] F. A. Dunand, S. Aime, A. E. Merbach, *J. Am. Chem. Soc.* **2000**, *122*, 1506.
[14] D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 9333.
[15] J. A. K. Howard, A. M. Kenwright, J. M. Moloney, D. Parker, M. Woods, M. Port, M. Navet, O. Rousseau, *Chem. Commun.* **1998**, 1381.
[16] M. Woods, Z. Kovacs, S. Zhang, A. D. Sherry, unpublished results. 2D ^{1}H exchange spectrospy (EXSY) was used to establish the mechanism of interconversion of the two diastereoisomeric coordination isomers of [Eu(2-(*p*-NO_2Bn)dota)]$^-$. The presence of only two of the four possible diastereoisomeric coordination isomers indicated that one exchange process had been "frozen out." The EXSY spectrum showed that the protons of the macrocycle remained in the same position, that is, axial to axial, equatorial to equatorial, which indicates that exchange occurs by arm rotation alone.
[17] R. S. Ranganathan, R. K. Pillai, N. Raju, H. Fan, H. Nguyen, M. F. Tweedle, J. F. Desreux, V. Jacques, *Inorg. Chem.* **2002**, *41*, 6846.
[18] R. S. Ranganathan, N. Raju, H. Fan, X. Zhang, M. F. Tweedle, J. F. Desreux, V. Jacques, *Inorg. Chem.* **2002**, *41*, 6856.
[19] O. Renn, C. F. Meares, *Bioconjugate Chem.* **1992**, *3*, 563; T. J. McMurry, M. Brechbiel, K. Kumar, O. A. Gansow, *Bioconjugate Chem.* **1992**, *3*, 108.
[20] W. D. Horrocks, Jr., D. R. Sudnick, *J. Am. Chem. Soc.* **1979**, *101*, 334.
[21] W. D. Horrocks, Jr., D. R. Sudnick, *Acc. Chem. Res.* **1981**, *14*, 384.
[22] A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams, M. Woods, *J. Chem. Soc. Perkin Trans. 2* **1999**, 2, 493.
[23] G. Gonzàlez, D. H. Powell, V. Tissières, A. E. Merbach, *J. Phys. Chem.* **1994**, *98*, 53.
[24] E. Toth, D. Pubanz, S. Vauthey, L. Helm, A. E. Merbach, *Chem. Eur. J.* **1996**, *2*, 1607.

