

HPLC separation of diastereomers of Ln^{III}-ethylenepropylene-triamine-pentaacetate complexes. Direct assessment of their water exchange rate†

László Burai, Éva Tóth* and André E. Merbach*

Ecole Polytechnique Fédérale de Lausanne, ICMB, BCH, Lausanne, Switzerland.

E-mail: eva.jakabtoth@epfl.ch; Fax: 41 21 693 9875; Tel: 41 21 693 9878

Received (in Cambridge, UK) 17th July 2003, Accepted 1st September 2003

First published as an Advance Article on the web 24th September 2003

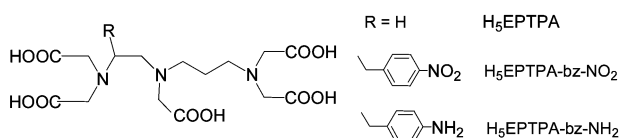
The diastereomers of two Ln^{III}-EPTPA derivatives have been separated by reversed-phase HPLC, and the water exchange rate on their Gd^{III} complexes has been directly determined by ¹⁷O NMR (H₅EPTPA = ethylenepropylene-triamine-pentaacetic acid).

The ligand ethylenepropylene-triamine-pentaacetate (EPTPA) (Scheme 1) has recently proved to be an ideal chelator of Gd^{III} for the development of high relaxivity, macromolecular contrast agents for Magnetic Resonance Imaging.¹ The water exchange rate on the Gd^{III} complex, which is one of the parameters to determine efficacy of contrast agents, is optimal to attain maximum relaxivities.² In addition, the sufficiently high thermodynamic stability of Gd^{III}EPTPA ensures safe *in vivo* application.¹

Introducing a pending arm in the 4-position of EPTPA generates a chiral centre. A second chiral centre arises when Gd^{III} is coordinated to the central nitrogen. Consequently, two diastereomers appear in solution (4*S*6*S*, 4*R*6*R* and 4*S*6*R*, 4*R*6*S*). Diastereomers may have different chemical and physical properties, thus their water exchange rate may also be different. Diastereomers can be separated on a RP-HPLC column as it was shown for Ln^{III} complexes of 4-aminobenzyl- and 4-ethoxybenzyl-DTPA (DTPA-bz-NH₂ and EOB-DTPA).^{3,4}

The nitrobenzyl derivative of EPTPA has recently been synthesized and the water exchange rate published on its Gd^{III} complex.¹ Here we report the HPLC separation of the two diastereomers of Ln^{III} complexes with two chiral EPTPA derivatives, EPTPA-bz-NO₂ and EPTPA-bz-NH₂ and the determination of the diastereomer ratio for the whole Ln³⁺ series.‡ For the first time, we measured individual water exchange rates on the two diastereomers of acyclic Gd^{III} complexes. For LnDOTA complexes it was previously observed that the exchange rate of the coordinated water may depend on the isomer conformation.⁵

The HPLC chromatograms of Ln(EPTPA-bz-NO₂)²⁻ and Ln(EPTPA-bz-NH₂)²⁻ solutions show two peaks (Fig. 1).§ As the starting ligands were racemic mixtures of 4*R* and 4*S* enantiomers, the two peaks represent the coelution of the 4*S*6*S*/4*R*6*R*, and 4*S*6*R*/4*R*6*S* enantiomers, respectively. Unfortunately, from the HPLC chromatogram one cannot decide which peak corresponds to which diastereomer. Therefore we simply assign the first peak in the Ln(EPTPA-bz-NO₂)²⁻ chromatogram to species **A** and the second to species **B**, similarly to assignments in the literature.⁴



Scheme 1 Structure of the ligands.

† Electronic supplementary information (ESI) available: Table S1–S4 and Figs. S1 and S2. See <http://www.rsc.org/suppdata/cc/b3/b308253a/>

The ratio of the **A** and **B** diastereomers is identical within the experimental error for the two EPTPA derivatives and does not change with pH (7 < pH < 10). However, it changes drastically along the lanthanide series (Fig. 2). This is rather remarkable since for the few DTPA-derivatives where diastereomer separation was performed by HPLC, the **A** : **B** ratio was always independent of the metal ion. (For instance, 65 : 35 was reported for Gd(EOB-DTPA)²⁻, 60 : 40 for La(EOB-DTPA)²⁻ and 60 : 40 for various Ln^{III}(DTPA-bz-NH₂)²⁻ complexes.)^{3,4} Moreover, in our case the **A** : **B** ratio has a maximum around the middle of the Ln^{III} series. Based on observation on Ln(DOTA)⁻ complexes,⁶ one possible explanation could be that with decreasing metal ion size there is no more space for the inner sphere water, thus what we see for the late lanthanides at the elution time of **B** is in reality a different species (without inner sphere water) eluted at the same time. The other possibility is that the size of the middle lanthanides matches the best with the specific steric conformation of diastereomer **A** and this gives rise to an increased stability of **A** over **B**. A maximum in the stability along the lanthanide series is not unusual for Ln^{III} complexes.⁷ Work is currently under progress to clarify this point.

It is also remarkable that on mixing a buffered ligand solution with Ln³⁺, the two isomers form in an equal ratio, independently of the metal. The **B** isomer is then slowly converted to **A** to reach equilibrium. This conversion, which can be conveniently followed by HPLC, is faster for the early, and slower for the late

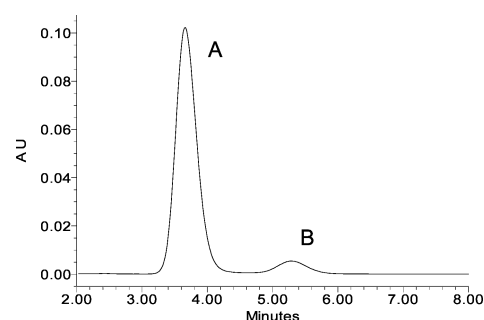


Fig. 1 Separation of the diastereomers **A** and **B** of Gd(EPTPA-bz-NO₂)²⁻.

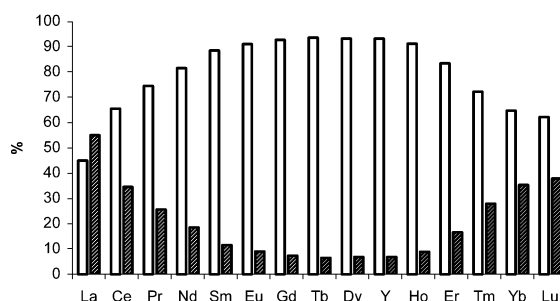


Fig. 2 The molar fraction of diastereomers **A** (white column) and **B** (dark column) for both Ln^{III}-EPTPA derivatives.

lanthanides. To our knowledge, such behaviour has never been observed.

For Gd(EPTPA-bz-NH₂)²⁻, the transformation of **B** to **A** was also monitored by recording ¹H NMR spectra as a function of time following the mixing of the ligand with Eu³⁺. In parallel, HPLC injections were done after the same periods of time. In the NMR spectrum two series of signals appear that we assign to the two diastereomers. Indeed, at each time point, the integral of their NMR peaks corresponds to the integral of the HPLC peaks (see ESI†). On the other hand, the **A** : **B** ratio of Gd(EPTPA-bz-NH₂)²⁻ determined from ¹H NMR does not change with temperature (0–50 °C) or pH (5 < pH < 9).

The fast formation and the subsequent, relatively slow transformation of **B** to **A** allow observing the difference in various properties of the two isomers. A parallel HPLC analysis of the samples after mixing ligand and metal will give the actual **A** : **B** ratio at any moment. We studied the water exchange on the two diastereomers of Gd(EPTPA-bz-NH₂)²⁻ and Gd(EPTPA-bz-NO₂)²⁻. The exchange rate published for Gd(EPTPA-bz-NO₂)²⁻ was evidently measured in an equilibrium solution (93 : 7 **A** : **B** ratio).¹ Now we followed the evolution of ¹⁷O 1/*T*₂ relaxation rates and chemical shifts after complex formation (25 °C). Concomitant to the transformation of **B** to **A**, the 1/*T*₂ relaxation rates increase with time for both complexes showing that the two diastereomers have different water exchange rates. Since the ¹⁷O chemical shifts are constant, we assume one inner sphere water for both diastereomers (data in ESI†).¶ To determine the water exchange rate for **A** and **B**, we fitted the experimental 1/*T*₂ values to the changing mole fraction of the bound water in **A** and **B** diastereomers (*P*_{*m*A} and *P*_{*m*B}):

$$\frac{1}{T_2} = \frac{1}{T_{2\text{sample}}} - \frac{1}{T_{2\text{ref}}} = \sum P_{mi} \frac{1}{\tau_{mi}} \frac{T_{2mi}^{-2} + \tau_{mi}^{-1} T_{2mi}^{-1} + \Delta\omega_m^2}{(\tau_{mi}^{-1} + T_{2mi}^{-1})^2 + \Delta\omega_m^2} \quad (1)$$

where *i* = **A** and **B**, $\sum P_{mi} = [\text{GdL}]/55.55$, τ_{mi} is the residence time of the inner sphere water molecules, and $1/\tau_{mi} = k_{\text{ex}i}$ is the water exchange rate. The *P*_{*m*} values were calculated for each time point from the **A** : **B** ratio obtained from the peak integrals in the corresponding HPLC chromatogram. $\Delta\omega_m$ is the chemical shift of the bound water, determined by the *A*/ħ hyperfine coupling constant, the *S* electron spin (7/2) and the *B* magnetic field:

$$\Delta\omega_m = \frac{g_L \mu_B S(S+1) B A}{3k_B T \hbar} \quad (2)$$

We assumed identical *A*/ħ for the two diastereomers of both Gd^{III} complexes ($-3.2 \times 10^6 \text{ rad s}^{-1}$).¹ As the system is in the fast exchange regime at 25 °C, 1/*T*_{2*m*} is influenced by the rates of both water exchange and 1/*T*_{1*e*} electronic relaxation.

$$\frac{1}{T_{2mi}} = \frac{S(S+1)}{3} \left(\frac{A}{\hbar} \right)^2 \tau_{\text{sl}i}; \frac{1}{\tau_{\text{sl}i}} = \frac{1}{\tau_{mi}} + \frac{1}{T_{1e}} \quad (3)$$

$$\left(\frac{1}{T_{1e}} \right)^{\text{ZFS}} = \frac{1}{25} \Delta^2 \tau_v \{ 4S(S+1) - 3 \} \left(\frac{1}{1 + \omega_s^2 \tau_v^2} + \frac{4}{1 + 4\omega_s^2 \tau_v^2} \right) \quad (4)$$

where ω_s is the Larmor frequency of the electron spin, Δ^2 is the trace of the zero field splitting (ZFS) tensor and τ_v is the correlation time for the modulation of the ZFS. These parameters have been previously determined for Gd(EPTPA-bz-NO₂)²⁻ from an ¹⁷O NMR and EPR study¹ and here we assume that they are identical for the **A** and **B** isomers. This was verified: we measured EPR peak-to-peak linewidths at X band as a function of the time after mixing the ligand with the Gd³⁺ solution and detected no change during the isomerization within the experimental error. Furthermore, the peak-to-peak linewidths were also similar for Gd(EPTPA-bz-NH₂)²⁻, thus we used the same electronic parameters for the two diastereomers in the fit of both Gd(EPTPA-bz-NO₂)²⁻ and Gd(EPTPA-bz-NH₂)²⁻ ($\Delta^2 = 0.4 \times 10^{20} \text{ s}^{-2}$; $\tau_v = 22 \text{ ps}$).¹

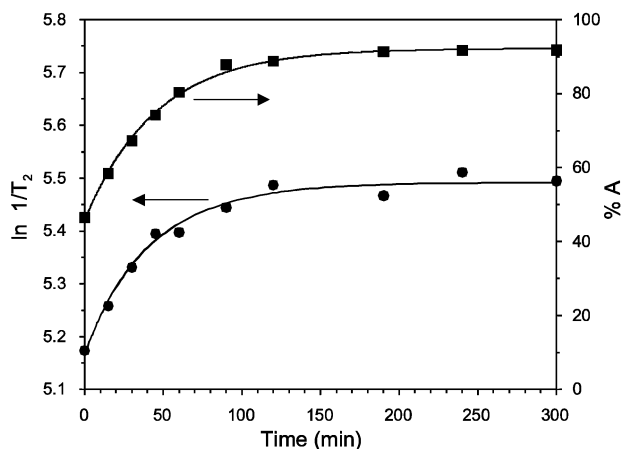


Fig. 3 Evolution of the ¹⁷O 1/*T*₂ relaxation rates at *B* = 4.7 T (circles) and the HPLC peak area for the **A** isomer (squares) as a function of the time for Gd(EPTPA-bz-NH₂)²⁻ after mixing the ligand with Gd³⁺ solution.

Fig. 3 shows the experimental ¹⁷O 1/*T*₂ relaxation rates and the fitted curves for Gd(DTPA-bz-NH₂)²⁻ (see ESI for experimental data and figure for Gd(EPTPA-bz-NO₂)²⁻†). The calculated *k*_{exⁱ}²⁹⁸ values are $(1.4 \pm 0.1) \times 10^8 \text{ s}^{-1}$ and $(2.6 \pm 0.2) \times 10^8 \text{ s}^{-1}$ for **A** and **B** of Gd(EPTPA-bz-NO₂)²⁻, and $(1.8 \pm 0.1) \times 10^8 \text{ s}^{-1}$ and $(4.4 \pm 0.2) \times 10^8 \text{ s}^{-1}$ for **A** and **B** of Gd(EPTPA-bz-NH₂)²⁻. For both complexes, the exchange rate approximately doubles from **A** to **B**. Based on the *k*_{exⁱ}²⁹⁸ values of the two Gd(EPTPA-bz-NO₂)²⁻ diastereomers we obtain $1.48 \times 10^8 \text{ s}^{-1}$ for the equilibrium state (93% **A**, 7% **B**) which agrees well with the average value published before, $1.50 \times 10^8 \text{ s}^{-1}$.¹ The average *k*_{exⁱ}²⁹⁸ for Gd(EPTPA-bz-NH₂)²⁻ is $1.98 \times 10^8 \text{ s}^{-1}$.

In conclusion, an HPLC analysis showed that the diastereomer ratio for Ln^{III} EPTPA-derivatives varies as a function of the cation size and has a maximum for the middle lanthanides. On the formation of these complexes the two diastereomers form in equal ratio and then one of them slowly converts into the other to reach equilibrium. This allowed us to assess water exchange for the first time on individual diastereomers. Their exchange rate is, though not spectacularly, different.

Notes and references

† The authors are very grateful to Sabrina Laus, Angélique Sour and Robert Ruloff for the synthesis of the ligands.

§ HPLC experiments were performed on a Waters 600 instrument equipped with a 10 μm C₁₈ XTerra® column (4.6 × 150 mm). The eluent was water containing 10 mM TRIS buffer and 5–10% acetonitrile (0.5–1 ml min⁻¹ flow rate). A dual wavelength UV-Vis detector was used for detection (220/280 nm for Ln(EPTPA-bz-NO₂)²⁻; 240/290 nm for Ln(EPTPA-bz-NH₂)²⁻).

¶ ¹⁷O NMR measurements were performed on Bruker 200 and 400 spectrometer at 298 K. 10 mM TRIS buffer (pH = 9.2) was used as reference. The samples contained 1–2% ¹⁷O enriched water.

- S. Laus, R. Ruloff, É. Tóth and A. E. Merbach, *Chem. Eur. J.*, 2003, **9**, 3555.
- É. Tóth, L. Helm and A. E. Merbach Relaxivity of Gadolinium(III) Complexes: Theory and Mechanism in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Eds. É. Tóth and A. E. Merbach, Wiley, Chichester, 2001, p. 45–120.
- C. H. Cummins, E. W. Rutter and W. A. Fordyce Jr., *Bioconjugate Chem.*, 1991, **2**, 180.
- H. Schmitt-Willich, M. Brehm, Ch. L. J. Ewers, G. Michl, A. Müller-Fahrnow, O. Petrov, J. Platzek, B. Radüchel and D. Sülzle, *Inorg. Chem.*, 1999, **38**, 1134.
- S. Aime, A. Barge, M. Botta, A. S. de Sousa and D. Parker, *Angew. Chem., Int. Ed.*, 1998, **37**, 2673.
- S. Aime, M. Botta, M. Fasano, M. P. M. Marques, C. F. G. C. Geraldes, D. Pubanz and A. E. Merbach, *Inorg. Chem.*, 1997, **36**, 2059.
- T. Moeller, D. F. Martin, L. C. Thompson, R. Ferrus, G. Feistel and W. J. Randall, *Chem. Rev.*, 1965, **65**, 1.