## Relaxivity and Transmetallation Stability of New Benzyl-Substituted Derivatives of Gadolinium–DTPA Complexes

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In our efforts of finding new specific contrast agents of higher relaxivity and selectivity, we have prepared the two new benzyl-functionalized DTPA ('diethylenetriamine pentaacetate') gadolinium complexes (*S*)-**3** and (*R*,*S*)-**4**, and compared their properties with those of the known regioisomers (*S*)-**2** and (*S*)-**1**. The theoretical fitting of the reduced transverse relaxation rates of the <sup>17</sup>O-nucleus of H<sub>2</sub>O gave values for the water-residence time ( $\tau_M$ ) of 86–143 ns at 310 K, values that are not limiting the proton relaxivity at body temperature. <sup>1</sup>H-NMRD (nuclear magnetic-relaxation dispersion) Profiles showed that the relaxivity of **1**–**4** ( $r_1$ =4.3–5.1 s<sup>-1</sup> mM<sup>-1</sup> at 20 MHz and 310 K) is higher than for the Gd–DTPA parent compound **5**. Transmetallation assessment demonstrated that all substituted compounds, except for (*S*)-**2**, are more stable than **5**. The highest stability towards Zn<sup>2+</sup>-induced transmetallation was achieved with complexes **3**, **1**, and **4** (in decreasing order). Apparently, the steric hindrance of the benzyl substituents in positions 5, 4, and 2, respectively, favorably reduces the accessibility of Zn ions. From a synthetic point of view, 4-substituted DTPA complexes of type **1** are more readily accessible than 5-substituted compounds of type **3**. Therefore, the former seem to be superior for linking substituted DTPA complexes to macromolecules or specific vectors.

**Introduction.** – Synthesis of paramagnetic complexes capable to target specific pathologies is often performed through amide linkages with vectorizing moieties. Such a functionalization, however, has an adverse effect on the exchange rate of coordinated  $H_2O$  molecules, which is slowed down compared to that of the parent compounds, such as gadolinium (Gd) complexes of either DTPA ('diethylenetriamine pentaacetate') or DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate). This effect inhibits the possible gain in relaxivity brought by the lengthening of the molecular tumbling time [1][2]. In this context, studies of the water-exchange rate of different C-functionalized complexes are pertinent.

Recently, several paramagnetic complexes carrying benzyl (Bn) groups have been synthesized and characterized by multinuclear NMR [3]. It has been observed that a Bn group in 4- position (trivial atom-numbering) has a beneficial influence on the H<sub>2</sub>O-exchange rate of the Gd complex. This group, however, can theoretically be attached at four different positions of the DTPA skeleton (see formulae 1-5). In this work, we report the relaxivity of the four possible regioisomerically substituted Gd–DTPA complexes 1-4, two of which have not been synthesized before.

**Results and Discussion.** -1. Synthesis. The syntheses of the Gd complexes (S)-1 and (S)-2 have been reported previously [3][4]. The synthesis of a DTPA derivative substituted in 5-position (complex of type 3) was described by *Brechbiel* [5]. For the synthesis of the corresponding Gd-free, benzyl-substituted ligand, the dipeptide 6,



obtained in *ca.* 30% yield by condensation of the Phe and Gly derivatives **7** and **8**, respectively, was transformed into the substituted diethylenetriamine derivative **9** (*Scheme 1*). Thereby, **8** was prepared from *N*-Boc-protected Phe by *N*-hydroxysuccinimide activation<sup>1</sup>) [6][7]. The poor coupling efficiency for the condensation of **7** and **8** was attributed to steric hindrance, since modification of the experimental conditions (reaction time, solvent, temperature) did not improve the yield. The reduction of **6** to (*S*)-**9** was achieved with borane, and the product (hydrochloride from) was purified by precipitation. Finally, multiple alkylation of (*S*)-**9** with *tert*-butyl 2-bromoacetate afforded the pentaester **10**, which was hydrolyzed with HCl to the final ligand (*S*)-**11**.

Scheme 1. Synthesis of the New Chiral Ligand 11 Required for the Preparation of the Gd Complex (S)-3



Two methods have been proposed for the insertion of a Bn group in 2-position of DTPA (ligand **12** for complexes of type **4**): either reductive amination of a protected diethylenetriamine derivative with an  $\alpha$ -keto acid (*Scheme 2, a*) or selective mono-deprotonation of protected DTPA, followed by alkylation (*Scheme 2, b*).

<sup>1)</sup> Similar yields were obtained for activation with 1-hydroxy-1*H*-benzotriazole (HOBt).

The reductive-amination procedure has been described by *Westerberg* for the synthesis of a (4-nitrophenyl)methyl derivative [8]. We, thus, adapted this method for the preparation of **12** (*Scheme 2, a*). The central N-atom of terminally Boc-protected diethylenetriamine, obtainable by 'Boc-on' methodology from the unprotected precursor, was alkylated at the central N-atom with 2-chloro-N,N-diethylacetamide. The following reductive amination with NaBH<sub>3</sub>CN was slow (4 d) and gave yields of only *ca.* 20%. Moreover, the complex mixture of products obtained required purification of **13** by HPLC. The latter was then alkylated with 2-bromoacetic acid, followed by ester hydrolysis under strongly alkaline conditions (7M NaOH solution, reflux). The overall yield of **12** was only *ca.* 4%. Therefore, we decided to investigate the second route shown in *Scheme 2, b*.

Scheme 2. Two Different Synthetic Approaches towards the New Ligand **12** (racemate) Required for the Preparation of the Gd Complex (R,S)-3. Route b (selective deprotonation and alkylation) was much more convenient than route a (reductive amination).



b)



The three-step synthesis of **12** via carbanion **14** turned out to be very convenient (*Scheme 2, b*) since it is not only fast, but avoids the difficulties inherent to polyamine monoalkylation. The carbanion **14** was obtained *in situ* at low temperature by treating the DTPA pentamethyl ester **15** with a strong base. The success of this step strongly depended on the nature of the base, the solvent, the electrophile, and the ratios of reactants used. Hence, we optimized the reaction conditions, using different benzyl halides, bases (lithium diisopropylamide; LDA), potassium hexamethyldisilazide (KHMDS), and solvents (THF, THF/HMPA ('hexamethylphosphortriamide')) in various ratios. The optimal conditions were met with the use of 2 equiv. of KHMDS and 3 equiv. of benzyl iodide (BnI) in the presence of HMPA. Interestingly, in all protocols [9][10], monosubstitution in 2-position has been observed predominantly, but the percentage of unreacted pentaester and of polyalkylation were not negligible. In our case, monoalkylation of **15** to **16** proceeded in 32% isolated yield. Finally, HCl-promoted ester hydrolysis (86%) of **16** afforded the desired ligand **12**.

2. Relaxometric Studies of Gd Complexes. 2.1. Qualitative and Quantitative Evaluation of the Water-Residence Time. The influence of the water-residence time,  $\tau_{\rm M}$ , on the proton relaxivity  $r_1$ , defined as the increase of relaxation rate in the presence of 1 mm complex, can be qualitatively assessed by the temperature dependence of the relaxivity at 20 MHz. For Gd complexes, a decrease in temperature induces an increase in relaxivity, when the residence time of a coordinated H<sub>2</sub>O molecule is smaller than the relaxation time of its H-nuclei. The observed increase of  $r_1$  for complexes 1-4 on lowering the temperature is, thus, characteristic of a nonlimiting H<sub>2</sub>O exchange (*Fig. 1*). As for the unsubstituted parent complex, Gd–DTPA (5), no significant limiting effect of  $\tau_{\rm M}$  was observed even at low temperatures.

The quantitative evaluation of  $\tau_{\rm M}$  was carried out through the analysis of the temperature dependence of the reduced transverse relaxation rate of the H<sub>2</sub>O <sup>17</sup>O-nucleus (*Fig. 2*). Obviously, the exchange rate of H<sub>2</sub>O was different for the 4-Bn-substituted complex (*S*)-1: the maximum of the curve was observed at lower temperatures for (*S*)-1 and for (*S*)-2, whereas for (*R*,*S*)-4 and (*S*)-3 it occured at a temperature close to that observed for 5.

The theoretical adjustment of the experimental data, performed as described previously [3][11][12], allowed for the determination of various parameters:  $A/\hbar$ , the hyperfine-coupling constant between the O-nucleus of bound H<sub>2</sub>O and Gd<sup>3+</sup>;  $\tau_v$ , the correlation time modulating the electronic relaxation of Gd<sup>3+</sup>;  $E_v$ , the activation energy related to  $\tau_v$ ; the *B*-parameter, which is related to the mean-square of the zero-field-splitting energy ( $B = 2.4\Delta^2$ ); and  $\Delta H^{\pm}$  and  $\Delta S^{\pm}$ , the enthalpy and entropy of activation, respectively, of the water-exchange process. Water-residence times and other parameters of the theoretical adjustment are given in *Table 1*.

The water-residence time of the Gd complex (R,S)-4 (308 ns at 298 K) was comparable to that of the Gd complexes of BOPTA<sup>2</sup>) (321 ns at 298 K) [13] and of COPTA<sup>3</sup>) (290 ns at 298 K) [14]. Complexes 1 and 2 were characterized by smaller values of  $\tau_{\rm M}$  than Gd–DTPA (5), whereas, surprisingly, 3 and 4 had  $\tau_{\rm M}$  values close to that of the parent compound. Substitution at the 4- and 7-position of DTPA, such as in

<sup>&</sup>lt;sup>2</sup>) BOPTA = 'Benzyloxypropionictetraacetate' (trivial name).

<sup>3)</sup> COPTA = 4-Carboxy-5,8,11-tris(carboxymethyl)-1-cyclohexyl-2-oxa-5,8,11-triazatridecan-13-oic acid.



Fig. 1. Temperature dependence of the proton longitudinal relaxivity of the Gd complexes 1-5

 

 Table 1. Parameters Derived by Theoretical Adjustment of the Experimental <sup>17</sup>O-NMR Transverse-Relaxation Rates of the Gd Complexes 1–5. For a description of the physical symbols, see text.

	( <i>S</i> )-1	(S)- <b>2</b>	( <i>S</i> )- <b>3</b>	( <i>R</i> , <i>S</i> )- <b>4</b>	5
$\tau_{\rm M}^{310}$ [ns]	$86\pm8$	$87\pm5$	$122\pm45$	$143\pm15$	$143\pm25$
$\Delta H^{+}$ [kJ mol <sup>-1</sup> ]	$50.1 \pm 0.1$	$46.2\pm0.1$	$50.5\pm0.9$	$46.4\pm0.2$	$51.5\pm0.3$
$\Delta S^{\ddagger}$ [J mol <sup>-1</sup> K <sup>-1</sup> ]	$51.8\pm0.4$	$39.0\pm0.2$	$49.9\pm2.8$	$35.7\pm0.3$	$52.1\pm0.6$
$A/\hbar$ [10 <sup>6</sup> rad s <sup>-1</sup> ]	$-3.2\pm0.1$	$-2.8\pm0.1$	$-2.8\pm1.1$	$-3.1\pm0.6$	$-3.4\pm0.1$
$B [10^{20} \text{ s}^{-2}]$	$2.80\pm0.06$	$2.79\pm0.12$	$2.73\pm0.13$	$2.38\pm0.13$	$2.60\pm0.06$
$\tau_{\rm v}^{310}$ [ps]	$10.3\pm0.3$	$15.6\pm0.7$	$15.5\pm0.9$	$15.6\pm1.0$	$11.5\pm0.3$
$E_{\rm v}  [\rm kJ \ mol^{-1}]$	$8.7\pm3.9$	$3.7\pm2.6$	$7.3\pm5.6$	$9.4\pm1.9$	$4.5\pm4.2$

complexes of types 1 and 2 seems, thus, to be best with regard to  $\tau_{\rm M}$  (smaller values). This observation may be rationalized by means of more-pronounced steric hindrance in the coordination spheres of 1 and 2 as compared to the novel compounds 3 and 4 (*Table 1*).

2.2. 1 H-NMRD Profiles. The NMRD (nuclear magnetic-relaxation dispersion) profiles of the Gd complexes 1-4, shown in Fig. 3, were rather similar, and were in agreement with an increased relaxivity of the Bn-substituted derivatives at both low and high fields as compared to the parent complex 5. The relaxivities of (R,S)-4 were slightly lower than those of (S)-1-(S)-3, especially at low field, but still higher than for Gd-DTPA (5).

The theoretical adjustment of the NMRD profiles [3] takes into account both outerand inner-sphere contributions. By analogy to other Gd–DTPA derivatives, the



Fig. 2. Logarithmic <sup>17</sup>O-NMR reduced tranverse-relaxation rates (ln  $(1/T_2^r)$ , in s<sup>-1</sup> (vertical axes); with  $1/T_2^r = 55.55/(T_2^p \cdot [Gd complex]))$  as a function of reciprocal temperature (1/T; in K; horizontal axes) for aqueous solutions of the Gd complexes a) (S)-1, b) (S)-2, c) (S)-3, and d) (R,S)-4. The calculated lines lacking any data points represent the parent compound Gd–DTPA (5).

number of H<sub>2</sub>O molecules in the first coordination sphere q was fixed to 1, the distance d of closest approach for the outer-sphere contribution was set to 0.36 nm, the relative translational correlation time was fixed to  $3.3 \times 10^{-9}$  m<sup>2</sup> s<sup>-1</sup>, and  $\tau_{\rm M}$  was fixed to the value determined by the <sup>17</sup>O-NMR experiments. The electronic relaxation time at very low magnetic field is given by  $\tau_{\rm SO} = 1/(5B \cdot \tau_{\rm V})$ . The resulting parameters are summarized in *Table 2*.

The increased relaxivity of complexes (S)-1 and (S)-2 has been reported [3] to arise mainly from both a slightly lower mobility and a reduced distance of interaction (r) between the H-atom of the coordinated H<sub>2</sub>O molecule and the Gd-ion. Indeed, for the above two complexes, a distance r of 0.31 nm resulted in fitted values of  $\tau_R$  (84 and

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Fig. 3. <sup>1</sup>*H*-*NMRD* (Nuclear magnetic-relaxation dispersion) *relaxivity profiles of the Gd complexes* 1-5 *in H*<sub>2</sub>*O*. The lines have been obtained by theoretical fitting of the data points.

Table 2. Proton Longitudinal Relaxivity ( $r_1$ ; in s<sup>-1</sup> mm<sup>-1</sup>; measured at 20 MHz/0.47 T) for the Gd Complexes **1–5**. Also given are  $\tau_M$ ,  $\tau_R$ ,  $\tau_{SO}$ ,  $\tau_V$ , and r values (see text) obtained by theoretical fitting of experimental <sup>1</sup>H-NMRD profiles.

	( <i>S</i> )-1	(S)- <b>2</b>	(S)- <b>3</b>	( <i>R</i> , <i>S</i> )- <b>4</b>	5
$r_1^{310}$ [s <sup>-1</sup> l mmol <sup>-1</sup> ]	4.8	5.1	4.6	4.3	3.9
$\tau_{\rm M}^{310} [{\rm ns}]^{\rm a})$	86	87	122	143	143
<i>r</i> [nm]	0.291 - 0.30	0.296 - 0.30	0.30	0.31	0.31
$\tau_{\rm R}^{310}$ [ps]	$61 - 72^{b}$ )	$68 - 72^{\circ}$	68	72	59
$\tau_{so}^{310}$ [ps]	$77 - 91^{b}$	$86 - 93^{\circ}$	90	87	82
$ au_{\rm V}^{310}$ [ps]	14-17 <sup>b</sup> )	27-35°)	19	16	23

<sup>a</sup>) Derived by <sup>17</sup>O-NMR spectroscopy. <sup>b</sup>) The lower  $\tau$  value refers to a distance *r* of 0.291 nm, the higher one to 0.300 nm. <sup>c</sup>) The lower  $\tau$  value refers to a distance *r* of 0.296 nm, the higher one to 0.300 nm.

87 ps, resp.) significantly larger than those obtained by <sup>2</sup>H-relaxometry of the corresponding labeled diamagnetic lanthanum complexes ( $\tau_R^{310} = 65 \pm 7$  ps for La analogs of (S)-1 and (S)-2). Fittings were, thus, performed with shorter distances *r* to account for these  $\tau_R$  values. Values of *r* of 0.291–0.300 nm (for (S)-1) and 0.296–0.300 nm (for (S)-2) agreed with the  $\tau_R$  values obtained by <sup>2</sup>H-relaxometry. Similarly, for complex (S)-3, a value of *r* equal to 0.300 nm and a  $\tau_R$  value of 68 ps were in agreement with the observed relaxivity enhancement. According to the recent report of *Caravan et al.* [15], *r* should be equal to 0.31±0.01 nm. However, our values of

0.300 nm seem to be more adequate. Interestingly, for complex (R,S)-4, a reduction of r was not observed.

2.3. Transmetallation Stability. Transmetallation of Gd complexes with  $Zn^{2+}$  in phosphate-buffer solution (pH7) has been shown to result in a decrease of the paramagnetic relaxation rate as a consequence of the precipitation of gadolinium phosphate [16]. This phenomenon was not observed for macrocyclic complexes, but was clearly encountered with Gd-DTPA (5) and its bisamide derivatives. Transmetallation assessment of the Gd complexes 1-4 showed that all Bn-substituted compounds, except for (S)-2, were more stable than 5 (Fig. 4). The observed initial increase in the relaxation rate was most likely due either to a slight decrease of the temperature during the mixing of the Gd complexes with Zn solutions, or to solvation phenomena such as increase in the solvent accessibility of the Gd ions during transmetallation. Because a 'free' Gd-ion is surrounded by eight  $H_2O$  molecules, its relaxivity is much higher relative to that of a chelated Gd-ion, which has only one  $H_2O$ molecule in its first coordination sphere. Consequently,  $R_1^p$  is increased. A few minutes later, the production of insoluble GdPO<sub>4</sub> induces a decrease of  $R_1^p$ . With regard to transmetallation, the highest stabilities were achieved, in decreasing order, for compounds with Bn substituents in the 5-, 4-, and 2-positions (i.e., complexes 3, 1, and 4, resp.). Surprisingly, substitution in 7-position (complex 2) had a contradictory effect on the transmetallation stability with respect to  $Zn^{2+}$  (*Fig. 4*).



Fig. 4. Transmetallation stability towards  $Zn^{2+}$  of the Gd complexes **1–5** in aqueous solution. The transmetallation properties are expressed by evolution of the ratio  $X = R_1^p(t)/R_1^p(t_0)$  as a function of time t ( $t_0 = 0$ : addition of  $Zn^{2+}$ ). High values for X mean high transmetallation stability.

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**Conclusions.** – We have undertaken a comparative study of four possible Bnsubstituted Gd–DTPA complexes with respect to both relaxivity and transmetallation stability. Gd–DTPA Complexes of types **1** and **2**, substituted on the central arm, *i.e.*, in 4- and 7-position (trivial atom-numbering; see formulae) exhibit a faster exchange of H<sub>2</sub>O molecules than those substituted in other positions. This finding is relevant for the design of novel NMR contrast agents upon coupling substituted Gd–DTPA complexes to macromolecules or specific vectors. Also, synthetically, coupling in 4-position is easier.

Our study indicates that the steric hindrance of the first coordination sphere depends on substituent position. Depending on its proximity to the coordinated H<sub>2</sub>O, substituents could accelerate solvent expulsion. Substituents close to the first coordination shell are known to be capable of modifying the number of H<sub>2</sub>O molecules coordinated to lanthanide complexes [17][18]. Therefore, it is not unlikely that substituents may also affect the residence time of H<sub>2</sub>O in the first coordination sphere. It has been reported that  $\tau_{\rm M}$  is reduced for various derivatives of Gd–DTPA complexes [11][12][19], and that amide derivatives of Gd–DTPA or Gd–DOTA are characterized by lower water-exchange rates than their unsubstituted congeners [20–23].

It should be mentioned that the differential effects on the water-exchange rates of substituted Gd–DTPA complexes could also reflect differential exchange rates of the single stereoisomers. Some recent work has, however, ruled out a major influence of the co-existence of stereoisomers in the case of bisamide derivatives of Gd–DTPA complexes [24].

In conclusion, given that the synthesis of 5-Bn-substituted compounds of type 3 is more tedious and that the relaxivities and transmetallation properties of 3 and 1 are not too different, the 4-position (*i.e.*, complexes of type 1) seems to be optimal to link these paramagnetic ligands to a macromolecule or to a tailored vector to produce potent contrast agents of both higher relaxivity and selectivity.

## **Experimental Part**

1. *General.* All chemicals were purchased from *Aldrich* (Bornem, Belgium) and were used without further purification. Products were purified by anion- (*Dowex 1X8-400*) or cation- (*Dowex 50X8-400*) exchange chromatography. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra: *Bruker AMX-300* instrument (300 and 75 MHz, resp.); chemical shifts  $\delta_{\rm H}$  and  $\delta_{\rm C}$  in ppm rel. to SiMe<sub>4</sub> (=0 ppm) and *t*-BuOH (Me resonance at 31.2 ppm), resp.; multiplicities stated as *s* (*singlet*), *d* (*doublet*), *t* (*triplet*), *q* (*quartet*), and *m* (*multiplet*). Electrospray-ionization (ESI) mass spectra were obtained on a *Q-tof-2* mass spectrometer (*Micromass*, Manchester, UK), using samples dissolved in MeOH/H<sub>2</sub>O.

2. <sup>17</sup>O-NMR Experiments. Spectra were recorded on a Bruker AMX-300 spectrometer, with 2-ml samples placed in 10-mm tubes (o.d.). The temperature was regulated by air or N<sub>2</sub> flow controlled by a BVT-2000 unit. <sup>17</sup>O-NMR Transverse-relaxation times  $T_2$  of distilled H<sub>2</sub>O (pH 6.5–7.0) were measured by means of Carr-Purcell-Meiboom-Gill sequence, and the data were subsequently processed by a two-parameter fit. The <sup>17</sup>O-NMR  $T_2$  values of coordination H<sub>2</sub>O of the complexes were obtained from line-width measurements. All <sup>17</sup>O-NMR spectra were proton-decoupled. The sample conc. was below 40 mm, *i.e.*, (S)-1, 38.7; (S)-2, 20.75; (S)-3, 20.84; and (R,S)-4, 19.07 mM.

3. <sup>1</sup>H-NMRD Profiles. Samples of 0.6 ml in 10-mm tubes (o.d.) were inserted into a Field Cycling Relaxometer (Field Cycling Systems, Honesdale, PA, USA). Magnetic fields of 0.24 mT and 1.2 T were applied, which correspond to proton Larmor frequencies of 0.01 and 50 MHz, resp. Additional relaxation rates at 1.4 and 7.05 T were determined on a Minispec Mq-60 and a Bruker AMX-300 spectrometer, resp. Relaxation rates were also measured at 0.47 T on a Minispec PC-20 apparatus (Bruker, Karlsruhe, Germany), thermostabilized by

means of tetrachloroethylene flow. Fitting of the <sup>1</sup>H-NMRD profiles was performed with data-processing software that uses different theoretical models for nuclear-relaxation phenomena (*Minuit*, CERN Library) [25][26].

4. *Transmetallation*. Transmetallation with  $Zn^{2+}$  was evaluated by the decrease of the longitudinal relaxation rate of H<sub>2</sub>O at 310 K and 20 MHz of buffered phosphate solutions (pH 7; [KH<sub>2</sub>PO<sub>4</sub>] = 26 mM, [Na<sub>2</sub>HPO<sub>4</sub>] = 41 mM), containing equimolar amounts (2.5 mM) of one of the Gd–DTPA complexes (1–5) and of  $Zn^{2+}$  [16].

5. Syntheses. 5.1. Synthesis of Ligand **11** for the Gd Complex (S)-**3** (see Scheme 1). (S)-2-Amino-3phenylpropanamide (L-Phe-NH<sub>2</sub>; **7**). L-Phenylalanine methyl ester hydrochloride (25.34 g, 177.5 mmol) was dissolved in anh. MeOH (150 ml), and the soln. was mixed with an equimolar amount of Et<sub>3</sub>N (16 ml). Then, Et<sub>2</sub>O (150 ml) was added under stirring to precipitate the ammonium salt. The soln. was maintained at  $-10^{\circ}$  for 2 h, and the salt was filtered off. The filtrate was evaporated under reduced pressure, and the remaining yellow oil was dissolved in anh. MeOH (300 ml). NH<sub>3</sub> Gas was bubbled under stirring into this soln. for 2 h, and the mixture was stirred for 2 d. The solvent was evaporated, the pale yellow residue was dissolved in H<sub>2</sub>O (100 ml), the resulting soln. was adjusted to pH 11, stirred overnight at 50°, and concentrated to a minimum. The sample was purified by anion-exchange chromatography (*Dowex AG 1X8-400*, HCOONa; column: 20 × 2.4 cm). The column was washed with H<sub>2</sub>O. At pH *ca.* 6, compound **7** was eluted. The soln. was treated several times with H<sub>2</sub>O (20 ml) and evaporated to eliminate formic acid: 95% of **7**. <sup>1</sup>H-NMR (D<sub>2</sub>O, 298 K): 2.75 – 3.05 (*m*, CH<sub>2</sub>); 3.65 (*dd*, CH); 7.25 (*m*, Ph).

2-[[(tert-*Butoxy*)*carbonyl*]*amino*]*acetic Acid* (*N*-Boc-Gly). Et<sub>3</sub>N (30 ml) was added to glycine (10 g, 133.21 mmol) in H<sub>2</sub>O (120 ml). Boc-on<sup>4</sup>) (36.15 g, 146.75 mmol) in 1,4-dioxane (80 ml) was added dropwise under stirring and an inert atmosphere. The green soln. turned yellow upon stirring for 4 h at r.t. The mixture was poured in H<sub>2</sub>O (250 ml) and extracted with Et<sub>2</sub>O (5 × 50 ml). The aq. phase was adjusted to pH 2 with 3M HCl and extracted with AcOEt (5 × 50 ml). The combined org. phase was evaporated, and the remaining yellow oil was dissolved in H<sub>2</sub>O (75 ml) and lyophilized to a white powder: 87% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 10.8 (br. *s*, OH); 4.0 (*s*, CH<sub>2</sub>); 5.2 (br. *s*, NH); 1.5 (*s*, *t*-Bu).

tert-*Butyl* N-*[2-[*(2,5-*Dioxopyrrolidin-1-yl*)*oxy]-2-oxoethyl]carbamate* (8). *N*-Boc-Gly (10 g, 57.1 mmol) and *N*-hydroxysuccinimide (7.66 g, 66.51 mmol) were mixed in THF (90 ml) under an inert atmosphere. Dicyclohexylcarbodiimide (DCC; 13.21 g, 64 mmol) in THF (40 ml) was added dropwise. The mixture was stirred at r.t. overnight. Then, AcOH (0.1 ml) was added to stop the reaction. The soln. was stirred for 1 h and was then filtered, and the filtrate was evaporated under reduced pressure. The residue was suspended in i-PrOH (100 ml) and stirred vigorously for 1 h. The resulting suspension was filtered, and the remaining solid was dried with a drying pistol at  $50^{\circ}$ : 68% of 8. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 2.7 (*s*, 2 CH<sub>2</sub>); 1.4 (*s*, *t*-Bu); 4.9 (br. *s*, NH); 4.2 (*s*, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 298 K): 168.2; 167.2; 150.3; 35.2; 79.4; 28.2; 24.6.

tert-*Butyl* (S)-N-[2-[(2-Amino-1-benzyl-2-oxoethyl)amino]-2-oxoethyl]carbamate (**6**). Compound **7** (9.32 g, 62.4 mmol) and Et<sub>3</sub>N (60 ml, 330.6 mmol) were dissolved in anh. MeCN (75 ml) and anh. MeOH (75 ml). Compound **8** (17 g, 62.4 mmol), dissolved in anh. THF (200 ml), was added dropwise to the mixture under an inert atmosphere, and the resulting soln. was stirred for 4 d at r.t. The mixture was evaporated, and the residue was suspended in MeCN (100 ml) and stirred for 2 h. The precipitate was filtered off, and the filtrate was evaporated. The resulting residue was dissolved in H<sub>2</sub>O (100 ml) and CHCl<sub>3</sub> (100 ml). The aq. phase was adjusted to pH 11 with a sat. Na<sub>2</sub>CO<sub>3</sub> soln. The mixture was stirred with brine (50 ml), and the two phases were separated. The aq. phase was extracted with CHCl<sub>3</sub> (5 × 25 ml). The combined org. phase was dried (MgSO<sub>4</sub>) and evaporated. The remaining solid was purified by column chromatography (CC) (SiO<sub>2</sub>; CHCl<sub>3</sub>/MeOH 1:1): 27% of **6**. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 6.0 (br. *s*, NH<sub>2</sub>); 5.4 (br. *s*, NH); 6.5 (br. *s*, NH); 1.4 (*s*, *t*-Bu); 3.4 (*s*, CH<sub>2</sub>); 156.3; 79.4; 28.8; 54.8; 37.1; 40.2.

(S)-N<sup>2</sup>-(2-Aminoethyl)-3-phenylpropane-1,2-diamine Tris(hydrochloride) (9). Compound 6 (4 g, 14.42 mmol) was dissolved in trifluoroacetic acid (TFA; 50 ml) at 0°, and then stirred for 5 h at r.t. The mixture was repeatedly evaporated, and the residue was redissolved in H<sub>2</sub>O (50 ml) to eliminate TFA. The residue was dissolved in H<sub>2</sub>O (50 ml), and the soln. was adjusted to pH 10 with aq. Na<sub>2</sub>CO<sub>3</sub> soln. This soln. was evaporated, and the residue was dissolved in MeOH. The soln. was filtered and evaporated to afford the corresponding Boc-deprotected intermediate (=2-[(2-aminoacetyl)amino]-3-phenylpropanamide; not shown

 <sup>&</sup>lt;sup>4</sup>) '2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile'; (systematic name: N-[(*tert*-butoxycarbonyl)oxy]benzenecarboximidoyl cyanide.

in *Scheme 1*). This intermediate was suspended in anh. THF (100 ml), and a BH<sub>3</sub> soln. (1M in THF, 150 ml) was added dropwise at  $-10^{\circ}$  under an inert atmosphere. The mixture was stirred for 1 h at this temp. and was then allowed to warm to r.t. The soln. was refluxed overnight. After cooling to 5°, anh. MeOH (50 ml) was slowly added (to quench excess BH<sub>3</sub>). The soln. was evaporated under reduced pressure, and the residue was again quenched with MeOH (50 ml, as above). The solvent was evaporated, and the remaining oil, dissolved in anh. EtOH (100 ml), was filtered. The filtrate was cooled in a bath of ice, saturated with HCl, and stirred for 2 h. The precipitating hydrochloride **9** was collected and dried with a drying pistol at 50°: 40% of **9**. <sup>1</sup>H-NMR (D<sub>2</sub>O, 298 K): 2.9–4.0 (*m*, CH, 4 CH<sub>2</sub>); 7.1–7.3 (*m*, Ph). <sup>13</sup>C-NMR (D<sub>2</sub>O, 298 K): 137.7; 129.8; 129.1; 127.1; 56.6; 43.3; 42.6 · 40.0 · 36.0

tert-*Butyl* (S)-*8-Benzyl-6,9,12-tris*[2-(tert-*butoxy*)-2-oxoethyl]-2,2-dimethyl-4-oxo-3-oxa-6,9,12-triazatetradecan-14-oate (**10**). Compound **9** (1.39 g, 4.58 mmol) was dissolved in *Hünig*'s base ((i-Pr)<sub>2</sub>NEt; 7.98 ml, 45.81 mmol) and anh. DMF (50 ml). *tert*-Butyl 2-bromoacetate (5.17 ml, 32.06 mmol), dissolved in anh. DMF (30 ml), was added dropwise at 5° under an inert atmosphere. The mixture was stirred at this temp. for 1 h, then at 50° for 48 h. The filtrate was evaporated under reduced pressure, and the residual oil was dissolved in AcOEt (50 ml) and H<sub>2</sub>O (50 ml). The aq. phase was extracted with AcOEt ( $3 \times 50$  ml). The combined org. phase was extracted with H<sub>2</sub>O (50 ml) and sat. aq. NaHCO<sub>3</sub> soln. (50 ml), dried (MgSO<sub>4</sub>), and evaporated. The residual oil was purified by CC (SiO<sub>2</sub>; Et<sub>2</sub>O): 46% of **10**. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 7.2 (*m*, Ph); 1.5 (*s*, 5 *t*-Bu); 2.5 – 3.0 (*m*, 4 CH<sub>2</sub>); 3.2 – 3.6 (*m*, 5 CH<sub>2</sub>); 3.7 (*m*, CH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 315 K): 28.4; 36.4; 66.6; 50.3; 53.7; 55.2; 56.0; 56.5; 62.1; 80.5; 81.1; 81.5; 139.5; 129.3; 128.3; 125.9; 172.9; 171.0; 170.8. ESI-MS: 765 ([*M* + H]<sup>+</sup>), 787 ([*M* + Na]<sup>+</sup>).

(S)-2-([1-Benzyl-2-[bis(carboxymethyl]amino]ethyl][2-[bis(carboxymethyl]amino]ethyl]amino]acetic Acid (11). Compound 10 (1.6 g, 2.09 mmol) was treated with conc. HCl (75 ml), and the soln. was stirred for 24 h at r.t. The mixture was filtered and evaporated. The residue was dissolved in a minimum of H<sub>2</sub>O, adjusted to pH 10 with a sat. aq. NaHCO<sub>3</sub> soln., and extracted with AcOEt ( $5 \times 30$  ml). The aq. phase was adjusted to pH 2 with 3M HCl, concentrated, and purified by cation-exchange chromatography (*Dowex AG 50W-X8*, H<sup>+</sup>-form; column:  $15 \times 2.4$  cm). The column was washed with H<sub>2</sub>O until reaching a pH of *ca*. 6. The product was then eluted with 2M aq. NH<sub>3</sub> soln. (500 ml). The soln. was evaporated, and the residue was dissolved in a minimum of MeOH. The soln. was filtered, and the product was precipitated with cold Et<sub>2</sub>O: 70% of 11. <sup>1</sup>H-NMR (D<sub>2</sub>O, 298 K, pH > 7): 7.3 (*m*, Ph); 2.3–3.5 (*m*, 9 CH<sub>2</sub>, CH). <sup>13</sup>C-NMR (D<sub>2</sub>O, 320 K, pH > 7): 180.6; 180.3; 179.4; 65.3; 61.8; 60.5; 59.3; 54.3; 53.3; 52.6; 140.3; 129.7; 129.2; 126.7; 32.3. ESI-MS: 550 ([*M* + 3Na]<sup>+</sup>).

5.2. Synthesis of the Ligand **12** for the Gd Complex (R,S)-**4** (see Scheme 2, b). Methyl 5,8,11-Tris(2-methoxy-2-oxoethyl)-3-oxo-2-oxa-5,8,11-triazatridecan-13-oate (**15**). To DTPA<sup>5</sup>) (5 g, 12.7 mmol), suspended in anh. MeOH (200 ml), was added at 0° under inert gas SOCl<sub>2</sub> (10 ml, 140 mmol) under stirring. The clear soln. was stirred overnight, and then refluxed for 4 h. The residual solvent was evaporated under reduced pressure. The residue was dissolved in AcOEt (100 ml), and a sat. aq. NaHCO<sub>3</sub> soln. was slowly added under stirring until a pH of *ca.* 7 was reached. The soln. was filtered, the phases were separated, and the aq. phase was extracted with AcOEt (5 × 30 ml). The combined org. phase was dried (MgSO<sub>4</sub>), filtered, evaporated, and dried to afford **15** (95%). Yellow oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 2.75 (*t*, 2 CH<sub>2</sub>); 2.85 (*t*, 2 CH<sub>2</sub>); 3.7 (*s*, 4 Me); 3.65 (*s*, Me); 3.6 (*s*, 4 CH<sub>2</sub>); 3.5 (*s*, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 298 K): 171.5; 171.8; 54.8; 54.6; 52.4; 51.8; 51.2; 51.0. ESI-MS: 464 ([*M* + H]<sup>+</sup>), 486 ([*M* + Na]<sup>+</sup>).

*Methyl* (R,S)-4-*Benzyl-5,8,11-tris(2-methoxy-2-oxoethyl)-3-oxo-2-oxa-5,8,11-triazatridecan-13-oate* (16). To a soln. of KHMDS<sup>6</sup>) (8.64 ml, 4.32 mmol) in toluene, diluted with anh. THF (10 ml), was added 15 (1 g, 2.16 mmol) in anh. THF at  $-78^{\circ}$  under an inert atmosphere over a period of 20 min. The yellow soln. was stirred at this temp. for an additional 20 min. Then, a soln. of BnI (0.77 ml, 6.48 mmol) [27] in a mixture of anh. THF (12 ml) and HMPA (0.108 ml) was added dropwise at  $-78^{\circ}$  over a period of 30 min, and this soln. was stirred for an additional 30 min. Then, the cooling bath was removed, and the mixture was stirred for 1 h at r.t., then overnight at 50°. The solvents were evaporated, and the residue was dissolved in AcOEt (50 ml) and sat. aq. NaHCO<sub>3</sub> soln. (50 ml). The aq. phase was extracted with AcOEt ( $3 \times 15$  ml), and the combined org. phase was dried (MgSO<sub>4</sub>) and evaporated. The remaining yellow oil was prepurified by CC (SiO<sub>2</sub>; AcOEt). The crude product fractions were combined, evaporated, and purified by CC (SiO<sub>2</sub>; AcOEt/hexane gradient): 32% of 16. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 7.2 (*m*, Ph); 2.65–2.85 (*m*, 4 CH<sub>2</sub>); 2.95 (*m*, CH<sub>2</sub>); 3.4 (*s*, CH<sub>2</sub>); 3.5–3.7 (*m*, 5 Me, 3 CH<sub>2</sub>, CH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 310 K): 172.7; 172.3; 172.1; 171.8; 138.1; 129.4; 128.4; 126.6; 66.3; 36.7; 55.2; 55.0; 53.2; 52.8; 52.7; 52.4; 52.3; 52.1; 51.6; 51.4; 50.6. ESI-MS: 554 ([*M*+H]<sup>+</sup>); 576 ([*M*+Na]<sup>+</sup>).

<sup>5)</sup> Systematic name: 2-(bis{2-[bis(carboxymethyl)amino]ethyl}amino)acetic acid.

<sup>6)</sup> Potassium hexamethyldisilazide.

(R,S)-2-[[2-[[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl](carboxymethyl)amino]-3phenylpropanoic Acid (**12**). Compound **16** (1.09 g, 1.97 mmol) was treated with conc. HCl (45 ml). The soln. was stirred for 24 h at r.t., filtered, and evaporated. The residue was dissolved in a minimum of H<sub>2</sub>O, and the pH was adjusted to 10 with a sat. aq. NaHCO<sub>3</sub> soln. The aq. phase was extracted with AcOEt (5 × 30 ml), concentrated, and adjusted to pH 2 with 3M HCl. The product was purified by ion-exchange chromatography (*Dowex AG* 50W-X8, H<sup>+</sup>-form; column: 15 × 2.4 cm). The column was washed with H<sub>2</sub>O until reaching a pH of *ca*. 6. Then, the product was eluted with 2M aq. NH<sub>3</sub> soln. (500 ml). The product fractions were evaporated, and the remainder was dissolved in a minimum of MeOH. The MeOH soln. was filtered and poured in cold ether, whereupon **12** (86%) precipitated. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 298 K): 6.8 (*m*, Ph); 2.4–3.9 (*m*, 9 CH<sub>2</sub>, CH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 310 K): 174.7; 174.2; 171.6; 170.6; 138.1; 131.7; 130.5; 126.7; 62.9; 57.7; 56.8; 56.6; 55.7; 54.6; 52.9; 52.7; 34.4. ESI-MS: 560 ([*M* + 2K]<sup>+</sup>).

5.3. *Gadolinium-Complex Formation*. The ligand (**11** or **12**; 0.5 mmol), dissolved in H<sub>2</sub>O (2 ml), was treated dropwise with an equimolar aq. soln. of  $GdCl_3 \cdot 6$  H<sub>2</sub>O, keeping the pH between 5.5 and 6.5 by means of NaOH. The stirred soln. was heated at 60° for 48 h. The medium was then adjusted to pH 9 with aq. NaOH soln. (precipitation of free  $Gd^{3+}$  as  $Gd(OH)_3$ ), and the resulting precipitate was filtered off. The filtrate was adjusted to pH 7, and then treated with *Chelex* resin for 2 h (elimination of residual free  $Gd^{3+}$ , as assessed by means of the xylenol-orange test). The final concentration of the Gd-complexes (compounds **1–5**) were determined on samples treated with HNO<sub>3</sub> by comparing their proton paramagnetic-relaxation rates at 20 MHz with those of reference samples containing known amounts of  $Gd^{3+}$ .

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