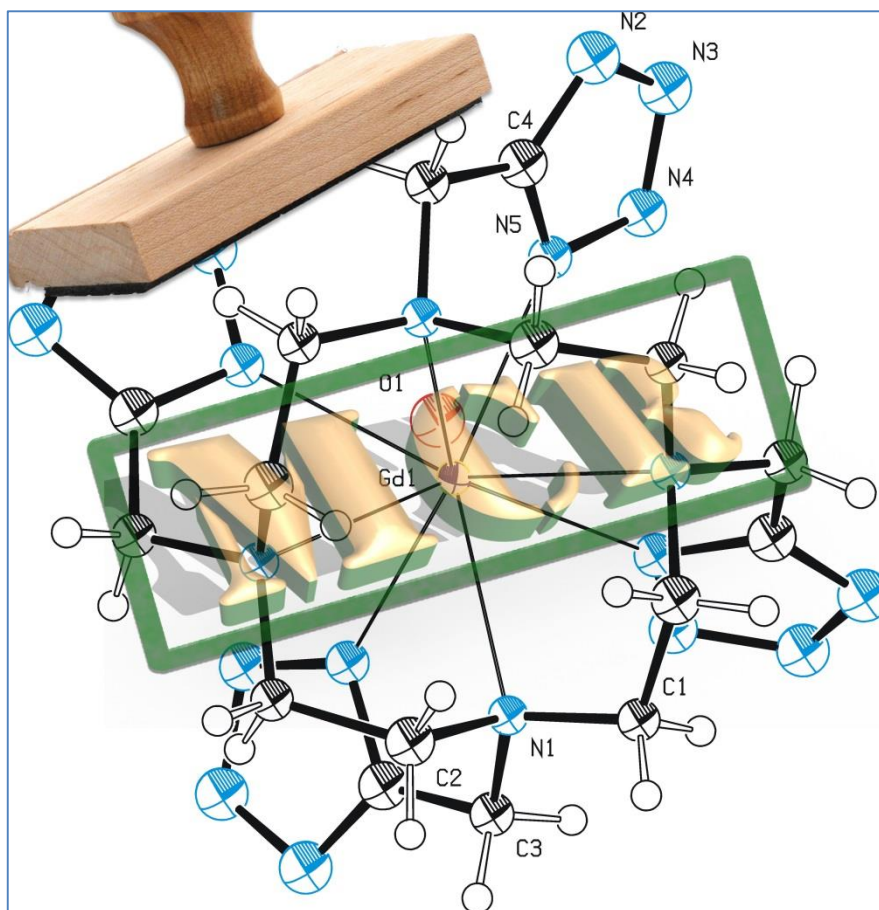# CHEMISTRY
A European Journal

Supporting Information

Gd-TEMDO: Design, Synthesis, and MRI Application**

André Boltjes+,[a] Annadka Shrinidhi+,[a] Kees van de Kolk,[b] Eberhardt Herdtweck,[c] and Alexander Dömling*[a]

chem_201600720_sm_miscellaneous_information.pdf
chem_201600720_sm_video.wmv

Contents

1. General remarks

Instruments employed: Electrothermal digital melting point apparatus; Bruker Avance DRX 500 MHz NMR spectrometer (B AV-500) equipped with Bruker Automatic Sample Changer (B ACS 60). For MRI; Bruker 9.4T 89mm bore scanner equipped with 1500mT/m gradientset. Thar SFC-MS equipped with autosampler and autoinjector; pH meter pHenomenal® with Thermo Scientific Orion ROSS Ultra pH electrode; Jasco V-660 UV-VIS Spectrophotometer. High resolution mass spectra were recorded using a LTQ-Orbitrap-XL (Thermo) at a resolution of 60000@m/z400.

Chemicals required: DOTA, paraformaldehyde, sodium hydroxide, potassium chloride, potassium hydrogen phthalate, ethylene diamine tetraacetic acid, KOH concentrate (Sigma-Aldrich); Arsenazo III (TCI); 1,4,7,10-tetraazacyclododecane, azidotrimethylsilane, gadolinium chloride anhydrous (ABCR); phenolphthalein, xylenol orange (Fluka); acetic acid (Acros), sodium acetate (Merck); hydrochloric acid, methanol, acetonitrile (Boomlab) were obtained commercially and used without any further purification. 3-Isocyanopropanenitrile was prepared in the laboratory from its corresponding formamide. Millipore water was used for the preparation of all solutions.

NMR spectra were recorded in $CDCl_3$ (with 0.03% TMS), DMSO-d_6 and D_2O (with 0.03% DSS) at either 500 MHz (δ_H) or 125 MHz (δ_C); the coupling constants (J) are in Hz. Abbreviations: mp (melting point), s (singlet); d (doublet); t (triplet); q (quartet); br (broad); dd (doublet of doublet), etc. The nomenclatures of all the compounds were derived by ChemDraw (CambridgeSoft).

2. Synthetic procedures and characterization for compound 3, 4, 7, 8 and precursor

Experimental Section

3,3',3'',3'''-((1,4,7,10-tetrakis((1H-tetrazol-5-yl)methyl)-1,4,7,10-tetraazacyclododecane (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(methylene))tetrakis(1H-tetrazole-5,1-diyl))tetrapropanenitrile (**7**)

In a 50 mL roundbottom flask was added paraformaldehyde (3.0 g, 100 mmol), 1,4,7,10-tetraazacyclododecane (0,861 g, 5 mmol), azidotrimethylsilane (6,6 mL, 50 mmol) and 3-isocyanopropanenitrile (2,0 g, 25,00 mmol) in methanol (15 mL) to give a yellow suspension. Stirring overnight, removing the solvent by decantation, redissolving in 20 mL acetonitrile,

filtering over celite and concentrating under reduced pressure yields 3,3',3'',3'''-(5,5',5'',5'''-((1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(methylene))tetrakis(1H-tetrazole-5,1-diyl))tetrapropanenitrile, (3.58 g, 100%) as an orange oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.75 (t, J = 6.4, 8H), 3.95 (s, 8H), 3.22 (t, J = 6.4, 8H), 2.69 (s, 16H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.9, 118.0, 50.9, 46.3, 42.5, 17.8 ppm. HRMS (ESI) m/z calculated [M+H]$^+$: 713,39405; found [M+H]$^+$: 713, 39435

*1,4,7,10-tetrakis((1H-tetrazol-5-yl)methyl)-1,4,7,10-tetraazacyclododecane (**3**)*
In a 100 mL roundbottomflask was added 3,3',3'',3'''-(5,5',5'',5'''-((1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(methylene))tetrakis(1H-tetrazole-5,1-diyl))tetrapropanenitrile (**7**) (2.0 g, 2,8 mmol) and NaOH (1,80 g, 22,5 mmol) in acetonitrile-water (5:1, 20 mL) to give a yellow solution. After stirring overnight at room temperature, the solvent was evaporated from the reaction mixture and the solid mass was dissolved in 75 mL water. The pH of solution was adjusted to 7.0 with aqueous HCl. This neutral solution was extracted with dichloromethane (2 x 25 mL) to remove organic impurities. The aqueous layer was lyophilized and redissolved in 100 mL methanol and undissolved solid mass was removed by filtration. The supernatant liquid was evaporated to dryness, redissolved in 5 mL water and the pH was adjusted 7.75 with aqueous NaOH. A white solid precipitated and was collected by filtration, washed with cold water and dried to obtain 1.26 g (86%) of TEMDO. m.p.>300°C. ^{1}H NMR (500 MHz, D_2O) δ 4.20 (s, 7H), 2.87 (s, 16H). ^{13}C NMR (125 MHz, D_2O) δ 160.0, 52.4, 49.7 ppm. HRMS (ESI) m/z calculated [M+H]$^+$: 501,28786; found [M+H]$^+$: 501,28754

*Gadolinium(III) 1,4,7,10-tetrakis((1H-tetrazol-5-yl)methyl)-1,4,7,10-tetraazacyclododecane (**8**)*
The Gd^{3+} complex of TEMDO was prepared by heating the 1:1.1 mixture of TEMDO ligand (**3**) (1.0 g, 2.0 mmol) and GdCl$_3$. 6H$_2$O (0.82 g, 2.2 mmol) in 20 mL Millipore water at 70°C for 10 days at pH 6.8. The remaining free Gd^{3+} was removed using Chelex 100 and the clean liquid was lyophilized to give pure Gd-TEMDO complex as a white solid (>98%, 1.28g). Complexes for Eu and La were prepared in the same manner. HRMS (ESI) m/z calculated [M+2H]$^+$: 656,18848; found [M+2H]$^+$: 656,18832

N-(2-cyanoethyl)formamide
A solution of 3-aminopropionitrile (10g, 143 mmol) in ethyl formate (250 mL, 143 mmol) was refluxed for 5 hours. The mixture was concentrated in vacuo, yielding 13,9g, 99% as a yellow oil. The product is obtained as a mixture of two amide rotamers. ^{1}H NMR (500 MHz,

DMSO-d$_6$): δ = 8.22 (s, 1H, major), 6.97 (s, 1H, major), 3.57 (tt, J=9.0, 4.3, 2H), 2.79 – 2.61 (m, 2H) ppm. ^{13}C NMR (125 MHz, CDCl$_3$) δ 162.1, 118.2, 37.9, 34.3, 20.5, 18.4 ppm.

3-isocyanopropanenitrile (**4**)

To a cold (0 °C) solution of N-(2-cyanoethyl)formamide (8.65g, 88mmol) and triethylamine (61 mL, 441 mmol) in DCM was added drop wise over 60 min. POCl$_3$ (8.2 mL, 88 mmol). After the addition the reaction was stirred at 0 °C for 2.5 hours. Then, carefully, 10% Na$_2$CO$_3$ was added followed by water (200 mL) to dissolve all solids. The organic layer was separated and the water layer extracted with DCM (3 × 25 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO$_4$, and concentrated *in vacuo*. The crude dark brown residue was purified by passing it through a plug of silica (100% DCM). After evaporation of all volatiles the product(4.2g, 59%) was obtained as a brown oil which solidified upon standing. ^{1}H NMR (500 MHz, CDCl$_3$) δ 3.73 (t, J = 6.7 Hz, 2H), 2.81 (t, J = 6.7 Hz, 2H) ppm. ^{13}C NMR (125 MHz, CDCl$_3$) δ 160.1, 115.8, 37.7, 37.6, 37.6, 18.8 ppm.

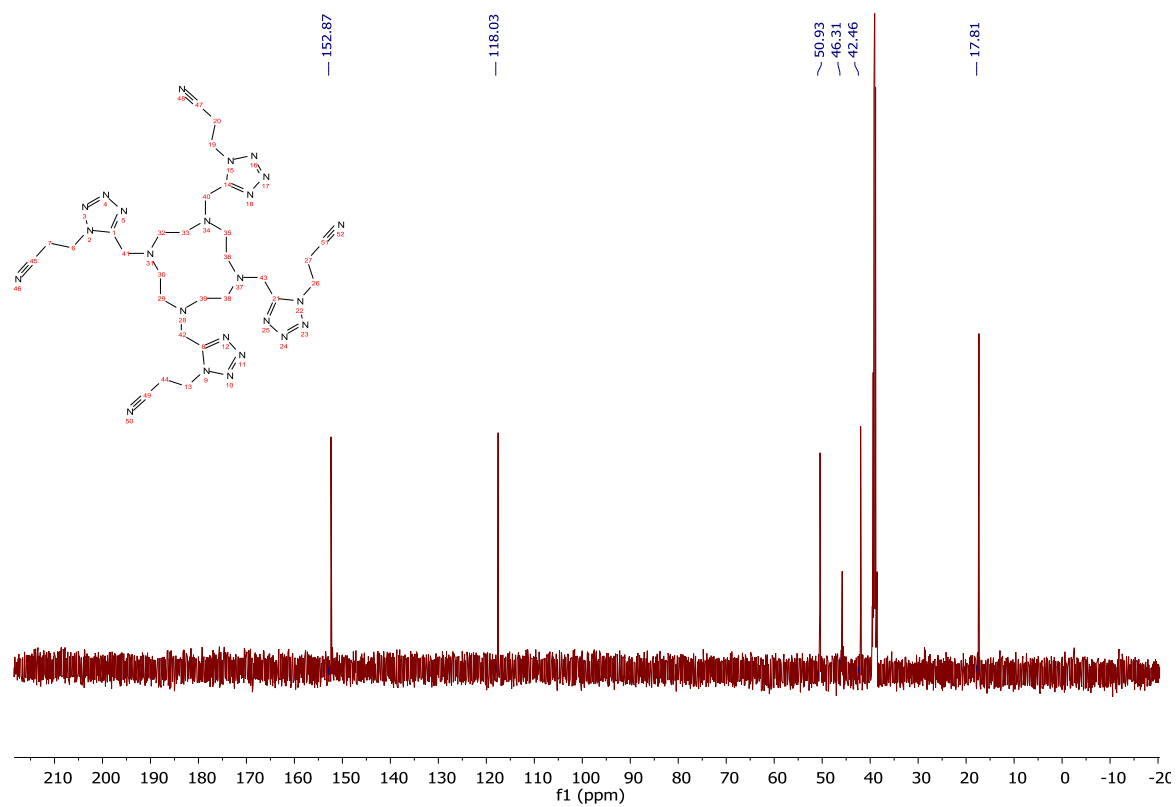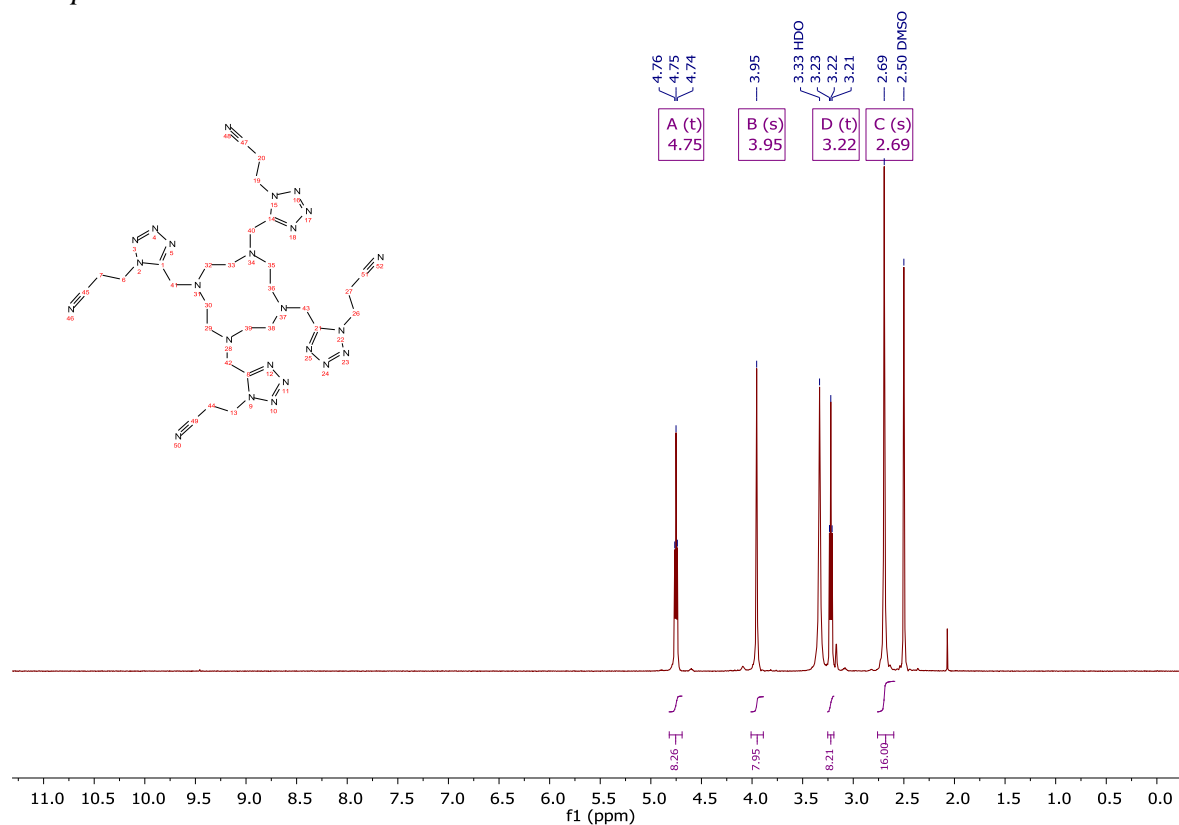# 3. ¹H NMR and ¹³C NMR spectra of compounds 3, 4 and 8
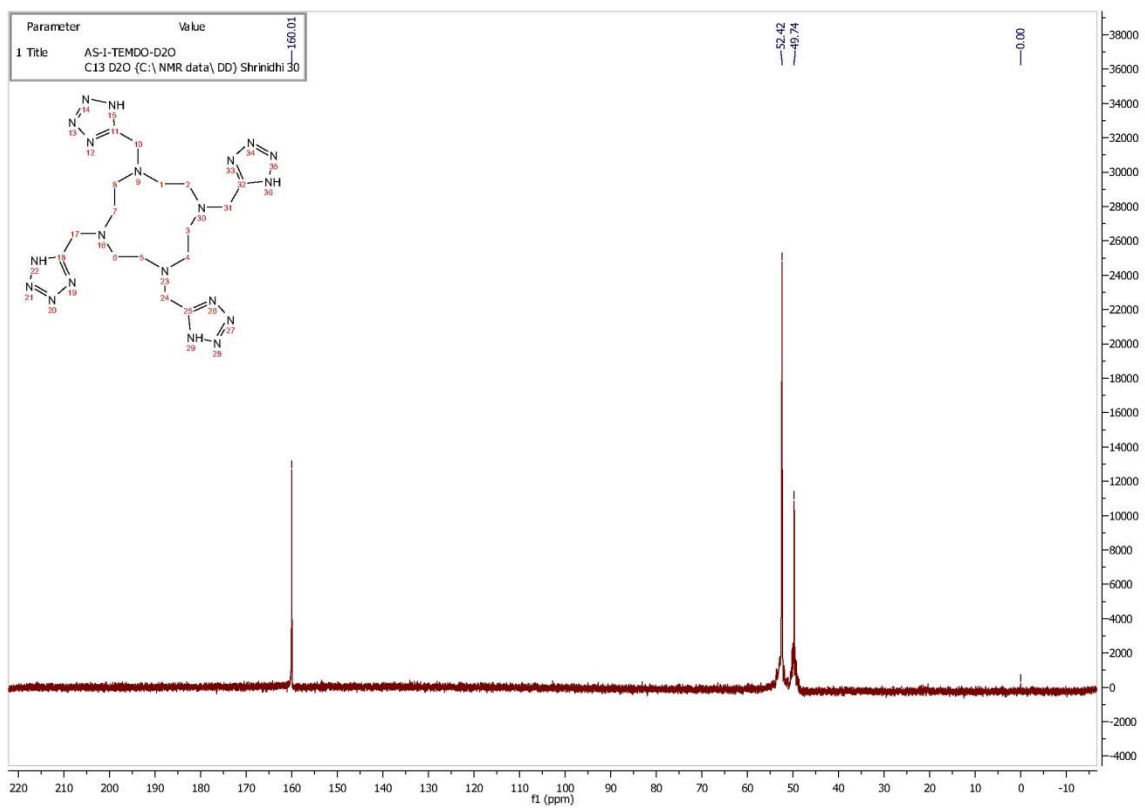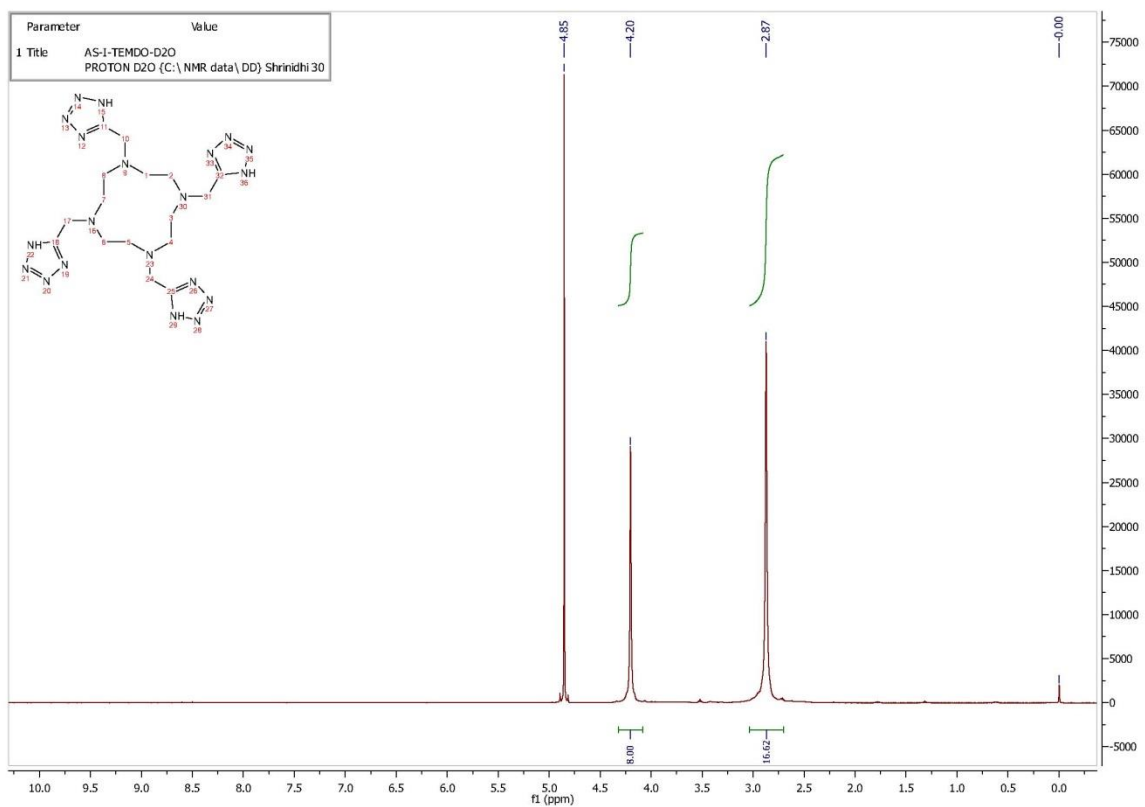
N-(2-cyanoethyl)formamide
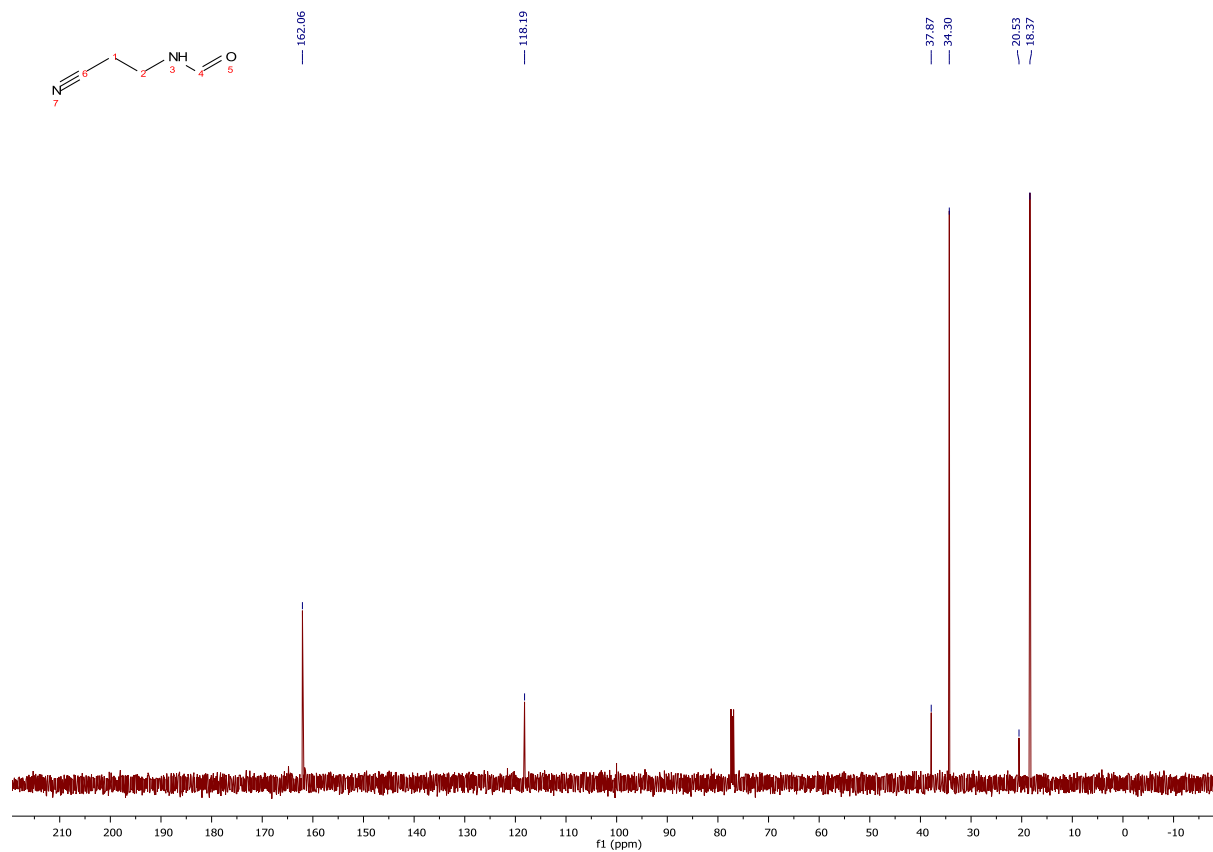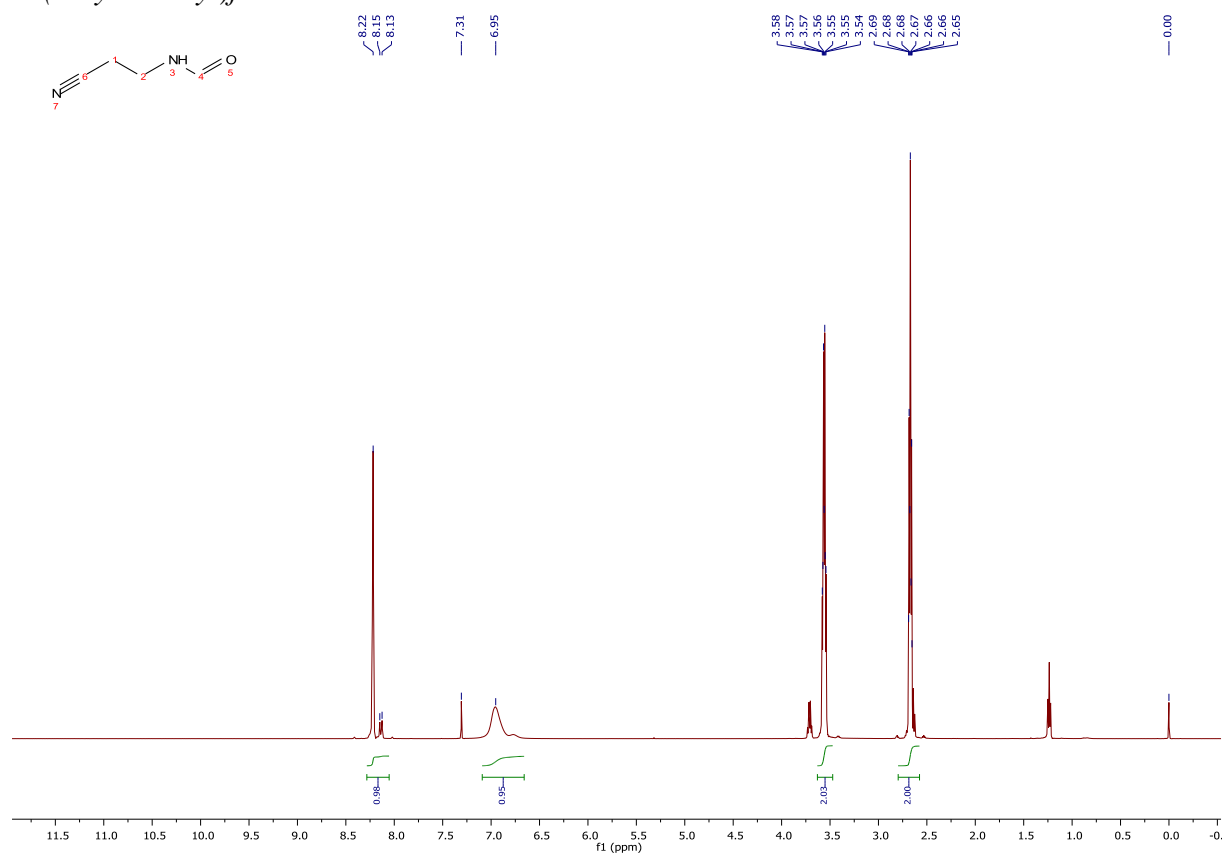
Compound (**4**)
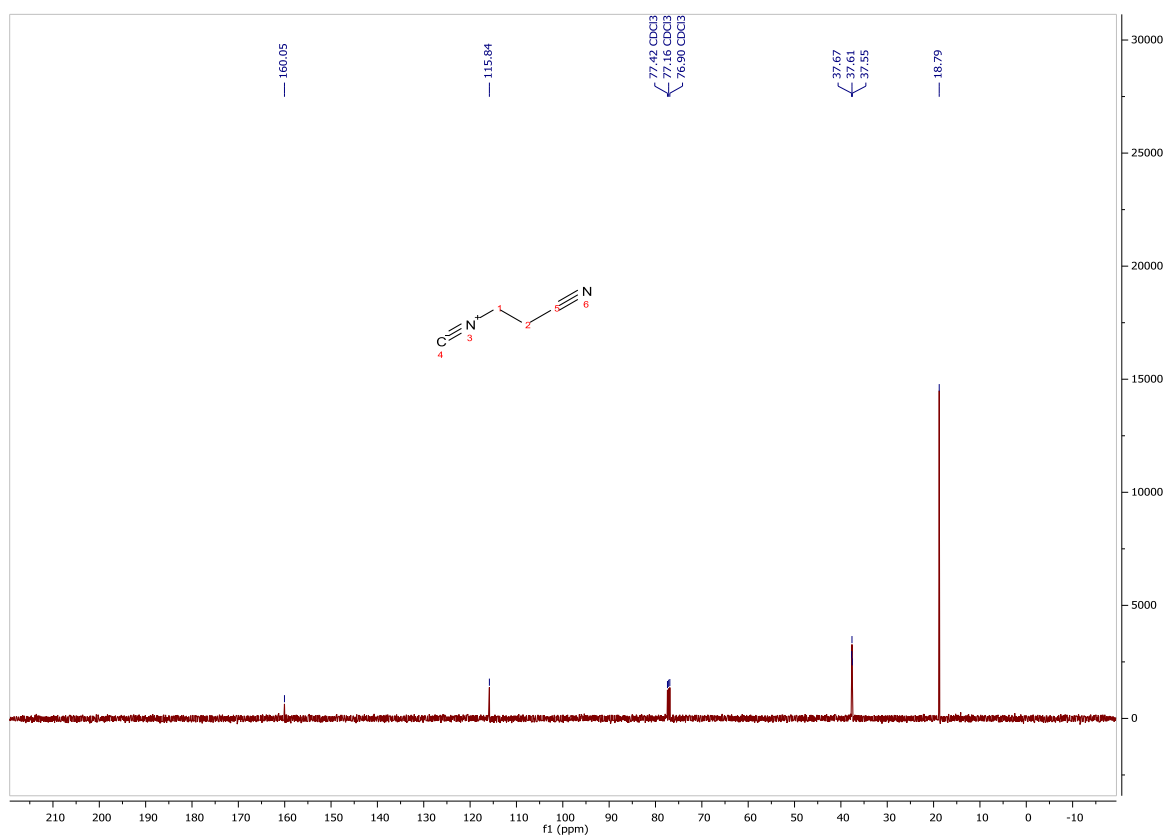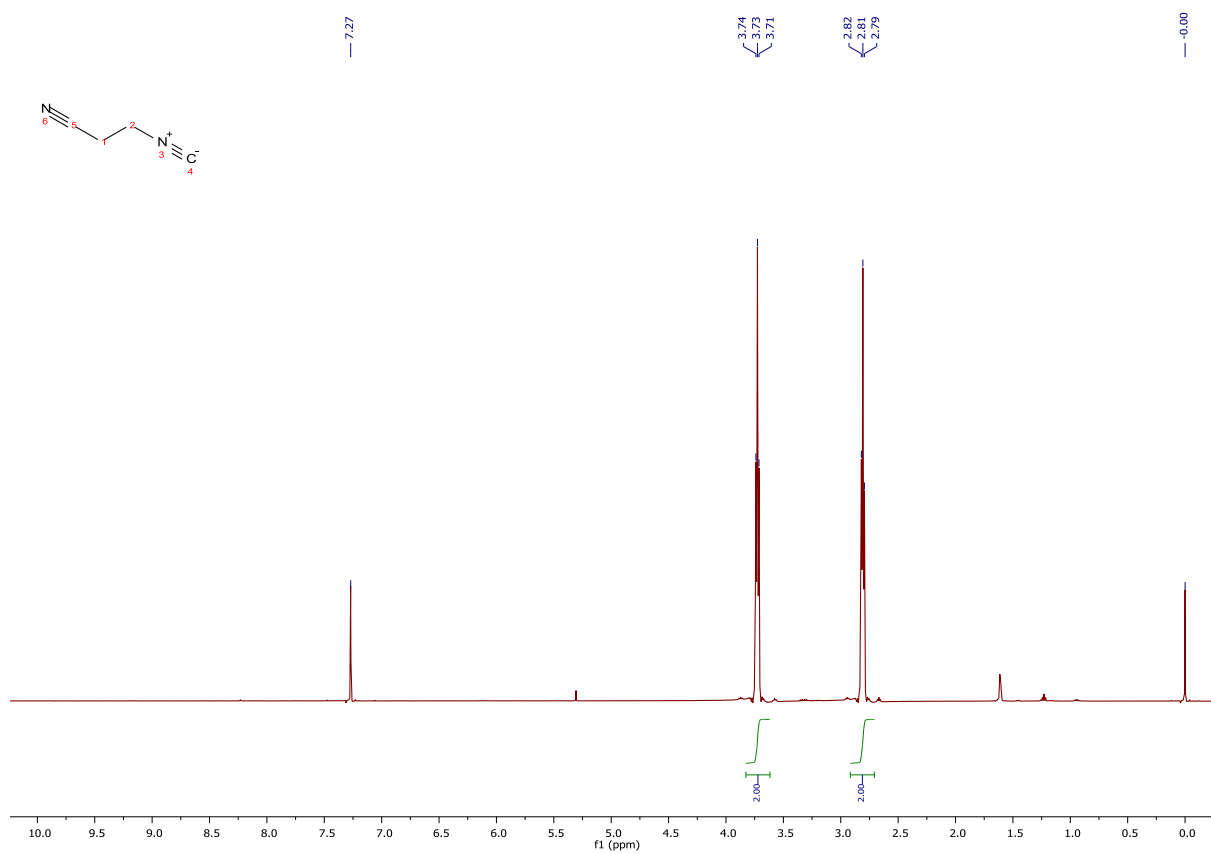
Compound 8

Compound 3

4. Kinetic studies

All kinetics experiments were studied by spectrophotometry at 20 °C in a thermostated cell holder with a Jasco UV-VIS Spectrophotometer. UV-VIS spectra were recorded from 200 nm to 800 nm in 1 cm quartz cell. The determination of rate constants for the formation (k_f) and and dissociation (k_d) of Gd-TEMDO complex were followed at 275 nm in 1.0M KCl.

2a. Kinetics of formation of Gd-TEMDO and Gd-DOTA (k_f):

The rates of the formation reactions were studied for Gd-TEMDO by spectrophotometry in the presence of 10-50 fold excess of Gd^{3+}at 20 °C. A constant pH 5.6 (acetate buffer) and ionic strength 1.0M were maintained to monitor the absorbance at 275nm, where complex was the only absorbing species. The formation of lanthanide complexes with DOTA and their derivatives generally follows a first-order reaction kinetics because the rate determining step is the deprotonation of the monoprotonated intermediate.[1] Therefore, the formation rates of [Gd-TEMDO] can be expressed as, $r_f = \dfrac{d[Gd-TEMDO]}{dt} = k_{obs}[TEMDO]_t \to (1)$, where k_{obs} (= k_f) is a first-order rate constant, $[TEMDO]_t$ is the total concentration of $H_xTEMDO^{(4-x)-}$ species. The plot of k_{obs} vs. $[Gd^{3+}]$ gives a saturation curve which indicates the formation of a reaction intermediate. The rates of complex formation are pH-dependent, slows down considerably with increasing the acid concentrations. The experimentally measured first-order rate constants, k_{obs} values, are proportional to the OH^- concentration, by the equation $k_{obs} = k_{OH}[OH^-]s^{-1} \to (2)$, where the k_{OH} ($M^{-1}s^{-1}$) rate constant characterizes the formation rate.

Procedure: From 10×10^{-3} M TEMDO solution, 0.1 mL was pippeted out and transferred into each of the five 3 mL vials and an aliquot (0.2, 0.4, 0.6, 0.8, 1.0 mL) of 50×10^{-3} M GdCl$_3$ was added to each, respectively. Added 0.2 mL 100×10^{-3} M acetate buffer to each and made up to 2 mL using 1M KCl. The change in wavelength of maximum absorbance (275 nm) was monitored against blank using the Parallel Time Course Measurement program for 24 h. From the plot of Abs vs time, slope of tangent at initial time period corresponds to the value of $\dfrac{d[Gd-TEMDO]}{dt}$ and $[TEMDO]_t = [TEMDO]_o$.

Table 1. Calculation table for TEMDO:

Initial rate kinetics				
Approximations: dAbs/dt = d[Gd-L]/dt and [Gd-L]$_t$ = [Gd-L]]$_0$				
Equations: d[Gd-L]/dt = kobs[Gd-L]] and k$_{obs}$ = k$_{OH}$ [OH$^-$]				
[Gd^{3+}] (M)	[TEMDO]$_0$ (M)	d[Gd-TEMDO]/dt in M^{-1} min^{-1}	d[Gd-TEMDO]/dt in M^{-1} s^{-1}	k$_{obs}$
0	0	0	0	0
0,005	0,0005	3,58E-07	5,97E-09	1,19E-05
0,01	0,0005	4,08E-07	6,80E-09	1,36E-05
0,015	0,0005	4,30E-07	7,17E-09	1,43E-05
0,02	0,0005	4,36E-07	7,27E-09	1,45E-05
0,025	0,0005	4,40E-07	7,33E-09	1,47E-05

Figure 1. Plot of [Gd^{3+}] vs k$_{obs}$ for Gd-TEMDO complex at pH = 5.6

pH	[OH$^-$]	Σ kobs (s^{-1})	from, kobs = k$_{OH}$ [OH$^-$],	k$_{OH}$ (M^{-1}s^{-1})
5,6	3,98E-09	1,38E-05		**3,47E+03**

Similarly, the formation rates were also calculated for Gd-DOTA for comparison purposes.

Table 2. Calculation table for DOTA:

[Gd^{3+}] (M)	[DOTA]$_0$ (mM)	d[Gd-DOTA]/dt in M^{-1} min^{-1}	d[Gd-DOTA]/dt in M^{-1} s^{-1}	k$_{obs}$
0	0	0	0	0
0,005	0,0005	4,93E-04	8,22E-06	1,64E-02
0,01	0,0005	6,07E-04	1,01E-05	2,02E-02
0,015	0,0005	6,47E-04	1,08E-05	2,16E-02
0,02	0,0005	6,59E-04	1,10E-05	2,20E-02
0,025	0,0005	6,74E-04	1,12E-05	2,25E-02

Figure 2. Plot of [Gd^{3+}] vs k$_{obs}$ for DOTA

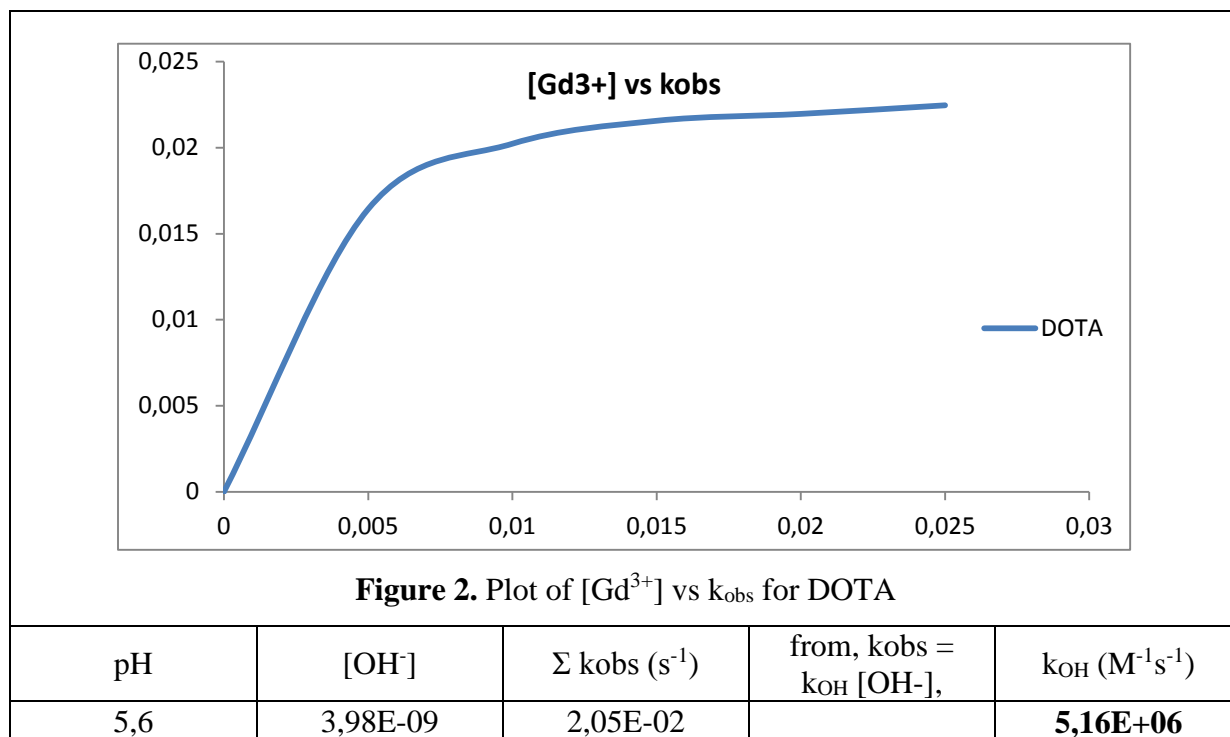| pH	[OH$^-$]	Σ kobs (s^{-1})	from, kobs = kOH [OH-],	kOH (M^{-1}s^{-1})
5,6	3,98E-09	2,05E-02		**5,16E+06**

Table 3. Formation rate constants of [Gd-TEMDO] and [Gd-DOTA] complexes (I = 1.0 M KCl, T = 20 °C)

Gd-L complex	$k_{obs}(s^{-1})^a$	$k_{OH}(M^{-1}s^{-1})^a$	$k_{OH}(M^{-1}s^{-1})$ at 25°C[b]
Gd-TEMDO	$1.38 \pm 0.5 \times 10^{-5}$	$3.47 \pm 0.5 \times 10^{3}$	---
Gd-DOTA	$2.05 \pm 0.5 \times 10^{-2}$	$5.16 \pm 0.5 \times 10^{6}$	5.90×10^{6}

[a]This work, [b]Ref. [2]

<u>2b. Kinetics of dissociation of Gd-TEMDO and Gd-DOTA (k_d):</u>

Gd-TEMDO complex required for the dissociation studies were synthesized according to the given procedure in main article. Similarly, Gd-DOTA complex was synthesized from commercially obtained DOTA ligand.

Since the lanthanide complexes DOTA and their derivatives are known to be unstable at pH<1.[3] Therefore, the dissociation kinetics of Gd-TEMDO was studied in 0.1-0.5M HCl (the sum of HCl and KCl concentration were 1.0M) at 20 °C. The acid assisted dissociation of Gd-TEMDO complex was monitored at 275nm, where complex was the only absorbing species. In the presence of excess acid, the dissociation rate of Gd-TEMDO complex can be written as,

$$r_d = -\frac{d[Gd-TEMDO]}{dt} = k_d[Gd-TEMDO]_t \rightarrow (3)$$, where k_d is pseudo-first order rate

constant. The dissociation rate is directly proportional to acid concentration as,

$k_d = k_0 + k_H[H^+] \rightarrow (4)$, where k_0 is characteristic for the dissociation of Gd-TEMDO and k_H is characteristic for the dissociation of Gd-TEMDO.

Procedure: From 100×10^{-3} M Gd-TEMDO solution, 0.2 mL was pippeted out and transferred into each of the five 3 mL vials and an aliquot (0.2, 0.4, 0.6, 0.8, 1.0 mL) of 1M HCl was added to each, respectively. The solution was made up to 2 mL using 1M KCl. The change in wavelength of maximum absorbance (275 nm) was monitored against blank using the Parallel Time Course Measurement program for 24 h. From the plot of Abs vs time, slope of tangent at initial time period corresponds to the value of $\dfrac{d[Gd-TEMDO]_t}{dt}$ and $[Gd\text{-}TEMDO]_t = [Gd\text{-}TEMDO]_o$. In the same way dissociation rates were studied for Gd-DOTA.

Table 4. Calculation table for TEMDO:

Initial rate kinetics				
Approximations: dAbs/dt = d[Gd-L]/dt and [Gd-L]t = [Gd-L]$_0$				
Equations: d[Gd-L]/dt = k$_{obs}$[Gd-L]] and k$_{obs}$ = k$_d$+k$_H$ [H$^+$]				
[Gd-TEMDO] (M)	[HCl] (M)	d[Gd-TEMDO]/dt in M-1 min-1	d[Gd-TEMDO]/dt in M-1 s-1	k$_{obs}$
	0			
1,00E-02	0,1	5,71E-09	9,52E-11	9,52E-09
1,00E-02	0,2	6,37E-09	1,06E-10	1,06E-08
1,00E-02	0,3	7,11E-09	1,19E-10	1,19E-08
1,00E-02	0,4	7,92E-09	1,32E-10	1,32E-08
1,00E-02	0,5	8,54E-09	1,42E-10	1,42E-08

Figure 3. Plot of k$_{obs}$ vs [H$^+$] for Gd-TEMDO complex

k$_{obs}$ = k$_d$+k$_H$ [H$^+$]	k$_H$ (M^{-1}sec^{-1})	**1,20E-08**		
	k$_d$ (sec^{-1})	8,28E-09		

Similarly, the dissociation rates were also calculated for Gd-DOTA as follows.

Table 5. Calculation table for DOTA:

[Gd-DOTA] (M)	[HCl] (M)	d[Gd-DOTA]/dt in M^{-1} min^{-1}	d[Gd-DOTA]/dt in M^{-1} s^{-1}	k_{obs}
	0			
1,00E-02	0,1	1,29E-07	2,14E-09	2,14E-07
1,00E-02	0,2	3,29E-07	5,49E-09	5,49E-07
1,00E-02	0,3	5,19E-07	8,66E-09	8,66E-07
1,00E-02	0,4	7,05E-07	1,18E-08	1,18E-06
1,00E-02	0,5	9,00E-07	1,50E-08	1,50E-06

$$y = 3{,}196E\text{-}06x - 9{,}823E\text{-}08$$
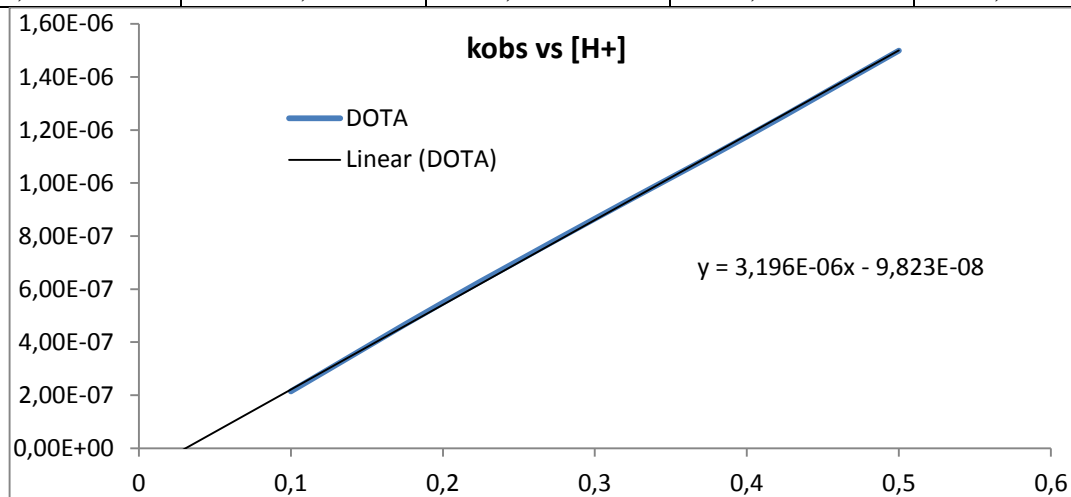
Figure 4. Plot of k_{obs} vs [H$^+$]

$k_{obs} = k_d + k_H$ [H$^+$]	k_H (M^{-1}sec^{-1})	**3,20E-06**	
	k_d (sec^{-1})	9,82E-08	

Table 6. Rate constants characterizing proton assisted dissociation of [Gd-TEMDO] and [Gd-DOTA] complexes (I = 1.0 M KCl, T = 20 °C)

Gd-L complex	$k_d (s^{-1})$ [a]	$k_H (M^{-1}s^{-1})$ [a]	$k_H (M^{-1}s^{-1})$ at 25°C [b]
Gd-TEMDO	$8.28 \pm 0.5 \times 10^{-9}$	$1.20 \pm 0.5 \times 10^{-8}$	---
Gd-DOTA	$9.82 \pm 0.5 \times 10^{-8}$	$3.20 \pm 0.5 \times 10^{-6}$	8.40×10^{-6}

[a]This work, [b]Ref. [4]

From Tables 5 and 6, it is clear that the Gd-TEMDO complex is 1.5×10^3 times slower in formation as well as 2.7×10^2 times slower in dissociation as compared to Gd-DOTA complex.

5. Equilibrium studies

The stability constant of the metal-ligand complex is described as, $K = \dfrac{[M.\mathbf{1}]}{[M].[\mathbf{1}]} \rightarrow (5)$, where

[M.**1**] is the equilibrium concentration of the metal-ligand complex, [M] is the equilibrium concentration of free metal ions and [**1**] is the equilibrium concentration of the ligand.

The stability constants (K) were determined as a product of the conditional stability constant of the metal-ligand complex (K') and the coefficient α (at pH 5.6).

The conditional stability constant of the metal - ligand is defined as, $K' = \dfrac{[M.\mathbf{1}]}{[M]\{C(\mathbf{1}) - [M.\mathbf{1}]\}} \rightarrow (6)$, where [M.**1**] is the equilibrium concentration of the metalligand complex, [M] is the equilibrium concentration of free metal ions and C(**1**) is the molar concentration of the ligand.

The coefficient α is defined as, $\alpha = \left(1 + \sum\limits_{i=1}^{n} [H^+]^i \times \beta_i \right) \rightarrow (7)$, where β_i is the overall

protonation constant of an $H_i\mathbf{1}$ species defined by equation $\beta_i = \dfrac{[H_n\mathbf{1}^{(4-i)-}]}{[\mathbf{1}^{4-}].[H^+]^i} \rightarrow (8)$ and $\mathbf{1}^{4-}$

represents the TEMDO anion.

The standard deviation is defined as, $s_i = \sqrt{\dfrac{1}{n-1} \times \sum (\beta_i - \overline{\beta_i})^2} \rightarrow (9)$, where $\overline{\beta_i}$ is the

average value of the constant β_i and n = 4.

Protonation constants (log β_i) and stability constant (log K) values were determined using the literature procedure.[5]

3a. Determination of protonation constant of Gd-TEMDO and Gd-DOTA (β_i's):

The protonation constants were calculated from the potentiometric data with the computer program LTGW ETITR,[5] derived from LETAGROP ETITR.[6] The obtained protonation constants of TEMDO and DOTA (Table 1) were used to calculate their coefficient α

The "static titration curve" for the mixtures of a 10^{-4} M solution of TEMDO with various amounts of carbonate-free KOH at constant ionic strength (0.1 M KCl) is depicted in Figure 5A. The pH range of the titrations was 2-10. Each point was prepared and measured three

times and the average value was used for further calculations. It was found that TEMDO reached its protonation equilibrium after 96 hours under experimental conditions. Maximum difference between the data of each point was 0.05 pH unit.

<u>Procedure</u>: Method of the static titration based on the long-term pH measurement of the studied reagents was applied for the determination of protonation constants of TEMDO. pH meter pHenomenal® with Thermo Scientific Orion ROSS Ultra pH electrode was used for static potentiometry at 20 °C. The static titration of TEMDO was performed with a set of various solutions. From 1×10^{-3} M TEMDO solution, 1 mL was pippeted out and transferred into each of the twenty-six 20 mL vials and an aliquot (0.0 to 2.5 mL, in 0.1 mL increments) of 2.5×10^{-3} M KOH was added to each, respectively. These experiments were carried out in a box with N_2 atmosphere to eliminate the effect of carbon dioxide. The pH values of the reaction mixtures were measured for 200 hours (96 hours is need for the reach of equilibrium). The pH electrode was calibrated using standard buffers (pH = 4.00, 7.00, 9.00) obtained from VWR chemicals. Similarly, the protonation constants were studied for Gd-DOTA (Figure 5B).
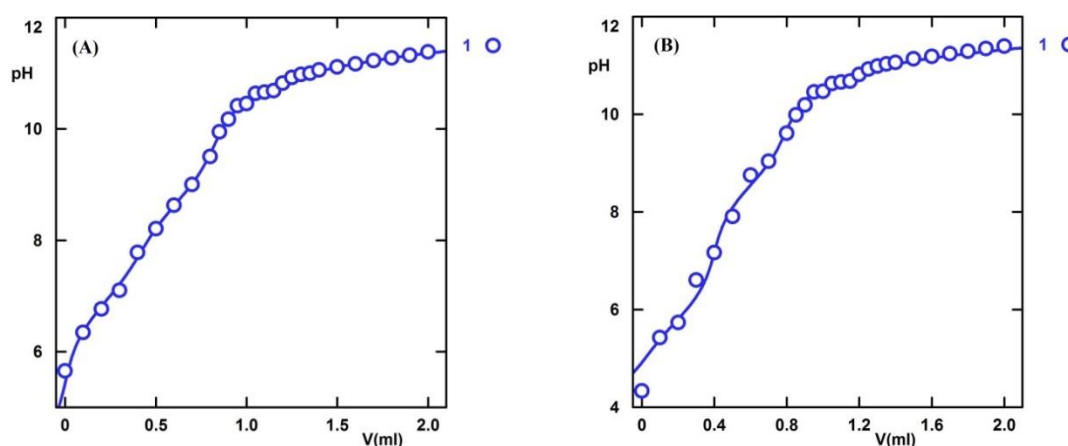

Figure 5A. Titration curve of TEMDO (in box under atmosphere of nitrogen). Reaction mixture: total volume 10 mL composed of TEMDO (C = 10^{-4} M), KOH (concentration from 0 to 4.8 x 10^{-4} M), KCl (I = 0.1 M), reaction time: 200 hours. The curves were calculated using the constants given in Table 3. **B.** Titration curve of DOTA (in box under atmosphere of nitrogen). Reaction mixture: total volume 10 mL composed of DOTA (C = 10^{-4} M), KOH (concentration from 0 to 4.8 x 10^{-4} M), KCl (I = 0.1 M), reaction time: 50 hours. The curves were calculated using the constants given in Table 3.

Table 7. Protonation constants ($\log \beta_i^H$) of TEMDO and DOTA ligands (I = 1.0 M KCl, T = 20 °C, values given in parentheses are standard deviations)

Ligand\Species	[HL]	[H₂L]	[H₃L]	[H₄L]	$\log \alpha$
TEMDO	10.0 (s=0.04)	14.9 (s=0.04)	23.4 (s=0.04)	30.3 (s=0.04)	13.0
DOTA[a]	12.05 (0.04)	21.96 (0.005)	26.52 (0.005)	30.5 (0.005)	15.5
DOTA[b]	12.09 (0.04)	21.85 (0.005)	26.4 (0.005)	30.5 (0.005)	---

[a]This work, [b]Ref. [7]

3b. Determination of Stability constant of Gd-TEMDO and Gd-DOTA (K):

The conditional stability constants of Gd-**Arsenazo III** (Gd-**2**) and Gd-Arsenazo III-TEMDO complexes were calculated from the spectrophotometric data by using least squares minimizing program LTGW-SPEFO,[5] derived from LETAGROP SPEFO.[8] The formation of a Gd-**TEMDO/DOTA** (Gd-**1**) complex was observed at optimum complexation conditions, i.e. pH 5.6.

The spectra of Arsenazo III and Gd-**2** complexes are depicted in Figure 6. The conditional stability constants of Gd-**2** were determined by titrating **2** with a solution of the Gd^{3+} ion. All solutions were prepared separately and the absorbencies were measured after 15 days (absorbencies refer to different GdL complexes). The conditional stability constants of Gd-**2** were calculated from data at wavelengths 662, 608, 556, 428 and 309 nm (Figure 7, Table 8; reaction time: 15 days at at 20 °C). These values could be used for calculating the conditional stability constants of a Gd-**1** complex from data at wavelengths 662 (663 for DOTA), 608, 556 and 309 nm (Figure 8A for TEMDO and Figure 8B for DOTA, Table 8; reaction time: 10 days at 65 °C followed by 5 days at 20 °C) because the pH values were identical in both cases (pH = 5.6). The values of the conditional stability constants of Gd-**1** complex K' were obtained by these calculations. Three sets of data were used for these calculation and the average values of constant K'.

Procedure: All equilibrium measurements were studied by spectrophotometry at 20 °C in a thermostated cell holder with a Jasco UV-VIS Spectrophotometer. UV-VIS spectra were recorded from 250 nm to 800 nm in 1 cm quartz cell using Spectra Measurement program. Conditional stability constants of Gd-**2** complexes were determined in pH = 5.6 sodium acetate buffer. The constants were determined by titration of **2** solution (0.050 mL, c = 1x10⁻³ M) with metal ion solution (0.010 mL to 0.300 ml, in 10 μL increments, c = 5x10⁻⁴ M) in

sodium acetate buffer (1.95 ml, c = 0.1 M; pH = 5.6) and the changes of the absorbance during the titration were measured.

This complexation was studied by UV-VIS titration of Gd-**2** complex (0.050 mL, c(AA) = $1x10^{-3}$ M; 0.050 mL, c(metal) = $1x10^{-3}$ M) in sodium acetate buffer (1.90 mL, c = 0.1 M; pH = 5.6) with a solution of the chelator, **1** (TEMDO or DOTA, 0.010 ml to 0.300 mL, in 0.010 ml increments, c = $5x10^{-4}$ M). The reaction mixture of ion metal solution with **2** solution was titrated by **1** solution with 0.010 mL additions. These reaction mixtures were maintained at 65 °C in sealed vials for 6 weeks followed by an additional 24 h at 20 °C to reach equilibration and the changes of the absorbance were measured.

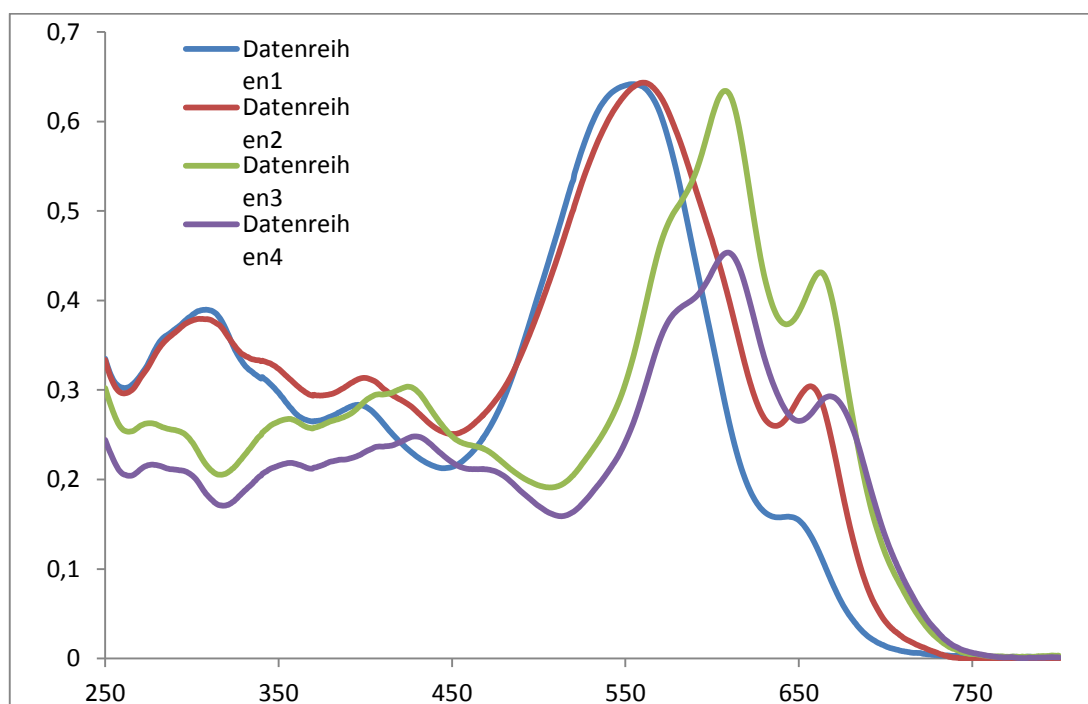**Figure 6.** Plot of absorbance vs. wavelength. UV-VIS spectrum of titration of Arsenazo III by GdCl$_3$ solution (T = 20 °C). Reaction mixture: Arsenazo III (C = $2.5x10^{-5}$M), sodium 0.1M sodium acetate buffer, pH = 5.6; Series 1 − **2** only, Series 2 − **2** + $2.49x10^{-6}$M GdCl$_3$, Series 3 − **2** + $9.80x10^{-6}$M GdCl$_3$, Series 4 − **2** + $6.52x10^{-5}$M GdCl$_3$.
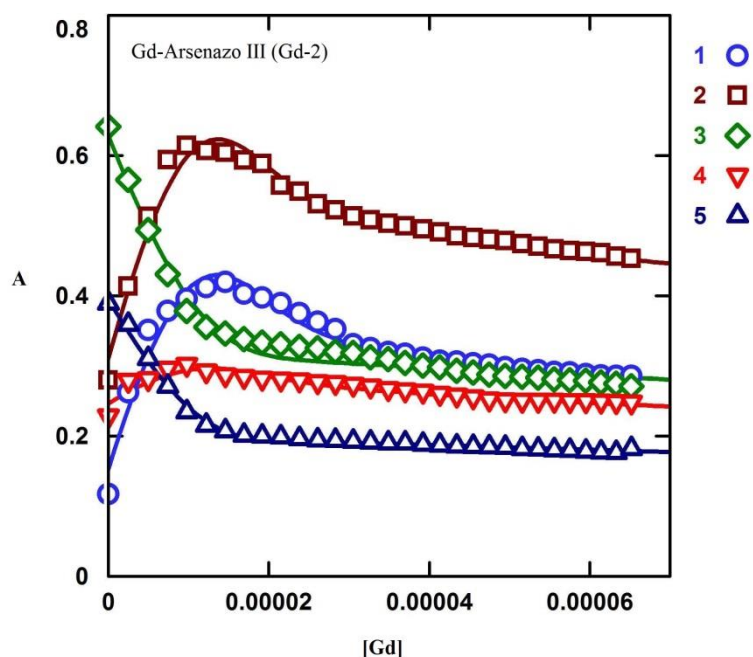
Figure 7. Plots of absorbance vs concentration of Gd^{3+}. Spectrophotometric titration of Arsenazo III solution by the solution of $GdCl_3$. $C(\mathbf{2})_0 = 2.35 \times 10^{-5}$ M, 0.1M sodium acetate buffer, pH = 5.6; λ (nm): ○- 662, ☐- 608, ◇- 556, ▽- 428, △- 309.

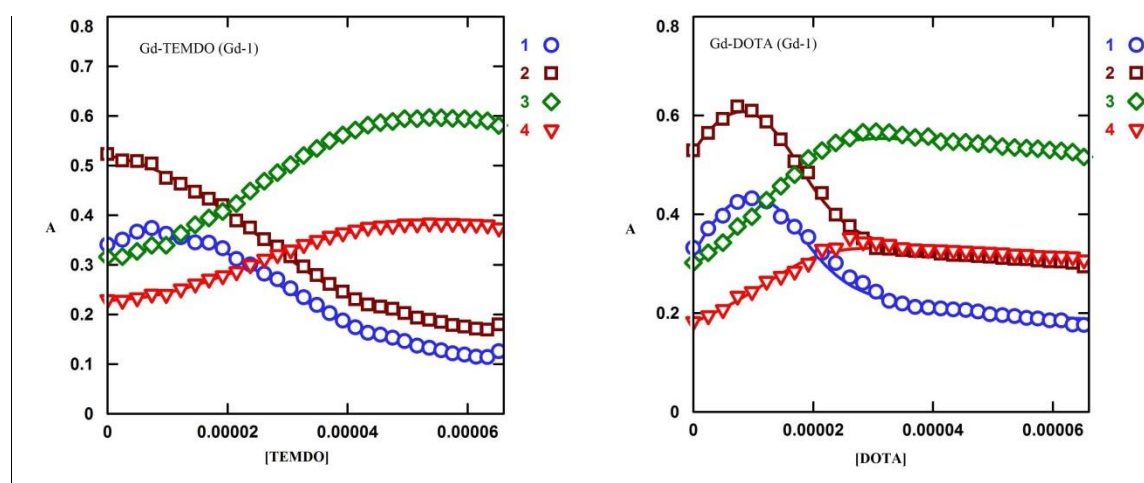**Figure 8.** Plots of absorbance vs concentration of **1**.

A- Spectrophotometric titration of Gd-**2** solution by the solution of TEMDO. $C(\mathbf{2})_0 = C(Gd)_0 = 2.35 \times 10^{-5}$ M. λ (nm): ○- 662, ☐- 608, ◇- 556, ▽- 309. Solid curves were calculated for constants given in Table 3.

B- Spectrophotometric titration of Gd-**2** solution by the solution of DOTA. $C(\mathbf{2})_0 = C(Gd)_0 = 2.35 \times 10^{-5}$ M. λ (nm): ○- 663, ☐- 608, ◇- 556, ▽- 309. Solid curves were calculated for constants given in Table 3.

Table 8. The conditional stability constants log β´ of Gd-Arsenazo III complex, conditional stability constants log K' and stability constants Log K of Gd-TEMDO complexes determined by UV-VIS Spectrophotometry (T = 20 °C).

Species	$\log \beta^{'}$	$\log K^{'}$	$\log K^{a}$	$\log K^{b}$

Gd-**2**₁	6.5 (s = 0.3)	---	---	
Gd-**2**₂	11.3 (s = 0.4)	---	---	
Gd-TEMDO	---	3.6 (s = 0.3)	16.6 (s = 0.3)	
Gd-DOTA	---	8.6 (s = 0.3)	24.1 (s = 0.2)	24.7

[a]This work, [b]Ref.[9]

Stability constant data from Table 8 shows that the Gd-TEMDO complex is less stable than Gd-DOTA.

6. Relaxivity measurements

MRI

A 400MHz (9.4 T) and 89 mm vertical bore scanner was used, equipped with 1500 mT/m gradient set (Bruker Biospin GmbH, Ettlingen, Germany)

Phantoms

Phantom tubes with a concentration range of 0, 0.125, 0.25 ,0.5, 1 and 2 mM in phosphate buffered saline (pH 7.50) at ambient temperature were used for relaxivity measurements.

Longitudinal T_1 measurement

T1 measurement was performed with a multiple spin echo saturation recovery method with variable TR. Slice excitation and refocussing were accomplished by hermite pulses resulting in 90 and 180 pulse lengths of 1.4 and 0.9ms. The following parameters were used: echo time (TE)=5.5ms;TR-array =100,150,300,500,900,1500,3000,6000,15000 and 20000ms;matrix size=128x128;FOV=3x3cm;slice thickness=1mm.The slice was positioned through the center of all phantom tubes, and images were acquired as single slice to prevent interslice modulation effects. The total acquisition time was 1h15min55s.

Data processing

For the T_1 fits, 8 TR values with a fixed echo time of 5.5ms were used to calculate T_1 ROI were drawn on the images using an image sequence analysis tool package (Bruker Paravision 4.0) using a three parameter fit function M(t)=A+M0(1-exp(-t/T1), were M0 is the equilibrium magnetization. All fits were performed using a non-linear least-squares algorithm.

Relaxivity of the contrast agent is defined as the efficiency by which an MRI contrast agent can accelerate the proton relaxivity rate in a homogeneous medium. The relaxation rate was calculated from the equation:(R1=1/T1(s-1). Relaxivity (r1) was calculated as the slope of the linear regression line of a plot of R1 versus concentration of MRI contrast agent.

Table 9. Gd-TEMDO relaxation rate determination

Conc (mM)	T_1 (ms)	R_1 (s^{-1})	r_1 (mM^{-1} s$^{-1)}$
0.1	1541,012	0.649	6.489
0.25	1145,698	0,873	3.491
0.5	788,6531	1.268	2.536
1	497,2361	2.011	2.011

Table 10. Gd-DOTA relaxation rate determination

Conc (mM)	T_1 (ms)	R_1 (s^{-1})	r_1 (mM^{-1} s$^{-1)}$
0.125	865.78	1.16	9.28
0.25	547.08	1.83	7.32
0.5	305.22	3.28	6.56
1	165.98	6.02	6.02

7. Crystallographic data

Single Crystal X-Ray Structure Determination of Ln-TEMDO complexes

General:

Data were collected on an X-ray single crystal diffractometer equipped with a CCD detector (Bruker APEX II, κ–CCD), a fine-focus sealed tube (Bruker AXS, D8) with MoK$_\alpha$ radiation (λ = 0.71073 Å), and a graphite monochromator by using the SMART software package.[1] The measurements were performed on a single crystal coated with perfluorinated ether. The crystal was fixed on the top of a cactus prickle (Opuntia ficus-india) and transferred to the diffractometer. The crystal was frozen under a stream of cold nitrogen. A matrix scan was used to determine the initial lattice parameters. Reflections were merged and corrected for Lorenz and polarization effects, scan speed, and background using SAINT.[2] Absorption corrections, including odd and even ordered spherical harmonics were performed using SADABS.[2] Space group assignments were based upon systematic absences, *E* statistics, and successful refinement of the structures. Structures were solved by direct methods with the aid of successive difference Fourier maps, and were refined against all data using WinGX[7] based on SIR-92 [3] in conjunction with SHELXL-97 [5]. C–*H* atoms were placed in calculated positions and refined using a riding model, with C–H distances of 0.99 Å, and U$_{iso(H)}$ = 1.2·U$_{eq(C)}$. O–*H* atoms were placed in calculated positions, with O–H distances of 0.84 Å, and U$_{iso(H)}$ = 1.5·U$_{eq(O)}$. Full-matrix least-squares refinements were carried out by minimizing $\Sigma w(F_o^2-F_c^2)^2$ with SHELXL-97[5] weighting scheme. Neutral atom scattering factors for all

atoms and anomalous dispersion corrections for the non-hydrogen atoms were taken from *International Tables for Crystallography*.[4] Images of the crystal structures were generated by PLATON. [6] CCDC 1039908 (La-TEMDO) contains the supplementary crystallographic data for this compound. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or via https://www.ccdc.cam.ac.uk/services/structure_deposit/

Special:

La-TEMDO: Full refinement was possible without running into problems. The atom O7 – crystal water was refined with an isotropic displacement parameter.
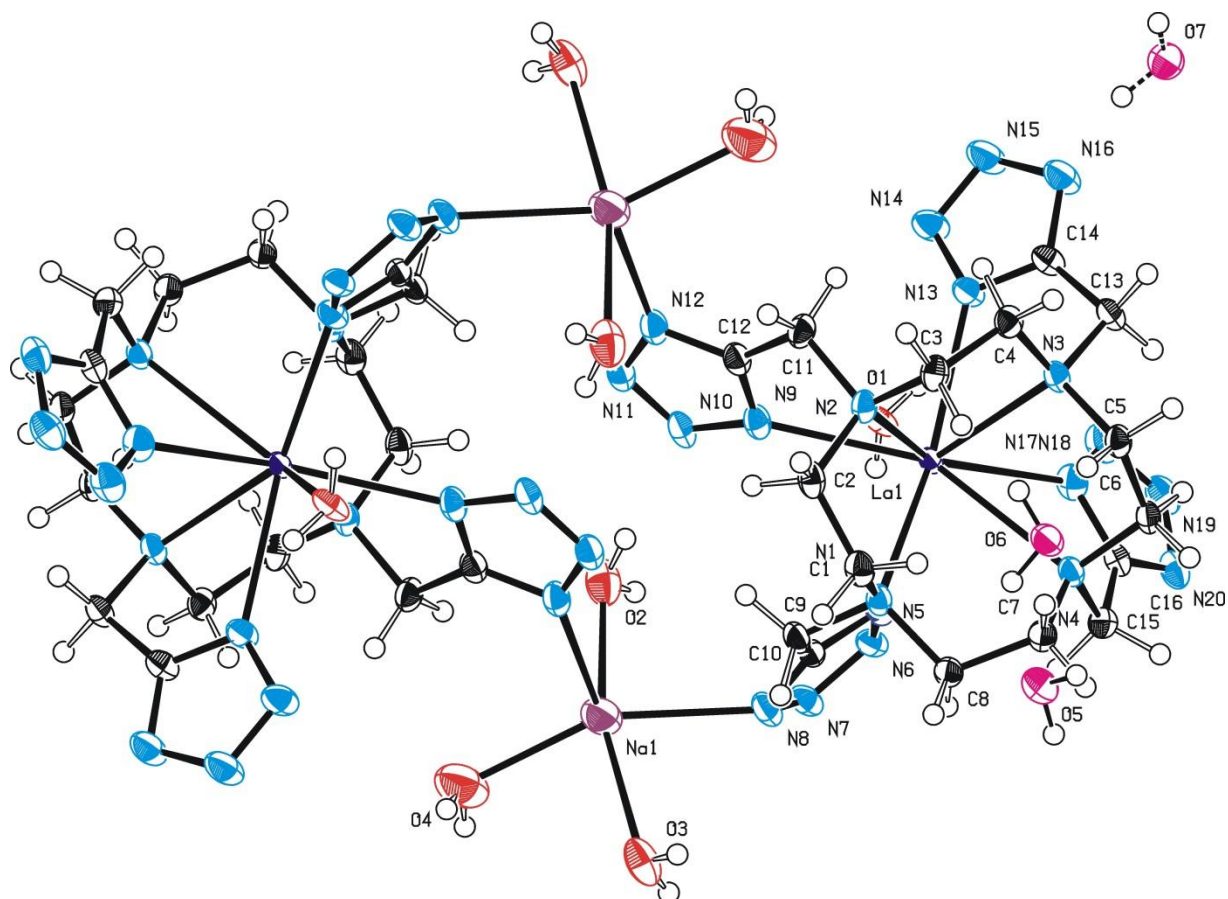
La-TEMDO

Figure 9. – Ortep drawing of compound **La-TEMDO** with 50% ellipsoids. [6]

Operator:	*** Herdtweck ***
Molecular Formula:	$C_{32} H_{70} La_2 N_{40} Na_2 O_{11}$
	$(C_{32} H_{64} La_2 N_{40} Na_2 O_8), 3(H_2 O)$
Crystal Color / Shape	Colorless fragment
Crystal Size	Approximate size of crystal fragment used for data collection:
	$0.13 \times 0.15 \times 0.25$ mm
Molecular Weight:	1515.08 a.m.u.
F_{000}:	1532
Systematic Absences:	h0l: h+l$\neq$2n; 0k0: k$\neq$2n
Space Group:	Monoclinic $\quad P\,2_1/n \quad$ (I.T.-No.: 14)
Cell Constants:	Least-squares refinement of 9670 reflections with the programs "APEX suite" and "SAINT" [1,2]; theta range $1.81° < \theta < 25.43°$; Mo(K$\overline{\alpha}$); $\lambda = 0.71073$ Å
	$a = \quad$ 11.1804(4) Å
	$b = \quad$ 17.6279(5) Å $\qquad \beta = \quad$ 91.9317(13)°

	$c =$ 14.6590(4) Å
	$V = 2887.46(15)$ Å^3; $Z = 2$; $D_{calc} = 1.743$ g cm^{-3}; Mos. $= 0.72$
Diffractometer:	Kappa APEX II (Area Diffraction System; BRUKER AXS); sealed tube; graphite monochromator; 50 kV; 30 mA; λ = 0.71073 Å; Mo(K$\overline{\alpha}$)
Temperature:	(-150±1) °C; (123±1) K
Measurement Range:	$1.81° < \theta < 25.43°$; h: -13/13, k: -21/21, l: -17/17
Measurement Time:	2×7.50 s per film
Measurement Mode:	measured: 10 runs; 4415 films / scaled: 10 runs; 4415 films φ– and ω–movement; Increment: $\Delta\varphi/\Delta\omega = 0.50°$; dx = 45.0 mm
LP - Correction:	Yes [2]
Intensity Correction	No/Yes; during scaling [2]
Absorption Correction:	Multi-scan; during scaling; $\mu = 1.564$ mm^{-1} [2]
	Correction Factors: T$_{min}$ = 0.6763 T$_{max}$ = 0.7452
Reflection Data:	84780 reflections were integrated and scaled
	2194 reflections systematic absent and rejected
	82586 reflections to be merged
	5322 independent reflections
	0.025 R$_{int}$: (basis F_o^2)
	5322 independent reflections (all) were used in refinements
	4968 independent reflections with $I_o > 2\sigma(I_o)$
	99.8 % completeness of the data set
	402 parameter full-matrix refinement
	13.2 reflections per parameter
Solution:	Direct Methods [3, 7]; Difference Fourier syntheses
Refinement Parameters:	In the asymmetric unit:
	44 Non-hydrogen atoms with anisotropic displacement parameters
	1 Non-hydrogen atoms with isotropic displacement parameters
Hydrogen Atoms:	In the difference map(s) calculated from the model containing all non-hydrogen atoms, not all of the hydrogen positions could be determined from the highest peaks. For this reason, the hydrogen

atoms were placed in calculated positions (d_{C-H} = 0.99 Å; d_{O-H} = 0.84 Å). Isotropic displacement parameters were calculated from the parent carbon atom (U_H = 1.2 U_C; U_H = 1.5 U_O). The hydrogen atoms were included in the structure factor calculations but not refined.

Atomic Form Factors:	For neutral atoms and anomalous dispersion [4, 5, 7]
Extinction Correction:	no
Weighting Scheme:	$w^{-1} = \sigma^2(F_o^2)+(a*P)^2+b*P$
	with a: 0.0299; b: 7.5055; P: [Maximum(0 or F_o^2)+2*F_c^2]/3
Shift/Err:	Less than 0.001 in the last cycle of refinement:
Resid. Electron Density:	+1.01 e_0^-/Å^3; -0.85 e_0^-/Å^3

R1: $\Sigma(||F_o|-|F_c||)/\Sigma|F_o|$

[$F_o > 4\sigma(F_o)$; N=4968]:	= 0.0260
[all reflctns; N=5322]:	= 0.0289

wR2: $[\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$

[$F_o > 4\sigma(F_o)$; N=4968]:	= 0.0669
[all reflctns; N=5322]:	= 0.0693
Goodness of fit: $[\Sigma w(F_o^2-F_c^2)^2/(NO-NV)]^{1/2}$	= 1.087
Remarks: Refinement expression $\Sigma w(F_o^2-F_c^2)^2$	

Gd-TEMDO (9)

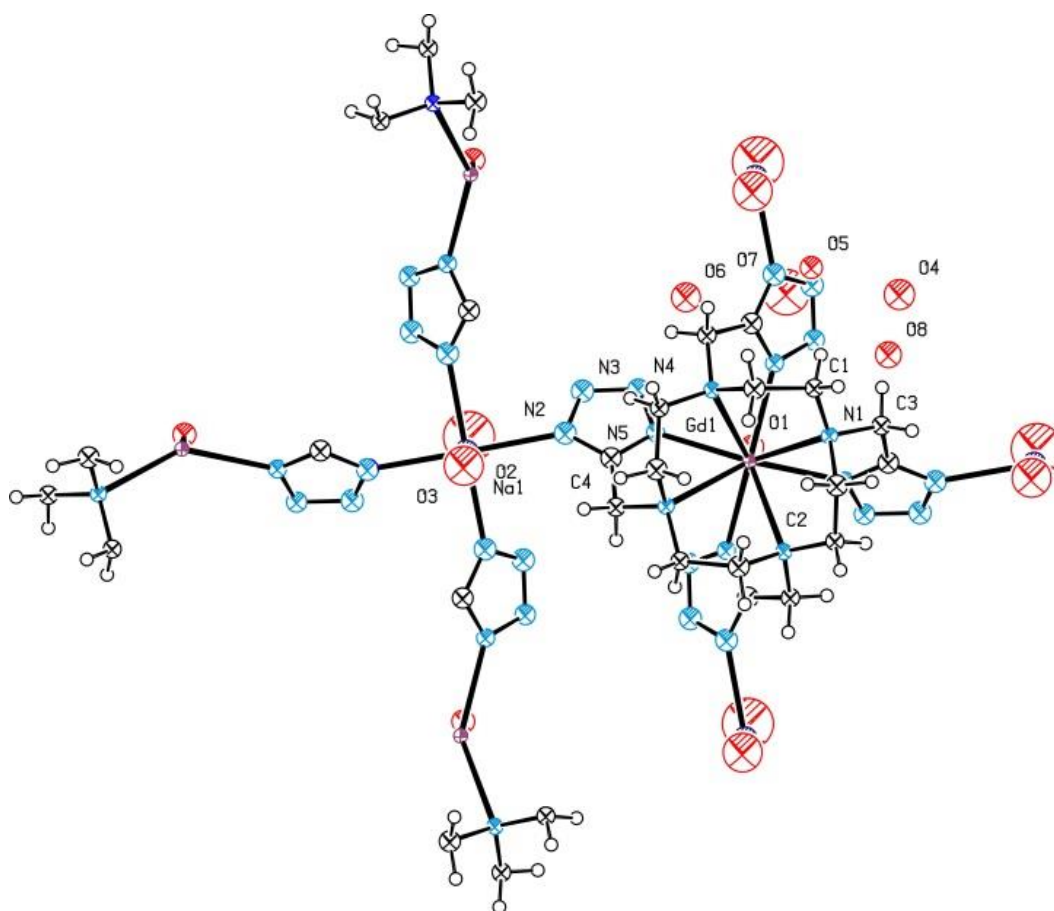**Figure 10.** – Ortep drawing drawing of compound **Gd-TEMDO** with 50% ellipsoids. [6]

Operator:	*** Herdtweck ***
Molecular Formula:	$C_{16} H_{54} Gd N_{20} Na O_{3+n}$
	$(C_{16} H_{30} Gd N_{20} Na O_3), n(H_2 O)$
Crystal Color / Shape	Colorless fragment
Molecular Weight:	946.79 a.m.u.
Systematic Absences:	h00: h≠2n
Space Group:	Tetragonal $P\,42_12$ (I.T.-No.: 90)
Cell Constants:	$a =$ 9.6950(4) Å
	$b =$ 9.6950(4) Å
	$c =$ 20.3549(9) Å
	$V = 1913.22(14)$ Å^3; $Z = 2$; $D_{calc} = 1.59$ g cm^{-3}

Eu-TEMDO
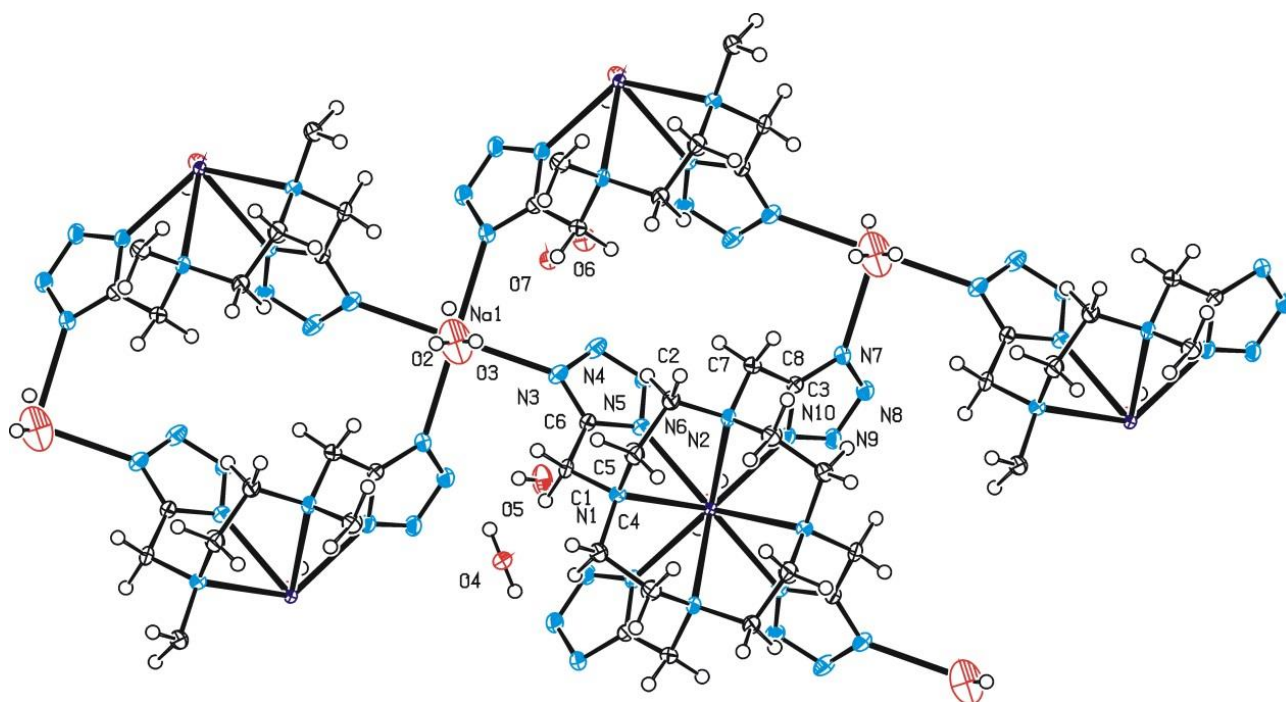
Figure 11. – Ortep drawing drawing of compound **10** with 50% ellipsoids.[6]

Operator:	*** Herdtweck ***
Molecular Formula:	$C_{16} H_{54} Eu N_{20} Na O_{3+n}$
	$(C_{16} H_{30} Eu N_{20} Na O_3)$, $n(H_2 O)$
Systematic Absences:	hkl: h+k≠2n; h0l: h+l≠2n
Space Group:	Monoclinic *C* 2/*c* (I.T.-No.: 15)
Cell Constants:	$a =$ 9.8064(2) Å
	$b =$ 3.71307(9) Å □ = 90.2319(10)°
	$c =$ 9.5809(2) Å
	$V = 3488.55(13)$ Å; $Z = 4$

8. References

[1]   L. Burai, I. Fábián, R. Király, E. Szilágyi, E. Brücher, *J. Chem. Soc., Dalton Trans.* **1998**, 243-248.

[2]   K. Kumar, M. F. Tweedle, *Inorg. Chem.* **1993**, *32*, 4193-4199.

[3]   a) A. E. Merbach, É. Tóth, *The chemistry of contrast agents in medical magnetic resonance imaging*, Wiley Online Library, **2001, p191**; b) Z. Baranyai, E. Brücher, T. Iványi, R. Király, I. Lazar, L. Zékány, *Helv. Chim. Acta* **2005**, *88*, 604-617; c) A. Pasha, G. Tircsó, E. T. Benyó, E. Brücher, A. D. Sherry, *Eur. J. Inorg. Chem.* **2007**, *2007*, 4340-4349.

[4]   X. Wang, T. Jin, V. Comblin, A. Lopez-Mut, E. Merciny, J. F. Desreux, *Inorg. Chem.* **1992**, *31*, 1095-1099.

[5]   Marie Reková, P. Vaňura, V. Jedináková-Křížová, *The Open Inorganic Chemistry Journal* **2009**, *3*, 26-32.

[6]   P. Brauner, L. G. Sillen, R. Whiteker, *Ark. Kemi* **1969**, *31*, 365-376.

[7]   S. Chaves, R. Delgado, J. J. R. F. Da Silva, *Talanta* **1992**, *39*, 249-254.

[8]   L. G. Sillen, B. Warnqvist, *Ark. Kemi* **1969**, *31*, 377-390.

[9]   a) W. P. Cacheris, S. K. Nickle, A. D. Sherry, *Inorg. Chem.* **1987**, *26*, 958-960; b) E. T. Clarke, A. E. Martell, *Inorg. Chim. Acta* **1991**, *190*, 37-46.