Electronic Supplementary Information

Paramagnetic lanthanide chelates for multicontrast MRI

Nevenka Cakić,[a] Tanja Savić,[a] Janice Stricker-Shaver,[a] Vincent Truffault,[b] Carlos Platas-Iglesias,[c] Christian Mirkes,[d] Rolf Pohmann,[d] Klaus Scheffler[d,e,*] and Goran Angelovski[a,*]

[a] MR Neuroimaging Agents, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
[b] Max Planck Institute for Developmental Biology, Tübingen, Germany.
[c] Departamento de Química Fundamental, Universidade da Coruña, A Coruña, Spain.
[d] High-Field Magnetic Resonance, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
[e] Department for Biomedical Magnetic Resonance, University of Tübingen, Tübingen, Germany

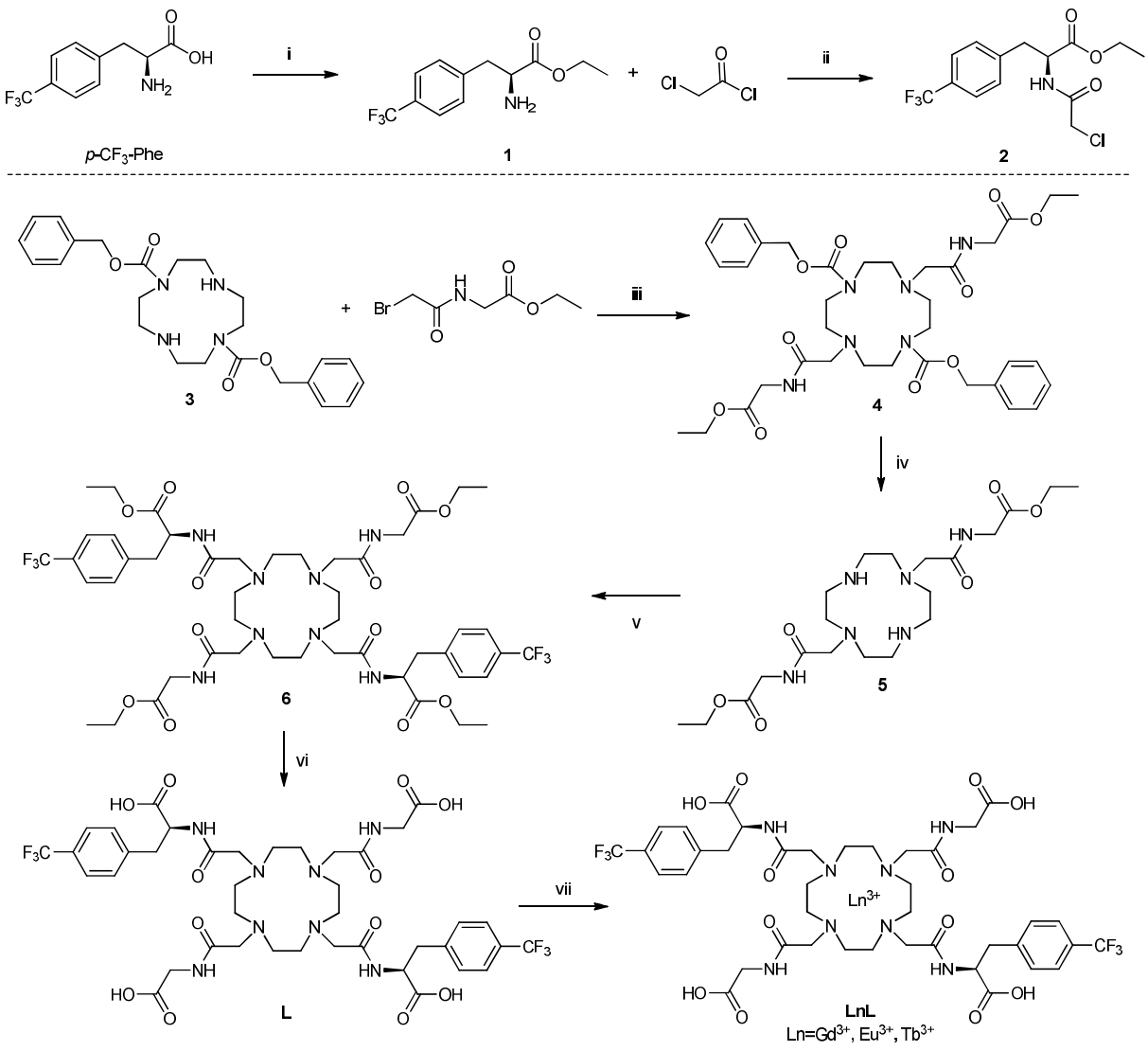
* Corresponding authors: klaus.scheffler@tuebingen.mpg.de, goran.angelovski@tuebingen.mpg.de

Contents

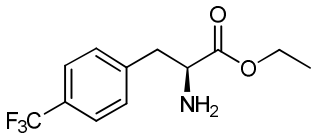
1. Synthetic procedures

General remarks. Commercially available reagents and solvents were used without further purification. 4-(trifluoromethyl)-L-phenylalanine (*p*-CF_3-Phe) was purchased from PepTech Corporation (Bedford, USA, Cat. No. AL224), while 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (**3**) was prepared according to previously reported procedure.[1] Purification of the synthesized compounds was performed using silica gel 60 (0.03–0.2 mm) from Carl Roth (Germany). All NMR spectra were acquired on a Bruker Avance III 300 MHz, processed using TopSpin 2.1 (Bruker GmbH), and analyzed with TopSpin 2.1 or ACD/SpecManager 9.0 (Advanced Chemistry Development, Inc.). The concentration of the complexes was determined using the bulk magnetic susceptibility shift (BMS).[2] Elemental analyses were performed on EuroEA 3000 Elemental Analyser (EuroVector SpA, Italy).

Optical rotations were performed on a Jasco P-1020 polarimeter. ESI-HRMS were performed on a Bruker BioApex II ESI-FT-ICR, equipped with an Agilent ESI-Source, measured via flow injection analysis. ESI-LRMS were performed on an ion trap SL 1100 system (Agilent, Germany). The synthetic procedure to obtain **L** is shown in the Scheme S1 with chemical structures of only the predominant macrocyclic S,S-stereoisomer.

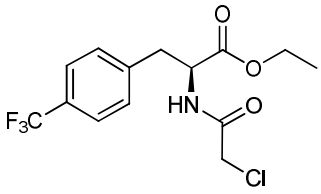


Scheme S1. Synthesis of the ligand **L** and complexes **LnL**. Reagents and conditions: i) $SOCl_2$, EtOH, 5-70 °C, 89 %. ii) Na_2CO_3, DMF, RT, 91 %. iii) K_2CO_3, CH_3CN, 70 °C, 67 %. iv) H_2, Pd/C, EtOH, RT, 91%. v) **2**, K_2CO_3, KI, 70 °C, 59 %. vi) NaOH, THF, RT, 94 %. vii) $LnCl_3$, NaOH, RT, 48 h.



(S)-2-Amino-3-(4-trifluoromethyl-phenyl)-propionic acid ethyl ester (1). The (S)-2-amino-3-(4-(trifluoromethyl)phenyl)propanoic acid (1.70 g, 7.29 mmol) was suspended in ethanol (10 ml), and cooled to 5 °C. Thionyl chloride (3 ml) was added slowly to the suspension over a period of 20 min, and the reaction mixture was then heated on the oil bath at 70 ^{o}C for 5 hours. After cooling of the reaction mixture, the excess of thionyl chloride and ethanol were removed by rotary evaporation. The product was recrystallized from the ethanol-ether mixture to provide the hydrochloride salt of **1** (1.94 g, 89 %) as a colorless solid.

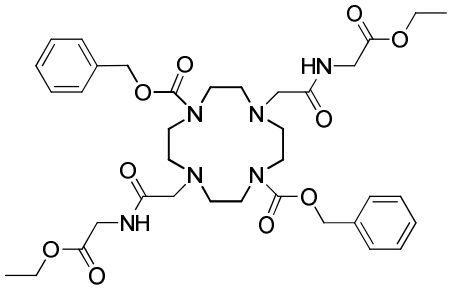
^{1}H NMR (300 MHz, D_2O): δ (ppm) 7.66 (d, *J*=7.7 Hz, 2 H), 7.40 (d, *J*=7.9 Hz, 2 H), 4.39 (t, *J*=6.8 Hz, 1 H), 4.20 (q, *J*=7.2 Hz, 2 H), 3.30 (d, *J*=7.0 Hz, 2 H), 1.15 (t, *J*=7.2 Hz, 3 H). **^{13}C{^{1}H} NMR (75 MHz, D_2O)**: δ (ppm) 169.3, 138.2, 130.0 129.4 (q, *J*=32.5 Hz), 125.9 (q, *J*=3.5 Hz), 124.2 (q, *J*=271.6 Hz), 63.6, 53.8, 39.5, 13.1. **^{19}F{^{1}H} NMR (282 MHz, D_2O)**: δ (ppm) -62.3. **EA**: for $C_{12}H_{14}F_3NO_2$·HCl (297.7 g/mol), calcd: C: 48.4%, H: 5.1%, N: 4.7%, found: C: 48.4%, H: 5.0%, N: 4.6%. $[\alpha]_D^{25}$ +4.102 (c 1.00, H_2O). **MP**: 209-211 ^{o}C. **ESI-LRMS**: for $C_{12}H_{15}F_3NO_2^+$ $[M+H]^+$: *m/z* calcd. 262.1, found 262.1.



(S)-2-(2-Chloro-acetylamino)-3-(4-trifluoromethyl-phenyl)-propionic acid ethyl ester (2). Sodium carbonate (380 mg, 3.59 mmol) was added to a suspension of (S)-ethyl 2-amino-3-(4-(trifluoromethyl)phenyl)propanoate hydrochloride (534 mg, 1.79 mmol) in dry DMF (2 mL). The mixture was stirred for 30 min at room temperature followed by dropwise addition (over a 1 min period) of chloroacetyl chloride (0157 ml, 223 mg, 1.97 mmol). The mixture was then further stirred for 1 h at room temperature after which time it was diluted with brine (50 mL) and extracted with EtOAc (2· 30 mL). The combined organic extracts were washed with brine (2×50 mL), dried, and concentrated. The crude product was purified by column chromatography (silicagel, EtOAc/hexane from 1:9 to 3:7) to give ethyl 2-(2-

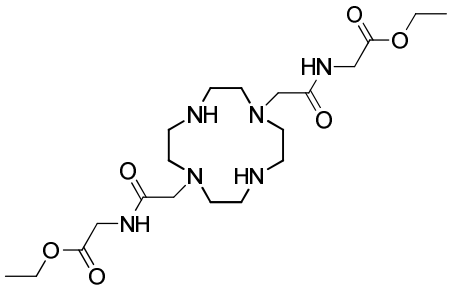
chloroacetamido)-3-(4-(trifluoromethyl)phenyl)propanoate (550 mg, 91 %) as a colorless solid. The analytical sample was prepared after recrystallization from CH_2Cl_2: *n*-hexane.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.56 (d, *J*=8.1 Hz, 2 H), 7.27 (d, *J*=7.9 Hz, 2 H), 7.07 (d, 1 H), 4.88 (dt, *J*=7.7, 6.0 Hz, 1 H), 4.19 (q, *J*=7.2 Hz, 2 H), 4.03 (s, 2 H), 3.25 (dt, *J*=7.7, 6.0 Hz, 1 H), 3.18 (dt, *J*=7.7, 6.0 Hz, 1 H), 1.24 (t, *J*=7.2 Hz, 3 H). **^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$)**: δ (ppm) 170.5, 165.7, 139.8, 129.7, 129.5 (q, *J*=32.5 Hz), 125.5 (q, *J*=3.5 Hz), 124.1 (q, *J*=271.8 Hz), 62.0, 53.3, 42.3, 37.7, 14.0. **^{19}F{^{1}H} NMR (282 MHz, $CDCl_3$)**: δ (ppm) -62.5. **EA**: for $C_{14}H_{15}ClF_3NO_3$ (337.72 g/mol), calcd: C: 49.8%, H: 4.5%, N: 4.1%, found: C: 49.5%, H: 4.5%, N: 4.0%. $[\alpha]_D^{25}$ +37.151 (c=1.00, CH_2Cl_2). **MP**: 74-76 ^oC. **ESI-LRMS**: for $C_{14}H_{16}ClF_3NO_3^+$ $[M+H]^+$: *m/z* calcd. 338.1, found 338.1.



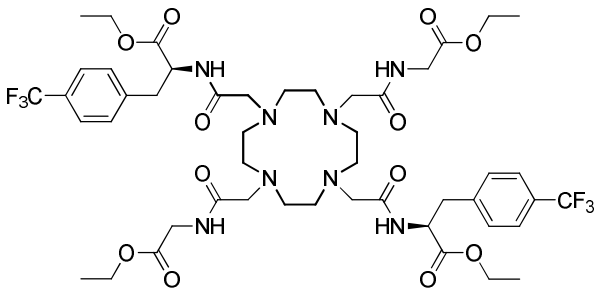
4,10-Bis-[(ethoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10tetraaza-cyclododecane-1,7-dicarboxylic acid dibenzyl ester (4). Macrocycle **3** (2.52 g, 5.72 mmol) was dissolved in acetonotrile (40 ml) and K_2CO_3 (3.79 g, 27.5 mmol) was added. The suspension was stirred for 15 min and then ethyl 2-(2-bromoacetamido)acetate (3.08 g, 13.73 mmol) in acetonotrile (10 ml) was added dropwise. The reaction mixture was heated at 70 ^oC for 18 hours. After cooling, the mixture was filtered, the solvent removed by rotary evaporation and the crude product was purified by column chromatography (silicagel, 4% MeOH in CH_2Cl_2) to give the desired product (2.785 g, 67%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.25 (s, 10 H), 5.02 (s, 4 H), 4.07 (q, *J*=7.1 Hz, 4 H), 3.84 (br. s., 4 H), 3.51-3.28 (br., 8 H), 3.14 (br. s., 4 H), 2.88-2.64 (br., 8 H), 1.17 (t, *J*=7.2 Hz, 6 H). **^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$)**: δ (ppm) 171.3, 169.7, 156.9, 136.5, 128.6, 128.3, 128.2, 67.4, 61.2, 58.3, 55.2, 55.0, 48.5, 48.0, 40.9, 14.2. **ESI-HRMS**: for $C_{36}H_{51}N_6O_{10}^+$ $[M+H]^+$: *m/z* calcd. 727.3661, found 727.3661.



(2-{7-[(Ethoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10tetraaza-cyclododec-1-yl}-acetylamino)-acetic acid ethyl ester (5). The macrocycle GA401 (2.414 g, 3.32 mmol) was dissolved in EtOH (50 ml) and 10% $Pd(OH)_2$ on carbon (500 mg) was added. The mixture was shaken for 18 hours under a hydrogen atmosphere (2.5 bar) in a Parr hydrogenator apparatus. The catalyst was removed by filtration through the cake of celite and the filtrate was concentrated to obtain the amine product (1.39 g, 91%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.06 (br. s., 2 H), 4.93 (br. s., 2 H), 4.17 (q, *J*=7.2 Hz, 4 H), 4.02 (br. s., 4 H), 3.29 (s, 4 H), 2.93-2.69 (m, 16 H), 1.27 (t, *J*=7.2 Hz, 6 H). **^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$)**: δ (ppm) 171.8, 170.5, 61.3, 60.3, 52.8, 46.5, 40.9, 14.1. **ESI-HRMS**: for $C_{20}H_{39}N_6O_6^+$ $[M+H]^+$: *m/z* calcd. 459.2926, found 459.2929.

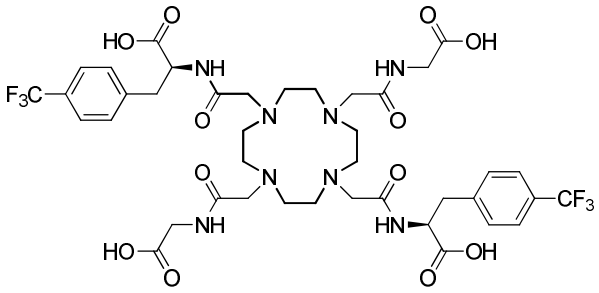


(S,S)-2-[2-(4,10-Bis-[(ethoxycarbonylmethyl-carbamoyl)-methyl]-7-{[1-ethoxycarbonyl-2-(4-trifluoromethyl-phenyl)-ethylcarbamoyl]-methyl}-1,4,7,10tetraaza-cyclododec-1-yl)-acetylamino]-3-(4-trifluoromethyl-phenyl)-propionic acid ethyl ester (6).

The macrocyclic secondary amine (409 mg, 0.892 mmol) was dissolved in acetonitrile (30 ml). K_2CO_3 (542 mg, 3.92 mmol) and KI (99 mg, 0.596 mmol) were added and the reaction mixture was stirred for 30 min. The solution of (S)-ethyl 2-(2-chloroacetamido)-3-(4-(trifluoromethyl)phenyl)propanoate in acetonitrile (10 ml) was added dropwise and the mixture was heated at 70 °C for 18 hours under a nitrogen atmosphere. Upon reaction completion, the solids were removed by filtration, the solvent was evaporated by rotary

evaporation and the residue was purified by column chromatography (silicagel, 3-5% gradient of MeOH in CH_2Cl_2) to give the product (555 mg, 59%) as an off-white amorphous solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.56 (d, *J*=8.1 Hz, 4 H), 7.42 (d, *J*=7.9 Hz, 4 H), 7.33 (d, *J*=8.3 Hz, 2 H), 7.29 (t, *J*=4.8 Hz, 2 H), 4.82 (dt, *J*=7.2, 7.1 Hz, 2 H), 4.11 (q, *J*=7.1 Hz, 8 H), 3.95 (d, *J*=3.6 Hz, 4 H), 3.19 (br. s., 8 H), 3.13-2.87 (broad, 4 H), 2.11 - 2.70 (broad, 16 H), 1.22 (t, *J*=7.2 Hz, 6 H), 1.20 (t, *J*=7.2 Hz, 6 H). **^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$)**: δ (ppm) 172.0, 171.4, 171.1, 170.0, 141.3, 130.1, 129.0 (q, *J*=32.2 Hz), 125.3 (q, *J*=3.5 Hz), 124.2 (q, *J*=271.8 Hz), 61.6, 61.5, 57.6, 56.5, 53.4, 50.7 (br), 41.0, 37.4, 14.1. **^{19}F{^{1}H} NMR (282 MHz, $CDCl_3$)**: δ (ppm) -62.1. $[\alpha]_D^{25}$ +8.366 (c=1.00, CH_2Cl_2). **ESI-HRMS**: for $C_{48}H_{66}F_6N_8NaO_{12}^+$ $[M+Na]^+$: *m/z* calcd. 1083.4597, found 1083.4606.



(S,S)-2-[2-(4,10-Bis-[(carboxymethyl-carbamoyl)-methyl]-7-{[1-carboxy-2-(4-trifluoromethyl-phenyl)-ethylcarbamoyl]-methyl}-1,4,7,10tetraaza-cyclododec-1-yl)-acetylamino]-3-(4-trifluoromethyl-phenyl)-propionic acid (L).

A solution of NaOH (2.0 M, ~ 1 ml) was added to a solution of tetraehtyl ester (450 mg, 0.424 mmol) in THF (1 ml) until pH 12 was reached. The mixture was vigorously stirred for 4 h at room temperature while adding NaOH to maintain pH. After reaction completion, THF was removed by rotary evaporation and the pH of the remaining aqueous solution was adjusted to 2 with HCl (1 M). The water was removed by rotary evaporation and the solid residue was triturated with EtOH to dissolve the product. The remaining solid (NaCl) was removed by filtration and the filtrate was evaporated to give the pure product (380 mg, 94%) as yellow amorphous solid.

^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 7.65 (d, *J*=7.9 Hz, 4 H), 7.51 (d, *J*=7.7 Hz, 4 H), 4.84 (br. s., 2 H), 4.07-3.83 (broad, 8 H), 3.73-2.98 (broad, 24 H). **^{13}C{^{1}H} NMR (75 MHz, CD_3OD)**: δ (ppm) 173.7, 172.9, 143.1, 131.3, 130.1 (q, *J*=32.2 Hz), 126.4 (q, *J*=3.5 Hz),

125.8 (q, *J*=271.8 Hz), 56.8, 56.0, 55.0, 42.1, 38.2. **$^{19}F\{^{1}H\}$ NMR (282 MHz, $CDCl_3$)**: δ (ppm) -63.6. $[\alpha]_D^{25}$ +10.547 (c 1.00, CH_3OH). **ESI-HRMS**: for $C_{40}H_{49}F_6N_8O_{12}^-$ $[M-H]^-$: *m/z* calcd. 947.3380, found 947.3396.

General procedure for the preparation of Ln^{3+} complexes: The Ln^{3+} complexes of **L** were prepared by mixing the ligand with the respective $LnCl_3$ salt in slight excess. The solution was stirred at RT for 48 h. The pH value was adjusted to 7.0–7.5 using NaOH (1 M). After 48 h, the mixture was stirred for 24 h at RT in presence of Chelex 100, maintaining the pH at 7.0-7.5, using HCl (1 M). The absence of free Ln^{3+} was verified by colorimetric assay using xylenol orange.[3]

Gadolinium complex, **GdL**: Obtained from **L** (30 mg, 0.032 mmol) and $GdCl_3 \cdot 6H_2O$ (1.2 equiv.) in quantitative yield. ESI-LRMS: for $C_{40}H_{46}F_6N_8O_{12}Gd^-$ $[M-4H]^-$: calc. 1102.2, found 1102.3.

Europium complex, **EuL**: Obtained from **L** (30 mg, 0.032 mmol) and $EuCl_3 \cdot 6H_2O$ (1.2 equiv.) in quantitative yield. ESI-LRMS: for $C_{40}H_{46}F_6N_8O_{12}Eu^-$ $[M-4H]^-$: calc. 1097.2, found 1097.2.

Terbium complex, **TbL**: Obtained from **L** (30 mg, 0.032 mmol) and $TbCl_3 \cdot 6H_2O$ (1.2 equiv.) in quantitative yield. ESI-LRMS: for $C_{40}H_{46}F_6N_8O_{12}Tb^-$ $[M-4H]^-$: calc. 1103.2, found 1103.3.

2. CEST NMR experiments

All CEST NMR experiments were recorded on a Bruker Avance III 300 MHz NMR spectrometer. The saturation transfer experiments were carried out at 25 ^oC or 37 ^oC by irradiating the sample at increments of 1 ppm, with 10% D_2O in H_2O to lock the deuterium frequency. Spectra were measured by recording the bulk water signal intensity as a function of the presaturation frequency.

For the concentration-independent (‘omega plots') and QUESP methods, data was collected by varying the saturation power whilst the saturation time remained constant (10 s and 3 s for

the omega plots and QUESP, respectively). The saturation field strengths varied between 425 and 1490 Hz, for the omega plots: 10, 12.5, 15, 17.5, 20, 22.5, 25, 30 and 35 μT; for QUESP: 10, 15, 17.5, 20, 22.5, 25, 27.5, 30 and 35 μT. For the QUEST experiments, data was collected by varying the saturation time and keeping the power constant (25 μT). The saturation times were 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 7.5 and 10 s. The QUEST and QUESP data were both fitted analytically with Scientist® 3.0 (Micromath, USA) using the previously published method (Eqs. 1 - 4), where χ_{CA} – the fractional concentration of the exchangeable protons of the contrast agent, t_{sat} the saturation time, α – the saturation efficiency, k_{ex} – the rate of proton exchange, $R_{1,2s}$ – the longitudinal and transverse relaxation rates during saturation of the solute, $R_{1,2w}$ – the longitudinal and transverse relaxation rates during saturation of the bulk water, ω_1 – the saturation power, M_s – the MR signal of bulk water after applying RF saturation pulse at the resonance frequency of exchangeable protons of contrast agent (ν_{CEST}) and M_o – the reference MR signal ($-\nu_{CEST}$).[4] The plots in the Figure S1 show the experimental points from QUEST and QUESP experiments and fitted curves with the following fixed parameters at 25/37 °C: R_{1w}=0.299/0.228 s^{-1}, R_{2w}=0.495/0.354 s^{-1}, obtained in independent experiments (inversion recovery and Car-Purcell-Meiboom-Gill experiment for R_{1w} and R_{2w}, respectively).

$$1-\frac{M_s}{M_o}=\frac{k_{ex}\times\alpha\times\chi_{CA}}{R_{1w}+k_{ex}\times\chi_{CA}}\times\left[1-e^{-(R_{1w}+k_{ex}\times\chi_{CA})\times t_{sat}}\right] \qquad (1)$$

$$\alpha=\frac{\omega_1^2}{\omega_1^2+p\,q} \qquad (2)$$

$$p=R_{2s}+k_{ex}-\frac{k_{ex}^2\times\chi_{CA}}{R_{2w}+k_{ex}\times\chi_{CA}} \qquad (3)$$

$$q=R_{1s}+k_{ex}-\frac{k_{ex}^2\times\chi_{CA}}{R_{1w}+k_{ex}\times\chi_{CA}} \qquad (4)$$

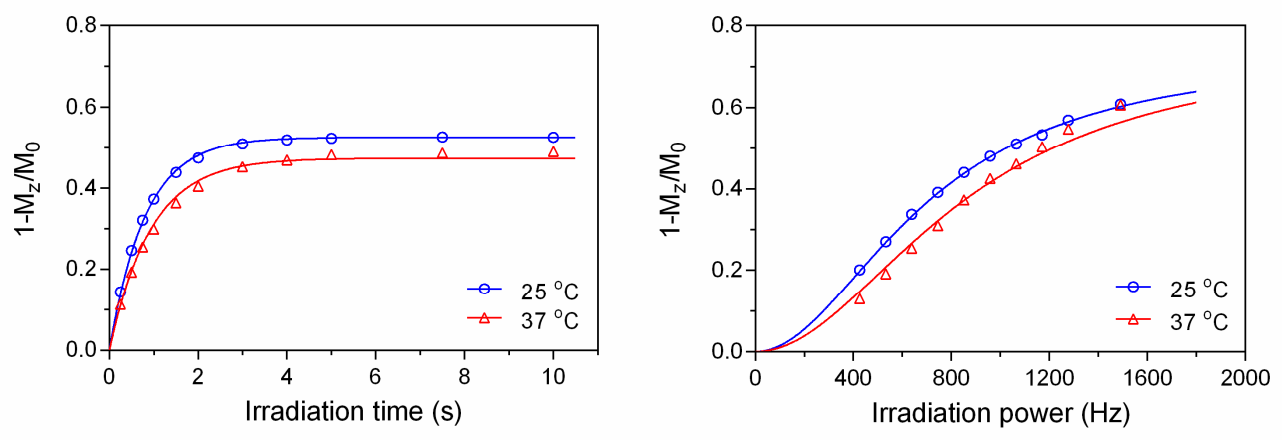


Figure S1. QUEST (left) and QUESP (right) results with **EuL** (15 mM) at 7 T and 25 or 37 °C. Values for the used saturation times, saturation power and performed fits are provided in the description above.

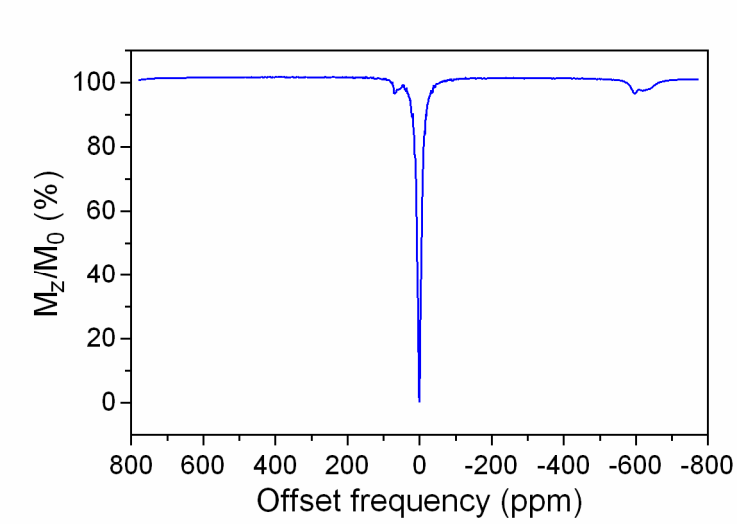


Figure S2. Z-spectrum of **TbL** (15 mM) obtained with following parameters B_1=25.0 µT, irradiation time = 3 s, 1 ppm resolution, 7 T. A weak CEST effect can be observed for the inner-sphere water molecule (-598 ppm) and amide protons (69 ppm).

3. ^{19}F NMR spectra

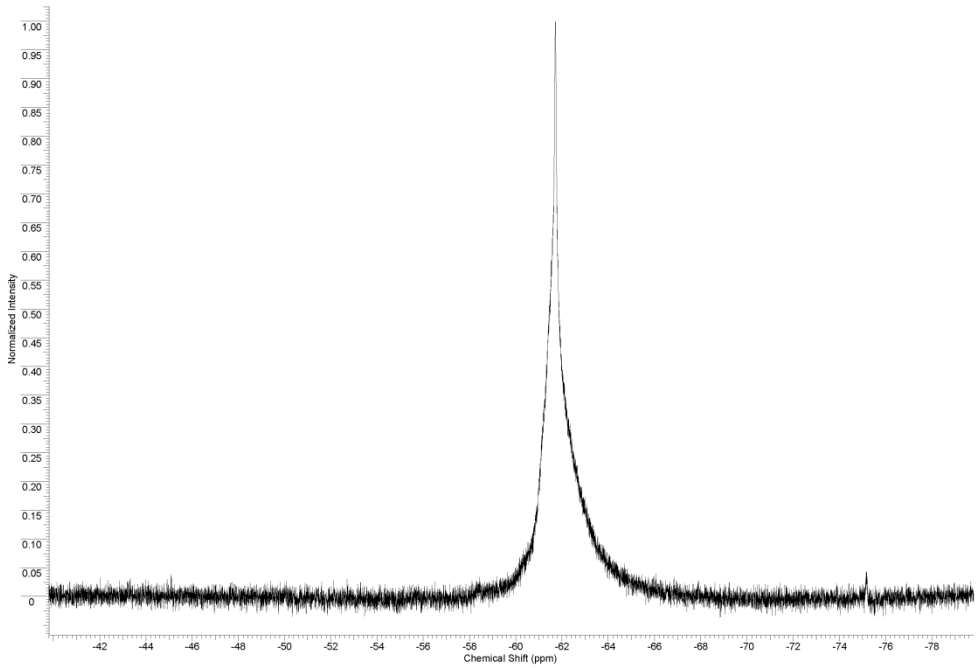


Figure S3. ^{19}F NMR spectrum of **GdL** in D_2O (282 MHz, 298 K).

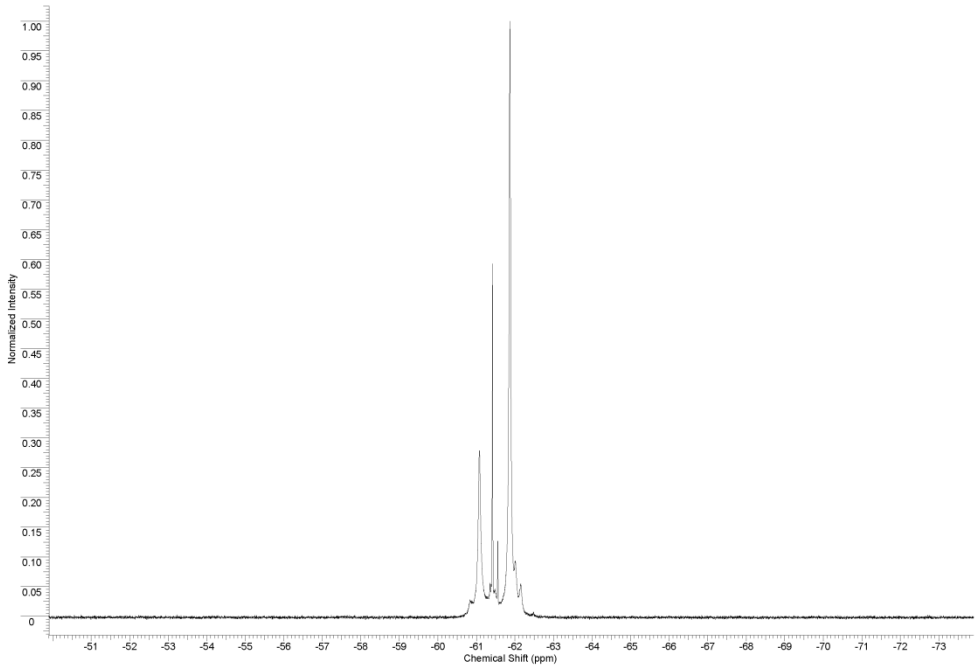


Figure S4. ^{19}F NMR spectrum of **EuL** in D_2O (282 MHz, 298 K).

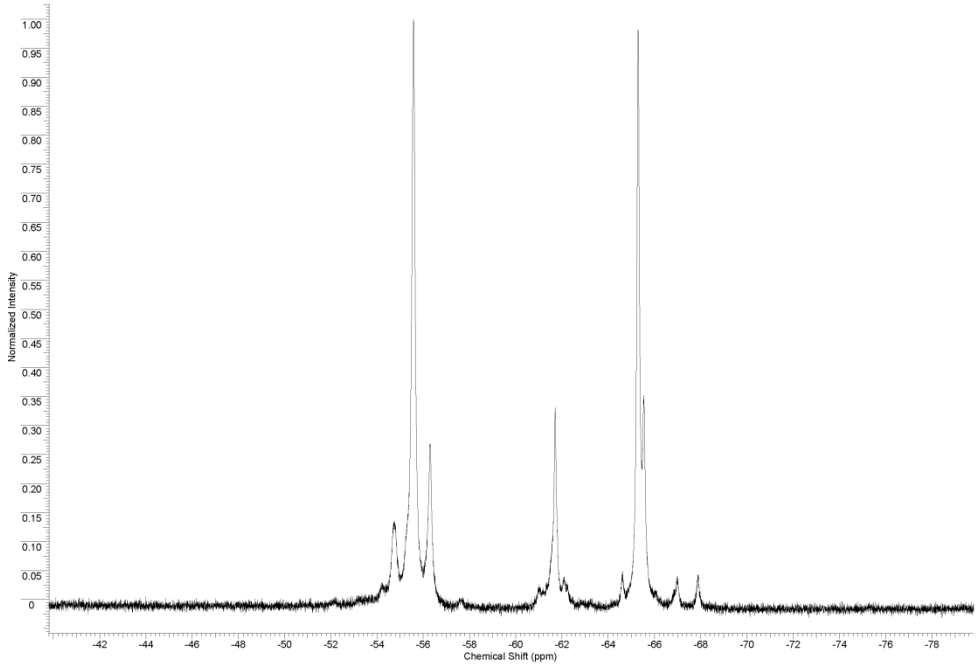


Figure S5. ^{19}F NMR spectrum of **TbL** in D_2O (282 MHz, 298 K).

4. NMR diffusion experiments

The determination of the diffusion coefficient *D* was performed using 2D – Diffusion Ordered NMR Spectroscopy (DOSY).[5] Experiments included 3 repetitions on **EuL** (15 mM, pH 7.3, 25 ^{o}C, δt= 2 ms, ΔT= 330 ms). Data analysis was done with TopSpin 2.1 using 16 linear points between 5–95 % gradient strength. The obtained value D=4.5±0.4 × 10^{-10} m^2s^{-1} is within the range of values previously reported by us on different monomacrocyclic systems.[6, 7]

5. MRI experiments

MRI experiments were performed on a Bruker 7T BioSpec 70/30 USR. A Bruker $^{1}H/^{19}F$ dual frequency volume coil was used for all (^{1}H and ^{19}F) *in vitro* experiments as well as for the ^{1}H *ex vivo*/*in vivo* measurements. The ^{19}F *ex vivo/in vivo* experiments were performed also using a Bruker surface coil and a custom-made micro coil.

MRI *in vitro*. Four vials were filled with: 1) **GdL**, 2) **EuL**, 3) **TbL** and 4) H_2O (all complexes 15 mM per Ln^{3+}), respectively and placed in a syringe containing saline solution. The ^1H scan protocol comprised a fast low angle shot (FLASH) imaging and a rapid acquisition with relaxation enhancement (RARE). The imaging parameters for the FLASH images were: echo time (TE) = 6.3 ms, repetition time (TR) = 100 ms, flip angle (FA) = 30°, resolution (RES) = 100x100 μm^2, slice thickness = 1.5 mm, total acquisition time (TA) = 5 min. The RARE images were acquired with a RARE factor = 8, TE = 40 ms, TR = 6000 ms, RES = 250×250 μm^2, TA = 1.5 min. The RARE sequence was also used to perform CEST imaging by adding a Gaussian presaturation pulse (B_1 = 18 μT, duration = 3 s) with an off-resonance frequency ranging from -30 kHz to +30 kHz. The pronounced shortening of T_1 and T_2 induced by **GdL** can clearly be seen in the T_1-weighted FLASH (Figure 3a) and T_2-weighted RARE images (Figure 3b). Only **EuL** exhibits a CEST effect, which can be observed by subtracting two RARE images which were presaturated at ±52 ppm with respect to the water resonance frequency.

The ^{19}F images were acquired using balanced steady state free precession (bSSFP) imaging. The scan parameters were: TE = 1.1 ms, TR = 2.2 ms, FA = 40°, number of excitations (NEX) = 15145, RES = 468x468 μm^2, slice thickness = 5 mm, TA=1 h. The three contrast agents are visible in the acquired images and exhibit a signal-to-noise ratio of about 8.

MRI *ex vivo* and *in vivo*. For these experiments, male Sprague-Dawley rats (150– 250 g, Charles River Laboratories) were used. The experiments were approved by the local authorities (Regierungspraesidium), and were in compliance with the guidelines of the European directive (2010/63/EU) for the care and protection of animals used for scientific purposes. Animals were housed individually prior to the experimental procedures with controlled light-dark cycle, temperature and humidity, with food and water provided ad libitum.

In each ^1H MRI experiment, the animal was initially anaesthetized with 5 % isoflurane (Forene, Abbott, Wiesbaden, Germany) and placed in a stereotactic frame (Stoelting Co., IL, US). Anaesthesia was then reduced to 1.5-2.0 % for maintenance. For ^{19}F MRI experiments, a mixture of medetomidine (0.4 mg/kg) and ketamine (60 mg/kg) (1:10) i.p. with robinul (0.05 mg/kg) s.c. as premedication was used. The depth of anesthesia was checked by the lack of withdrawal to a firmly pinched hind toe. The body temperature of the animal was maintained at 37.0 ± 1.0 °C by a rectal probe with a feedback controlled heat pad (50-7221-F,

Harvard Apparatus, MA, US). The eyes were protected with bepanthen eye ointment to keep the cornea from drying. Bregma and lambda were used for alignments and for locating the injection site. To target the somatosensory cortex, a position 3.4 mm right for **GdL** and left for **EuL** of midline, 0.2 mm anterior to bregma, and 1.5 mm below the pial surface was chosen as the site of injection. A burr hole was drilled at the skull directly above the injection site, and the contrast agent was delivered with a Hamilton syringe by a precision pump (70-4507, Harvard Apparatus) over the period of 20 min. The needle was retracted stepwise 10 min post-injection to avoid leakage of the contrast agent. In the case of *in vivo* experiments, the animal was sutured and transferred to the scanner where breathing rate, heart rate and blood oxygen saturation were also monitored during the scan. For *ex vivo* experiments, the animal was euthanized post-injection and transferred to the scanner for image acquisition.

MR images were acquired from 0.5-3.3 hours after intracranial injection of contrast agent using FLASH and RARE sequences with or without inversion recovery. Image analysis was performed in MATLAB (MathWorks, USA).

T_1-weighted ^{1}H and ^{19}F MRI experiments were performed with **GdL** (15 mM). T_1-weighted ^{1}H MRI was performed using the FLASH sequence with the following parameters: field-of-view (FOV) = 37.66×49.12 mm^2, matrix size (MTX) = 96×96, 1 slice, 1.29 mm thickness, TR = 25 ms, TE = 1.5 ms, FA = 20°, NEX = 48, TA = 1 min 55 s. ^{19}F MRI was performed using non-fluorine containing anesthesia (see above). In all experiments a reference tube containing a solution of NaF (192 mM) and Dotarem® (20 mM) was placed between the animal's head and the RF coil in order to have comparable T_1 and T_2 relaxation times as the contrast agent. Imaging was performed using the following sequences: FLASH, balanced steady state free precession (bSSFP), 2D, 3D ultrashort TE (UTE), zero TE (ZTE) and chemical shift imaging (CSI) with the scans lasting up to 3.3 hrs.

T_1-weighted and CEST MRI experiments were performed with **EuL** (15 mM). Following intracranial injection under constant anesthesia, the animal was euthanized and positioned in the scanner. Firstly, the anatomical scan was performed (IR-RARE): FOV = 60×60 mm^2, MTX=256×192, 5 slices, 0.5 mm thickness. TR/TE = 10000/9.7 ms, Rare factor = 8, inversion time = 8000 ms, NEX = 1, TA = 4 min. Thereafter, pairs of CEST images at frequencies with and without water saturation were acquired (RARE) with the following parameters: FOV = 60×60 mm^2, MTX = 120×120, 1 slice, 0.5 mm thickness, TR/TE = 5095.3/10 ms, Rare factor = 8, saturation pulse t_{sat} = 5 s, B_1=11.9 μT. The saturation offsets

were initially screened with 5 ppm, thereafter with 1 and 0.5 ppm resolution, respectively. The experiments at optimized saturation offset were performed with NEX = 12, TA = 15 min 17 s. CEST image analysis comprised firstly defining a region of interest (ROI), centered at the injection point with addition of surrounding pixels using the T_1-weighted image. Thereafter, the CEST contrast image was quantified using the asymmetric magnetization transfer ratio, $MTR_{asym} = (S_b\text{-}S_{CEST})/S_b \times 100$, where S represents the signal intensity of a given ROI at resonance of CEST (S_{CEST}) or background (S_b) signals, respectively.

5. References

1. Z. Kovacs and A. D. Sherry, *Synthesis*, 1997, 759-763.
2. D. M. Corsi, C. Platas-Iglesias, H. van Bekkum and J. A. Peters, *Magn. Reson. Chem.*, 2001, **39**, 723-726.
3. A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol. Imaging*, 2006, **1**, 184-188.
4. M. T. McMahon, A. A. Gilad, J. Y. Zhou, P. Z. Sun, J. W. M. Bulte and P. C. M. van Zijl, *Magn. Reson. Med.*, 2006, **55**, 836-847.
5. A. Macchioni, G. Ciancaleoni, C. Zuccaccia and D. Zuccaccia, *Chem. Soc. Rev.*, 2008, **37**, 479-489.
6. J. Henig, I. Mamedov, P. Fouskova, E. Tóth, N. K. Logothetis, G. Angelovski and H. A. Mayer, *Inorg. Chem.*, 2011, **50**, 6472-6481.
7. S. Gündüz, T. Savić, R. Pohmann, N. K. Logothetis, K. Scheffler and G. Angelovski, *ACS Sensors*, 2016, **1**, 483-487.