Gadolinium DO3A derivatives mimicking phospholipids; preparation and *in vitro* evaluation as pH responsive MRI contrast agents

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A series of Gd-DO3A derivatives (**8a**–**d**) mimicking phospholipids have been prepared. Two of the complexes, Gd-HADB-DO3A (**8a**) and Gd-HADO-DO3A (**8b**), have been evaluated as pH responsive MRI contrast agents *in vitro*. The T_1-relaxivity (r_1) of Gd-HADO-DO3A (**8b**) increased 142% on changing the pH from 6 to 8. The pH dependence is thought to arise from the formation of supramolecular structures caused by deprotonation of the amphiphilic complex at alkaline pH.

Introduction

Paramagnetic materials have been investigated as MRI contrast agents (CAs) for more than two decades.[1] These materials enhance the contrast of the image indirectly by lowering the magnetic relaxation time of the water protons in the surrounding tissues.[2,3] Gd(III) is particularly suited for this purpose because of its favourable magnetic properties (seven unpaired electrons). The aqua ion itself is too toxic for human use. This can be circumvented by chelation by a polydentate ligand. The most frequently used Gd(III) based CAs are stable, hydrophilic poly(aminocarboxylate) derived complexes with rapid extracellular distribution and renal elimination. Depending on the denticity one or more water molecules might be directly coordinated to the paramagnetic centre.

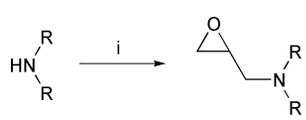
Gd complexes with amphiphilic properties have previously been synthesised and evaluated as blood-pool and liver imaging agents. Long chain amides and esters of DTPA (diethylenetriaminepentaacetic acid) are the most common.[4] More recently amphiphilic complexes based on the DO3A (1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane) structure have been reported.[5,6] These complexes are able to form supramolecular systems such as micelles, mixed micelles and liposomes in aqueous solutions, in the presence or absence of surfactants and phospholipids. The formation of these systems increases the efficacy (T_1-relaxivity) of the contrast agent due to an increase in the rotational correlation time (τ_R) of the Gd complex.

In the present work Gd–DO3A complexes mimicking the phospholipid structure have been prepared in order to achieve CAs able to form stable and rigid supramolecular systems. Macrocyclic chelates were chosen for the studies because of their favourable stability and hence reduced probability for intracellular dechelation.[7]

Results and discussion

Synthesis

The oxiranes **2b**–**d** have previously been reported in the literature.† However, especially for the oxiranes **2a**–**d** the experimental data are inadequate. The procedure of Gever[8] was, with minor changes, used for the synthesis of these compounds (Scheme 1). Reaction between the dialkylamines **1a**–**d** and



1a-d **2a-d**

a R = C_4H_9, **b** R = C_8H_{17}, **c** R = $C_{12}H_{25}$, **d** R = $C_{16}H_{33}$

Scheme 1 *Reagents and conditions*: i, 1) epichlorohydrin, H_2O, rt or Δ, 2) NaOH, H_2O, rt.

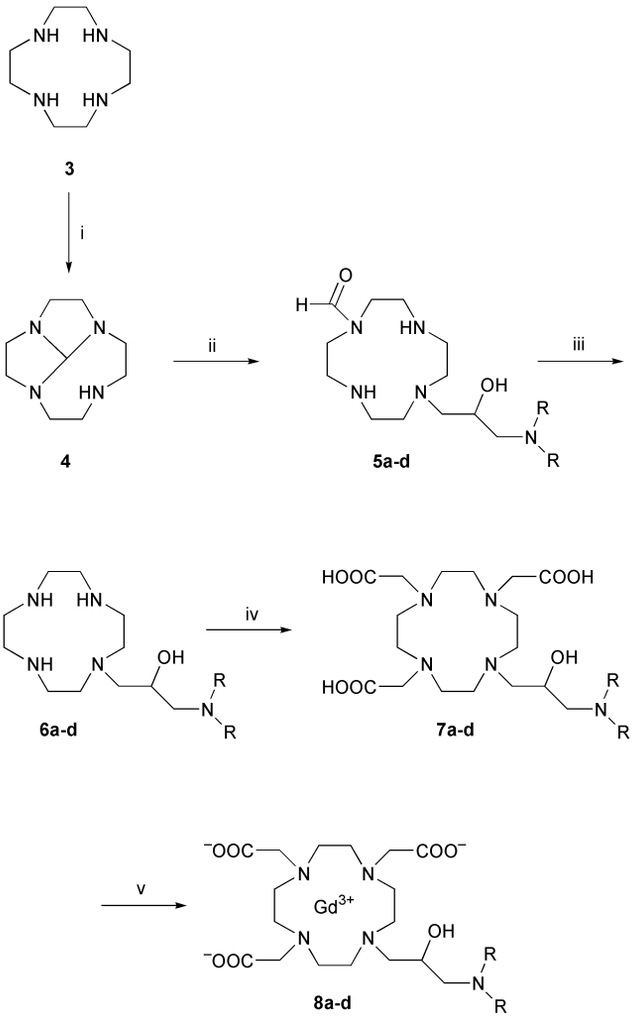
epichlorohydrin, in the presence of small amounts of water, followed by treatment with aqueous sodium hydroxide furnished the oxiranes **2a**–**d** in good yields. To obtain homogeneous reaction mixtures the temperature had to be raised in correspondence with the melting points of the most lipophilic dialkylamines.

The synthetic pathway to N-substituted DO3A derivatives followed a method developed by Platzek *et al.*[9] (Scheme 2). The protected derivative 1,4,7,10-tetraazatricyclo[5.5.1.0]tridecane (**4**) was obtained by reaction of 1,4,7,10-tetraazacyclododecane (cyclen) (**3**) with dimethylformamide dimethyl acetal.[10] The protected cyclen **4** was reacted with the oxiranes **2a**–**d** at 120 °C in the absence of solvent. The formyl derivatives **5a**–**d** were isolated upon hydrolysis with aqueous methanol in 40–65% yields after chromatography. The ^{13}C NMR spectra showed a higher number of signals than expected because of restricted rotation around the amide bond.

Hydrolyses of the amides **5a**–**d** were, except for the most lipophilic compound **5d**, performed with sodium hydroxide in methanol–water. Poor solubility of the amide **5d** resulted in partial hydrolysis only. However, hydrolysis with hydrochloric acid in methanol–water furnished the monosubstituted cyclen **6d** in 71% yield.

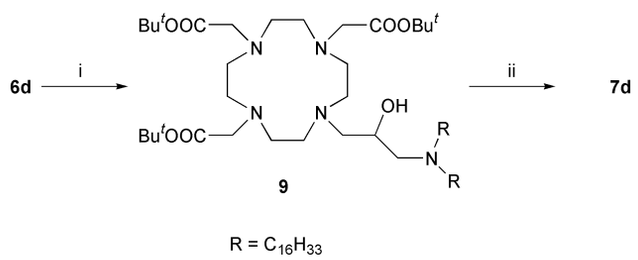
Carboxymethylation was achieved by reacting the monosubstituted cyclen derivatives **6a**–**d** with sodium chloroacetate in water–ethanol at pH 10. The high lipophilicity of the DO3A derivatives **7a**–**d** made ion exchange purification difficult. However, pure materials were obtained by flash chromatography on

† A search for oxiranes **2a**–**d** in *Chemical Abstracts* resulted in 21 references distributed as follows: **2a**: 17 references, **2b**: 1 reference, **2c**: 2 references and **2d**: 1 reference. Of these 12 were patents with inadequate experimental data, and the remaining 9 were papers in other languages than English.



Scheme 2 *Reagents and conditions*: i, dimethylformamide dimethyl acetal, toluene, 120 °C; ii, 1) **2a**–**d**, 120 °C, 2) MeOH, H_2O, rt; iii, NaOH or HCl, MeOH, H_2O, Δ; iv, $ClCH_2COONa$, EtOH, H_2O, pH 10, 70 °C; v, Gd_2O_3, H_2O, 90 °C.

silica gel. The yields were between 40 and 45% for the derivatives **7a**–**c**. Due to the low yield of the derivative **7d** (16%), the carboxymethylation was performed with *tert*-butyl bromoacetate followed by deprotection in neat TFA (Scheme 3).



Scheme 3 *Reagents and conditions*: i, $BrCH_2COOC(CH_3)_3$, Na_2CO_3, THF, H_2O, rt; ii, TFA, rt.

Complex patterns and overlapping peaks made the NMR spectra difficult to interpret. Electrospray MS showed the expected $[M + H]^+$ ions.

The Gd complexes **8a**–**d** were prepared by heating the corresponding ligand with Gd_2O_3 in water to 90 °C. Purification of all the complexes but the least lipophilic one, complex **8a**, was achieved by continuous extraction of alkaline (pH 10), aqueous solutions with chloroform for 24 hours. Complex **8a** was purified by the addition of small amounts of anion and cation exchange resin to an aqueous solution, followed by filtration. The electrospray MS data confirmed the presence of the Gd complexes.

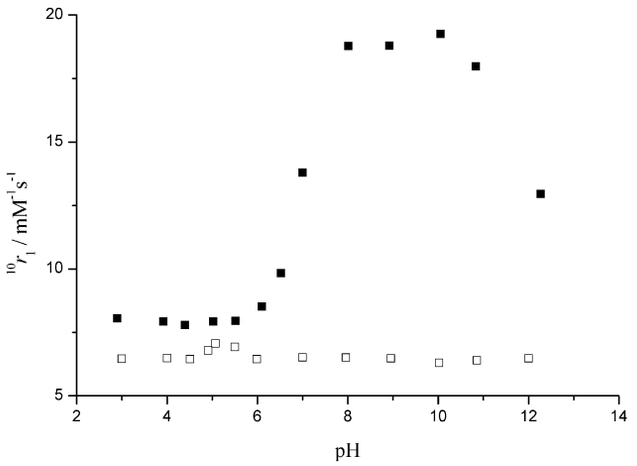


Fig. 1 The pH dependence of the T_1-relaxivity (r_1) of Gd-HADB-DO3A **8a** (□) and Gd-HADO-DO3A **8b** (■) (10 MHz, 25 °C).

In vitro relaxometry

The presence of a tertiary amino group in the side chains of the complexes **8a**–**d** led us to investigate the influence of pH on the T_1-relaxivities (r_1) in aqueous solutions. Because of favourable aqueous solubility Gd-HADB-DO3A (**8a**) and Gd-HADO-DO3A (**8b**) were chosen for these studies. The complexes were titrated in aqueous solution with HCl and NaOH. The T_1-relaxivities (r_1) as a function of pH are shown in Fig. 1.

The T_1-relaxivity (10 MHz, 25 °C) of Gd-HADB-DO3A (**8a**) was found to be nearly constant at about 6.5 mM^{-1} s^{-1} in the pH range 3–12, except for a small increase between pH 4.5 and pH 6. The T_1-relaxivity at the maximum (pH 5) was 7.1 mM^{-1} s^{-1}.

The T_1-relaxivity (10 MHz, 25 °C) of Gd-HADO-DO3A (**8b**) showed a marked pH dependence. At low pH (3–6) the relaxivity was about 7.9 mM^{-1} s^{-1}. The slightly higher value compared to Gd-HADB-DO3A might be attributed to an increase in the rotational correlation time (τ_R) of this complex caused by a higher molecular weight and/or possible formation of dimers or small clusters.

At higher pH (8–10) the relaxivity was found to be about 19.1 mM^{-1} s^{-1}, representing an increase of 142%. This large increase is thought to arise from the formation of colloidal aggregates, due to the higher lipophilicity of the deprotonated (neutral) complex compared to the protonated (positively charged) species. This will give rise to an increased τ_R of the complex and thus a higher relaxivity.

At high pH (10–12) the relaxivity decreased. This is presumably due to competition of hydroxide ions with water for the last coordination site on the gadolinium.

The properties of Gd-HADO-DO3A make it a valuable model for further development of pH responsive MRI contrast agents. Such contrast agents will have a large potential because of the reported reduced values of extracellular pH for tumours compared to healthy tissue.[11] Further experiments will be performed to investigate the mechanisms of this pH dependence.

Experimental

Synthesis

Reagents were obtained from Aldrich Chemical Co. Inc., USA or Fluka Chemie AG, Switzerland and used as received. 1,4,7,10-Tetraazacyclododecane (cyclen) was purchased from Macrocyclics Inc., USA. Dihexadecylamine (**1d**) was prepared

by reacting hexadecylamine with palmitoyl chloride, followed by reduction with lithium aluminium hydride.

NMR spectra were obtained on a Bruker Spectrospin Avance DPX300 (Bruker GmbH, Germany). Electron impact (EI) mass spectra were obtained using a Fisons VG ProSpec (70 eV and 220 °C) (Micromass Ltd., England). Electrospray (ES) FT-ICR-MS mass spectra were recorded on a Bruker BioApex 4.7 T (Bruker GmbH, Germany). Elemental analyses were carried out by Ilse Beetz Microanalytisches Laboratorium, Germany.

General procedure for the synthesis of oxiranes 2a–d. A mixture of dialkylamine (100 mmol), epichlorohydrin (120 mmol) and water (15 mmol) was stirred at 30–70 °C for 5–16 h. The mixture was washed with aqueous potassium carbonate (20%, 20 ml). Sodium hydroxide (36%, 20 ml) was added to the oily phase and the mixture was stirred at 30–70 °C overnight. The phases were separated and the oil dissolved in diethyl ether (100 ml), dried ($MgSO_4$) and evaporated *in vacuo* to yield the products as oils.

N,N-Dibutyl(oxiranylmethyl)amine (***2a***). The reaction between dibutylamine and epichlorohydrin was performed according to the general procedure. Yield: 13.67 g (74%). Mass spectrum (EI): *m/z* (relative intensity) 185 (7%) [M^+, $C_{11}H_{23}NO$], 142 (100), 100 (23); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 7.2 Hz, 6H), 1.20–1.40 (m, 8H), 2.30–2.50 (m, 6H), 2.60–2.70 (m, 2H), 2.90–3.00 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.92, 20.50, 29.05, 45.17, 50.87, 54.22, 56.66.

N,N-Dioctyl(oxiranylmethyl)amine (***2b***). The reaction between dioctylamine (12.07 g, 50 mmol) and epichlorohydrin (5.55 g, 60 mmol) was performed according to the general procedure. Yield: 10.42 g (70%). Mass spectrum (EI): *m/z* (relative intensity) 297 (1%) [M^+, $C_{19}H_{39}NO$], 285 (88), 280 (100), 254 (40), 156 (34); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 6.7 Hz, 6H), 1.15–1.30 (m, 20H), 1.35–1.50 (m, 4H), 2.35–2.58 (m, 6H), 2.62–2.75 (m, 2H), 2.95–3.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.03, 22.61, 27.01, 27.48, 29.27, 29.52, 31.81, 45.32, 50.96, 54.67, 56.79.

N,N-Didodecyl(oxiranylmethyl)amine (***2c***). The reaction between didodecylamine (2.48 g, 7.0 mmol) and epichlorohydrin (0.78 g, 8.4 mmol) was performed according to the general procedure. Yield: 2.38 g (83%). Mass spectrum (EI): *m/z* (relative intensity) 409 (3%) [M^+, $C_{27}H_{55}NO$], 366 (10), 254 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 6.7 Hz, 6H), 1.15–1.30 (m, 36H), 1.35–1.50 (m, 4H), 2.30–2.55 (m, 6H), 2.60–2.80 (m, 2H), 2.95–3.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.10, 22.66, 26.91, 27.49, 29.34, 29.62, 31.89, 45.35, 50.95, 54.60, 56.75.

N,N-Bis(hexadecyl)(oxiranylmethyl)amine (***2d***). The reaction between dihexadecylamine (2.33 g, 5.0 mmol) and epichlorohydrin (0.56 g, 6.0 mmol) was performed according to the general procedure. The crude product was further purified by applying it to a short column of silica gel. Elution with $CHCl_3$–MeOH–NH_3 (25%) 90 : 10 : 1 yielded 2.18 g (84%). Mass spectrum (EI): *m/z* (relative intensity) 521 (3%) [M^+, $C_{35}H_{71}NO$], 478 (12), 310 (100), 254 (88); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 6.7 Hz, 6H), 1.10–1.30 (m, 52H), 1.35–1.50 (m, 4H), 2.30–2.55 (m, 6H), 2.60–2.75 (m, 2H), 2.95–3.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.08, 22.67, 26.98, 27.51, 29.35, 29.59, 29.65, 29.69, 31.91, 45.33, 50.93, 54.66, 56.79.

General procedure for the synthesis of formyl derivatives 5a and 5b. A solution of 1,4,7,10-tetraazacyclododecane (cyclen) (15.0 mmol) and *N,N*-dimethylformamide dimethyl acetal (17.3 mmol) in toluene (30 ml) was heated to 120 °C under argon. After 2 h the toluene–MeOH azeotrope formed during the reaction was distilled off. The remaining toluene was evaporated *in vacuo* at 70 °C. *N,N*-Dialkyl(oxiranylmethyl)amine (16.5 mmol) was added and the mixture was heated to 120 °C under argon. After 16 h the reaction mixture was cooled to room temperature and MeOH–water (3 : 1, 20 ml) was added. The mixture was stirred at room temperature for 3 h and then the solvents were evaporated *in vacuo*. The residue was purified by flash chromatography (SiO_2, EtOH–THF–NH_3 (25%) 1 : 5 : 1) to yield the products as oils.

1-Formyl-7-[2-hydroxy-3-(N,N-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane (***5a***). The reaction was performed according to the general procedure with *N,N*-dibutyl(oxiranylmethyl)amine (3.06 g, 16.5 mmol). Yield: 3.52 g (61%). Mass spectrum (EI): *m/z* (relative intensity) 385 (<1%) [M^+, $C_{20}H_{43}N_5O_2$], 367 (8), 243 (100), 225 (47), 142 (52); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81 (t, 7.2 Hz, 6H), 1.10–1.35 (m, 4H), 1.35–1.50 (m, 4H), 2.10–2.40 (m, 9H), 2.45–2.70 (m, 10H), 3.00–3.70 (m, 9H), 8.05 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.92, 20.41, 29.06, 43.89, 46.78, 46.89, 46.93, 47.41, 49.80, 50.52, 51.95, 54.03, 58.65, 59.54, 65.80, 164.58.

1-Formyl-7-[2-hydroxy-3-(N,N-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane (***5b***). The reaction was performed according to the general procedure with *N,N*-dioctyl(oxiranylmethyl)amine (3.27 g, 11.0 mmol). Yield: 3.26 g (65%). Mass spectrum (EI): *m/z* (relative intensity) 497 (<1%) [M^+, $C_{28}H_{59}N_5O_2$], 479 (5), 284 (31), 280 (42), 254 (54), 243 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81 (t, 6.7 Hz, 6H), 1.10–1.25 (s, 20H), 1.30–1.40 (m, 4H), 2.10–2.80 (m, 18H), 2.85–3.25 (m, 4H), 3.30–3.65 (m, 6H), 8.08 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 22.53, 26.96, 27.36, 29.20, 29.46, 31.73, 43.96, 46.84, 47.01, 47.54, 49.85, 50.56, 52.08, 54.39, 58.73, 59.68, 65.83, 164.59.

1-Formyl-7-[2-hydroxy-3-(*N,N*-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (5c). A solution of 1,4,7,10-tetraazacyclododecane (cyclen) (0.87 g, 5.1 mmol) and *N,N*-dimethylformamide dimethyl acetal (0.69 g, 5.8 mmol) in toluene (10 ml) was heated to 120 °C under argon. After 2 h the toluene–MeOH azeotrope formed during the reaction was distilled off. The remaining toluene was evaporated *in vacuo* at 70 °C. *N,N*-Didodecyl(oxiranylmethyl)amine (2.28 g, 5.6 mmol) was added and the mixture was heated to 120 °C under argon. After 16 h the reaction mixture was cooled to room temperature and MeOH–water (3 : 1, 7 ml) was added. The mixture was stirred at room temperature for 3 h and then the solvents were evaporated *in vacuo*.

The residue was dissolved in water (50 ml) and extracted with diethyl ether (3 × 50 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was dissolved in *n*-hexane–ethyl acetate (3 : 2, 3 ml) and applied to a short column of alumina (neutral). The column was washed with *n*-hexane–ethyl acetate (3 : 2, 100 ml) and the product was eluted with MeOH (70 ml). Yield: 1.26 g (41%) of a yellow oil. Mass spectrum (EI): *m/z* (relative intensity) 609 (<1%) [M^+, $C_{36}H_{75}N_5O_2$], 591 (10), 392 (25), 243 (77), 225 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, 6.7 Hz, 6H), 1.10–1.20 (m, 36H), 1.25–1.35 (m, 4H), 2.15–2.55 (m, 8H), 2.56–2.80 (m, 7H), 2.85–3.00 (m, 3H), 3.40–3.60 (m, 4H), 4.20–4.80 (m, 5H), 8.10 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.94, 22.51, 26.80, 27.28, 29.18, 29.48, 29.50, 31.74, 43.93, 46.34, 46.64, 46.89, 47.94, 48.71, 50.93, 52.29, 54.15, 58.60, 60.65, 65.82, 164.52.

1-Formyl-7-[2-hydroxy-3-(*N,N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (5d). A solution of 1,4,7,10-tetraazacyclododecane (cyclen) (627 mg, 3.6 mmol) and *N,N*-dimethylformamide dimethyl acetal (499 mg, 4.2 mmol) in toluene (10 ml) was heated to 120 °C under argon. After 2 h the toluene–MeOH azeotrope formed during the reaction was distilled off. The remaining toluene was evaporated *in vacuo* at 70 °C. *N,N*-Dihexadecyl(oxiranylmethyl)amine (2.09 g, 4.0 mmol) was added and the mixture was heated to 120 °C under argon. After 16 h the reaction mixture was cooled to room temperature and MeOH–water (3 : 1, 8 ml) was added. The mixture was stirred at room temperature for 3 h and then the

solvents were evaporated *in vacuo*. The residue was dissolved in chloroform (75 ml) and washed with saturated brine (50 ml). The aqueous phase was extracted with chloroform (50 ml) and the combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. Flash chromatography (SiO_2, EtOH–THF–NH_3 (25%) 1 : 5 : 1) of the residue yielded an oil that was further purified on a short column of alumina. Washing with *n*-hexane–ethyl acetate 1 : 1 was followed by elution with EtOH–THF–NH_3 1 : 5 : 1 to yield the product as a yellow oil. Yield: 1.05 g (40%). Mass spectrum (EI): *m*/*z* (relative intensity) 703 (<1%) [$M^+ - 18$], 477 (65), 379 (85), 254 (100), 225 (46); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81 (t, 6.7 Hz, 6H), 1.10–1.20 (m, 52H), 1.25–1.40 (m, 4H), 2.10–2.90 (m, 16H), 3.10–3.80 (m, 8H), 8.09 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 22.55, 27.00, 27.35, 29.24, 29.58, 30.19, 31.80, 43.97, 46.67, 46.95, 47.33, 47.45, 49.47, 50.80, 52.30, 54.32, 58.73, 60.11, 65.85, 164.46.

General procedure for the synthesis of monosubstituted cyclen derivatives 6a–c. Sodium hydroxide (61.0 mmol) was added to a solution of the formyl derivative (6.1 mmol) in MeOH–water (3 : 1, 15 ml) and the temperature was raised to 70 °C. After 16 h the solvents were evaporated *in vacuo*. The residue was dissolved in water (10 ml) and extracted with dichloromethane (50 ml + 2 × 20 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to yield the products as oils.

*1-[2-Hydroxy-3-(N,N-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane (**6a**).* The reaction was performed according to the general procedure with 1-formyl-7-[2-hydroxy-3-(*N*,*N*-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane. Yield: 1.96 g (90%). Mass spectrum (EI): *m*/*z* (relative intensity) 357 (<1%) [M^+, $C_{19}H_{43}N_5O$], 215 (53), 197 (100), 168 (47), 142 (82); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (t, 7.2 Hz, 6H), 1.10–1.24 (m, 4H), 1.25–1.35 (m, 4H), 2.10–2.75 (m, 22H), 3.60–3.70 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.92, 20.40, 29.09, 45.25, 46.06, 46.97, 52.30, 53.77, 58.96, 59.28, 65.48.

*1-[2-Hydroxy-3-(N,N-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane (**6b**).* The reaction was performed according to the general procedure with 1-formyl-7-[2-hydroxy-3-(*N*,*N*-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane (1.65 g, 3.1 mmol). Yield: 1.37 g (94%). Mass spectrum (EI): *m*/*z* (relative intensity) 469 (1%) [M^+, $C_{27}H_{59}N_5O$], 284 (49), 280 (71), 254 (80), 215 (52), 197 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (t, 6.6 Hz, 6H), 1.10–1.20 (m, 20H), 1.25–1.40 (m, 4H), 2.20–2.45 (m, 9H), 2.50–2.60 (m, 8H), 2.65–2.80 (m, 4H), 3.40–3.70 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.95, 22.51, 26.89, 27.32, 29.18, 29.45, 31.71, 44.98, 45.85, 46.73, 52.07, 54.09, 58.98, 59.22, 65.45.

*1-[2-Hydroxy-3-(N,N-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (**6c**).* The reaction was performed according to the general procedure with 1-formyl-7-[2-hydroxy-3-(*N*,*N*-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (1.15 g, 1.9 mmol). Yield: 0.85 g (77%). Mass spectrum (EI): *m*/*z* (relative intensity) 581 (2%) [M^+, $C_{35}H_{75}N_5O$], 392 (41), 366 (57), 215 (63), 197 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (t, 6.7 Hz, 6H), 1.10–1.20 (m, 36H), 1.25–1.40 (m, 4H), 2.20–3.70 (m, 23H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.99, 22.56, 26.99, 27.34, 29.23, 29.53, 29.55, 31.80, 45.34, 46.24, 47.09, 52.44, 54.11, 59.10, 59.33, 65.47.

1-[2-Hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (6d). 1-Formyl-7-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (440 mg, 0.6 mmol) was dissolved in MeOH (10 ml) and hydrochloric acid (37%, 1 ml) was added. The mixture was refluxed for 16 h, cooled to room temperature and evaporated *in vacuo*. Water (25 ml) was added to the residue and the pH was adjusted to 12 by addition of sodium hydroxide (6 M). The aqueous solution was extracted with dichloromethane (30 ml × 3), and the combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. Yield: 300 mg (71%). Mass spectrum (EI): *m*/*z* (relative intensity) 478 (6%), 464 (7), 268 (26), 254 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 6.7 Hz, 6H), 1.10–1.25 (m, 52H), 1.30–1.45 (m, 4H), 2.10–2.45 (m, 8H), 2.50–2.65 (m, 6H), 3.60–3.80 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.05, 22.63, 27.00, 27.43, 29.31, 29.61, 29.65, 31.87, 45.16, 46.10, 46.91, 52.38, 54.17, 59.09, 59.34, 65.65.

General procedure for the synthesis of DO3A derivatives 7a–d. Monosubstituted cyclen derivative (4.7 mmol) in EtOH (40 ml) was added to a solution of sodium chloroacetate (23.5 mmol) in water (40 ml). The pH of the solution was adjusted to 10 by the addition of sodium hydroxide (1 M). The mixture was heated to 70 °C with stirring for 48 h, maintaining a pH of 10. The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography on silica gel to yield the products as colourless solids.

*1,4,7-Tris(carboxymethyl)-10-[2-hydroxy-3-(N,N-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane (**7a**).* The reaction was performed according to the general procedure with 1-[2-hydroxy-3-(*N*,*N*-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane. Mobile phase: $CHCl_3$–MeOH–NH_3 (25%) 9 : 4 : 1. Yield: 1.09 g (44%). Mass spectrum (ES): *m*/*z* 532 [[M + H]$^+$, $C_{25}H_{50}N_5O_7$]; ^{1}H NMR (300 MHz, CD_3OD): δ 0.92 (t, 7.2 Hz, 6H), 1.20–1.35 (m, 4H), 1.36–1.50 (m, 4H), 2.00–2.20 (m, 5H), 2.21–2.35 (m, 4H), 2.36–2.60 (m, 6H), 2.65–2.90 (m, 5H), 2.91–3.05 (m, 2H), 3.10–3.30 (m, 3H), 3.40–3.60 (m, 2H), 3.61–3.70 (m, 1H), 4.20–4.30 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.46, 21.64, 30.05, 49.76, 51.91, 53.45, 54.30, 54.36, 55.42, 57.27, 60.39, 60.56, 60.65, 61.24, 65.87, 179.26, 179.43, 179.60.

*1,4,7-Tris(carboxymethyl)-10-[2-hydroxy-3-(N,N-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane (**7b**).* The reaction was performed according to the general procedure with 1-[2-hydroxy-3-(*N*,*N*-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane (536 mg, 1.1 mmol). Mobile phase: $CHCl_3$–MeOH–NH_3 (25%) 9 : 4 : 1. Yield: 300 mg (41%). Mass spectrum (ES): *m*/*z* 644 [[M + H]$^+$, $C_{33}H_{66}N_5O_7$]; ^{1}H NMR (300 MHz, CD_3OD): δ 0.89 (t, 6.6 Hz, 6H), 1.20–1.35 (m, 20H), 1.40–1.55 (m, 4H), 2.00–2.20 (m, 4H), 2.21–2.35 (m, 3H), 2.40–2.60 (m, 4H), 2.65–3.10 (m, 6H), 3.20–3.25 (m, 3H), 3.40–3.70 (m, 3H), 4.15–4.35 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.51, 23.73, 27.70, 28.55, 30.51, 30.67, 33.03, 51.89, 53.45, 54.34, 55.73, 57.20, 60.55, 61.27, 65.91, 179.36.

*1,4,7-Tris(carboxymethyl)-10-[2-hydroxy-3-(N,N-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (**7c**).* The reaction was performed according to the general procedure with 1-[2-hydroxy-3-(*N*,*N*-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (750 mg, 1.3 mmol). Mobile phase: MeOH. Yield: 439 mg (45%). Mass spectrum (ES): *m*/*z* 756 [[M + H]$^+$, $C_{41}H_{82}N_5O_7$]; ^{1}H NMR (300 MHz, CD_3OD): δ 0.89 (t, 6.4 Hz, 6H), 1.20–1.35 (m, 38H), 1.40–1.70 (m, 4H), 2.00–2.40 (m, 8H), 2.45–3.00 (m, 16H), 3.10–3.60 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.56, 23.75, 25.67, 28.05, 30.50, 30.60, 30.69, 30.74, 30.78, 30.82, 33.09, 44.89, 51.54, 52.36, 53.46, 54.35, 54.71, 55.19, 59.70, 60.34, 60.57, 60.84, 65.40, 179.24, 179.53, 179.82.

*1,4,7-Tris(carboxymethyl)-10-[2-hydroxy-3-(N,N-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (**7d**).* The reaction was performed according to the general procedure with 1-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (462 mg, 0.7 mmol). Mobile phase: $CHCl_3$–MeOH–NH_3 (25%) 9 : 6 : 1. Yield 95 mg (16%). Mass spectrum (ES): *m*/*z* 435 [[M + 2H]$^{2+}$, $C_{49}H_{99}N_5O_7$]; ^{1}H NMR (300 MHz, CD_3OD): δ 0.90 (t, 6.7 Hz, 6H), 1.20–1.40 (m, 52H), 1.60–1.80 (m, 4H), 2.00–2.40 (m, 5H), 2.50–2.90 (m, 7H), 3.00–3.25 (m, 8H), 3.35–3.80 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 14.61, 23.79, 27.91, 30.42, 30.55, 30.76, 30.80, 30.85, 30.90, 33.13, 45.00–51.00 (small peaks), 51.19, 53.46, 54.15, 54.53, 55.78, 60.36, 60.74, 64.15, 178.84, 179.13.

1,4,7-Tris(*tert*-butoxycarbonylmethyl)-10-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (9). *tert*-Butyl bromoacetate (328 mg, 1.7 mmol) was added to 1-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (290 mg, 0.4 mmol) and sodium carbonate (178 mg, 1.7 mmol) in a mixture of THF and water (30 : 1, 7 ml). The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated *in vacuo*. The residue was dissolved in dichloromethane (20 ml) and washed with aqueous sodium carbonate (5%, 15 ml × 2). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was submitted to flash chromatography (SiO_2, CH_2Cl_2–MeOH 9 : 1) to yield 130 mg (30%) of a yellow oil. Mass spectrum (ES): *m*/*z* 1036 [[M + H]$^+$, $C_{61}H_{122}N_5O_7$]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.78 (t, 6.7 Hz, 6H), 1.00–1.25 (m, 45H), 1.30–1.50 (m, 19H), 1.80–2.40 (m, 9H), 2.60–3.25 (m, 11H), 3.40–4.20 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.95, 22.49, 27.72, 27.99, 29.17, 29.38, 29.47, 29.51, 31.72, 45.00–65.00 (small peaks), 81.49, 81.72, 84.33, 171.99.

1,4,7-Tris(carboxymethyl)-10-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (7d). Trifluoroacetic acid (1 ml) was added to 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (110 mg, 0.1 mmol) under argon. The solution was stirred at room temperature for 16 h and then evaporated *in vacuo*. Water (10 ml) was added to the residue and the resulting suspension was concentrated to dryness *in vacuo*. This was repeated once and the residue was dried under vacuum (50 °C). Yield 85 mg (98%).

Gadolinium 10-[2-hydroxy-3-(*N*,*N*-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (Gd-HADB-DO3A) (8a). A suspension of 1,4,7-tris(carboxymethyl)-10-[2-hyd roxy-3-(*N*,*N*-dibutylamino)propyl]-1,4,7,10-tetraazacyclododecane (170 mg, 0.32 mmol) and gadolinium oxide (58 mg, 0.16 mmol) in water (10 ml) was heated to 90 °C for 7 h. Activated charcoal was added and the suspension filtered hot. Some anion and cation exchange resin was added to the cooled solution and the pH adjusted to 7. The suspension was filtered after 1 h and evaporated *in vacuo* to yield 140 mg (64%) of a slightly yellow solid. Mass spectrum (ES): *m*/*z* 687 [[M + H]$^+$, $C_{25}H_{47}N_5O_7Gd$]; Anal. Calcd. (found) for $C_{25}H_{46}N_5O_7Gd\cdot 7H_2O$: C, 36.95% (36.94); H, 7.39 (7.06).

General procedure for the synthesis of the Gd-complexes 8b–d. A suspension of the DO3A derivative (0.23 mmol) and gadolinium oxide (0.12 mmol) in water (10 ml) was heated to 90 °C for 16 h. The mixture was cooled to room temperature, filtered and the pH of the filtrate was adjusted to 10 by addition of 1 M NaOH. The solution was continuously extracted with chloroform for 20 h. The organic phase was evaporated *in vacuo* to yield the products as hygroscopic solids.

*Gadolinium 10-[2-hydroxy-3-(N,N-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (Gd-HADO-DO3A) (**8b**).* The reaction was performed according to the general procedure with 1,4,7-tris(carboxymethyl)-10-[2-hydroxy-3-(*N*,*N*-dioctylamino)propyl]-1,4,7,10-tetraazacyclododecane. Yield: 120 mg (65%). Mass spectrum (ES): *m*/*z* 799 [[M + H]$^+$, $C_{33}H_{63}N_5O_7Gd$]; Anal. Calcd. (found) for $C_{33}H_{62}N_5O_7Gd\cdot 5.5H_2O$: C, 44.15% (44.11); H, 8.14 (7.52); N, 7.80 (8.00).

*Gadolinium 10-[2-hydroxy-3-(N,N-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (Gd-HADD-DO3A) (**8c**).* The reaction was performed according to the general procedure with 1,4,7-tris(carboxymethyl)-10-[2-hydroxy-3-(*N*,*N*-didodecylamino)propyl]-1,4,7,10-tetraazacyclododecane (197 mg, 0.26 mmol). Yield: 155 mg (65%). Mass spectrum (ES): *m*/*z* 911 [[M + H]$^+$, $C_{41}H_{79}N_5O_7Gd$]; Anal. Calcd. (found) for $C_{41}H_{78}N_5O_7Gd\cdot 3H_2O$: C, 51.01% (50.98); H, 8.78 (8.38); N, 7.26 (7.19).

*Gadolinium 10-[2-hydroxy-3-(N,N-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (Gd-HADHD-DO3A) (**8d**).* The reaction was performed according to the general procedure with 1,4,7-tris(carboxymethyl)-10-[2-hydroxy-3-(*N*,*N*-dihexadecylamino)propyl]-1,4,7,10-tetraazacyclododecane (92 mg, 0.11 mmol). Yield: 80 mg (71%). Mass spectrum (ES): *m*/*z* 1024 [[M + H]$^+$, $C_{49}H_{95}N_5O_7Gd$].

In vitro relaxometry

The relaxation measurements were performed at 10 MHz on a Spinmaster FFC (Fast Field Cycling) NMR relaxometer (Stelar s.r.l, Mede (PV), Italy). The T_1 relaxation rates (R_1) were obtained by the inversion recovery method at 25 °C. The T_1 relaxivity (r_1) was obtained by the relationship given in eqn. (1),

$$r_1 = \frac{R_1^{obs} - R_1^{m}}{C} \quad (1)$$

where R_1^{obs} and R_1^{m} are the relaxation rates in s^{-1} of the sample and the matrix respectively, and C is the Gd concentration in mM.

The r_1 values were plotted against pH to give the pH dependent relaxivity relationship as shown in Fig. 1. Analyses were performed in triplicate at each pH.

The measurements were performed on aqueous solutions of Gd-HADB-DO3A (**8a**) and Gd-HADO-DO3A (**8b**) with gadolinium concentrations of 1.49 mM and 1.52 mM, respectively. The pH of the solutions was adjusted by addition of minimal amounts of HCl or NaOH.

Determination of gadolinium concentration

Nitric acid and water was added to the chelate solution to a final concentration of 2.5 M HNO_3. After incubation at ambient temperature for 24 h the T_1 relaxivity was measured at 10 MHz (25 °C). The T_1 relaxivity of a standard solution of 2.12 mM $GdCl_3$ in 2.5 M HNO_3 was measured at the same conditions. The gadolinium concentration was then determined from eqn. (1).

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