Synthesis and physicochemical characterisation of new amphiphilic gadolinium DO3A complexes as contrast agents for MRI

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Received (in Lund, Sweden) 9th November 1999, Accepted 25th February 2000

Two approaches were employed in the syntheses of four 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecanes (**4**) with alkyl chain lengths from 4 to 16 carbons. Physicochemical properties, such as critical micelle concentration (CMC), micelle size, partition coefficient (*P*) between water and octan-1-ol and T_1 relaxivity (r_1), were investigated for the corresponding gadolinium (Gd) complexes. The Gd complexes containing the shortest alkyl chains (**5a** and **5b**) showed properties typical of water-soluble Gd complexes. On the other hand, the long-chained chelates (**5c** and **5d**) were found to possess amphiphilic properties and to form micelles. The relaxivities of these amphiphilic complexes were found to be concentration dependent, consistent with the formation of micelles. An unexpectedly high relaxivity was measured for compound **5d** below its CMC. This feature is probably caused by cluster formation due to low solubility in water.

Introduction

Vascular imaging is a large diagnostic area addressing directly or indirectly major diseases including cardiovascular diseases and cancer. Due to a rapid extracellular distribution and renal elimination, the commercialised low-molecular weight hydrophilic gadolinium (Gd) contrast agents are not the best candidates for vascular imaging.[1]

One key feature of improved vascular agents would be their ability to be retained in the bloodstream during the time of data acquisition, eliminating the need for repetitive injections. Several approaches have been considered in preclinical and clinical studies.[2–4] One particularly attractive concept is to exploit *in vivo* the high affinity of lipophilic Gd chelates towards plasma albumin.[5]

Liposomes, a class of particulate system, have also been extensively investigated as carriers for contrast materials.[6] The main focus has centred on their potential as liver contrast agents. However, small polymer-coated, Gd-labelled liposomes have shown promising potential as long-circulating contrast agents. In the design of a liposomal paramagnetic chelate, the latter should be attached rigidly to the surface of the membrane if high T_1 relaxivity is desired. Also, the use of a macrocyclic chelate for liposome association would improve the safety profile of the liposomal agent, minimising any dechelation *in vivo*, intravascularly or intracellularly.[7]

In the present work, the syntheses and characterisation of new amphiphilic Gd 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A) derivatives have been carried out for future incorporation into the liposome lamella. The T_1 relaxivities were determined for aqueous solutions of the amphiphilic Gd–DO3A derivatives with different concentrations in order to determine any correlation with micelle formation.

Experimental

Synthesis

Reagents were obtained from either Aldrich Chemical Co. Inc., USA or Fluka Chemie AG, Switzerland and used as received. 1,4,7,10-Tetraazacyclododecane (cyclen) (**1**) was purchased from Macrocyclics Inc., USA. 1,4,7-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A) (**3**) was prepared according to the literature.[8]

NMR spectra were obtained on Bruker Spectrospin Avance DPX200 or DPX300 instruments (Bruker GmbH, Germany). NMR spectra of the DO3A derivatives were recorded at 75 °C. 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 instrument (Bruker GmbH, Germany). Elemental analyses were carried out by Ilse Beetz Microanalytisches Laboratorium, Germany. ICP-AES analyses were obtained using an ICP-AES Perkin-Elmer Plasma 2000 instrument (Perkin-Elmer Inc., USA).

General procedure for the syntheses of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (4) from DO3A (3) (Scheme 1). A solution of DO3A (**3**) in water or in a mixture of water and methanol was adjusted to pH 12 by the addition of sodium hydroxide (5 M). 1,2-Epoxyalkane was added (neat or dissolved in methanol) and stirred for the designated time and temperature until TLC (propan-2-ol–ethyl acetate–NH_3 (25%) 4:2:1) indicated complete disappearance of DO3A. The solution was cooled and transferred to an ion exchange column (Amberlite IRA-410, formate form) which was subsequently washed with water. The column was eluted with formic acid (3 M). The fractions containing the product were combined and evaporated *in vacuo*. Remaining formic acid was removed by dissolving the residue in water (20 ml) followed by evaporation *in vacuo*. The last process was repeated three times. The compounds appeared as white solid materials.

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (HB-DO3A) (**4a**) (Scheme 1).* DO3A (**3**) (0.87 g, 2.5 mmol) in water (10 ml) was reacted with 1,2-epoxybutane (0.36 g, 5.0 mmol) according to the general procedure. The reaction mixture was stirred for 6 h at ambient temperature. Yield: 0.49 g (47%). Mass spectrum (ES^+): *m/z* 419

DOI: 10.1039/a908979i

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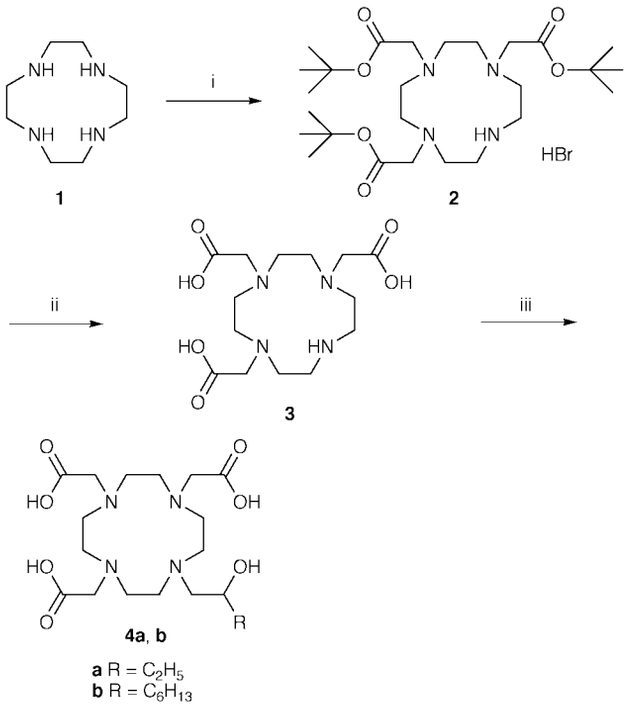


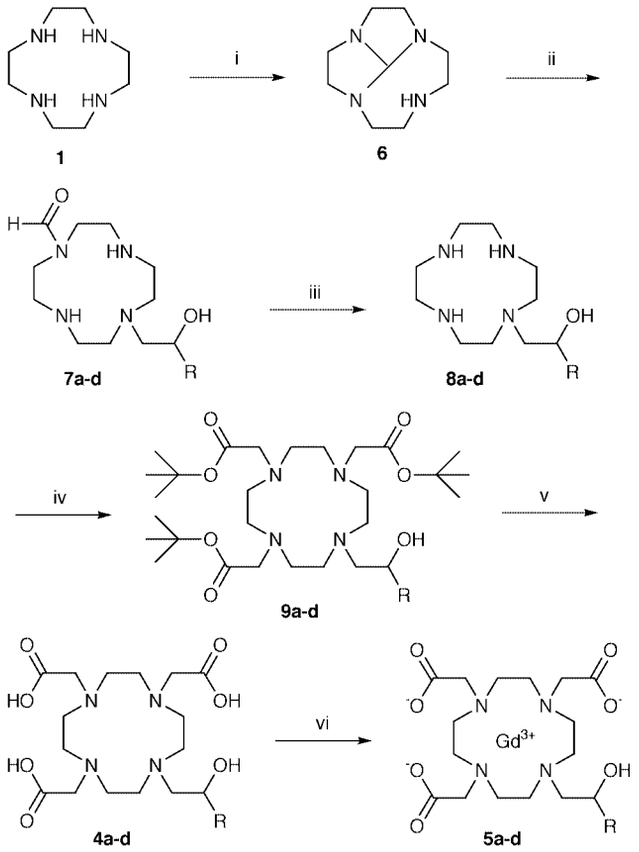
Scheme 1 Synthesis of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane by alkylation of DO3A. *Reagents and conditions*: i, $BrCH_2COOC(CH_3)_3$, DMA, rt.; ii, CF_3COOH, rt.; iii, epoxide, H_2O, MeOH.

[[M + H]$^+$, $C_{18}H_{35}N_4O_7$]. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89 (t, 7.2 Hz, 3H), 1.46 (t, 6.7 Hz, 2H), 3.12–3.54 (m, 23H), 3.89 (s, 1H), 4.18 (s, 2H), 8.05 (s (br)). ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.87, 27.50, 47.65, 47.77, 50.65, 51.27, 52.72, 54.01, 58.87, 65.81, 167.00, 171.62.

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (HO-DO3A) (**4b**) (Scheme 1).* DO3A (**3**) (1.04 g, 3 mmol) in water–methanol (25 ml, 4 : 1) was reacted with 1,2-epoxyoctane (1.15 g, 9 mmol) in methanol (5 ml) according to the general procedure. The reaction mixture was stirred for 72 h at 50 °C. Yield: 0.42 g (30%). Mass spectrum (ES$^+$): *m/z* 475 [[M + H]$^+$, $C_{22}H_{43}N_4O_7$]. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, 6.9 Hz, 3H), 1.25–1.40 (m, 10H), 2.70–3.50 (m, 21H), 3.91 (s, 1H), 6.27 (s (br)). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.39, 21.55, 24.20, 28.26, 30.81, 35.01, 47.58, 49.60, 51.77, 51.82, 54.42, 54.51, 57.99, 64.77, 170.96, 171.59.

General procedure for the synthesis of 1-formyl-7-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (7) (Scheme 2). A solution of 1,4,7,10-tetraazacyclododecane (**1**) (1.00 equiv.) and *N*,*N*-dimethylformamide dimethyl acetal (1.15 equiv.) in toluene was heated to 120 °C under argon atmosphere. The methanol formed during the reaction was removed by distillation before the reaction mixture was evaporated *in vacuo* at 70 °C. 1,2-Epoxyalkane (1.10–1.30 equiv.) was added and the reaction mixture was heated at 120 °C under argon atmosphere for 16 h. The mixture was cooled to room temperature, methanol–water (3 : 1) was added and the mixture was stirred for 3 h before it was evaporated *in vacuo*. The residue was purified by flash chromatography on silica to give the products as yellow oils.

*1-Formyl-7-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**7a**) (Scheme 2).* Reaction of 1,4,7,10-tetraazacyclododecane (**1**) (4.31 g, 25.00 mmol) with *N*,*N*-dimethylformamide dimethyl acetal (3.43 g, 28.75 mmol) and 1,2-epoxybutane (1.98 g, 27.50 mmol) was performed according to the general procedure. Ethanol–THF–NH_3 (25%) 1 : 3 : 1 was used as eluent. Yield: 2.72 g (40%). Mass spectrum (EI): *m/z* (relative intensity) 272 (1) [M$^+$, $C_{13}H_{28}N_4O_2$], 213 (7) [$C_{10}H_{21}N_4O$], 116 (100). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (t, 6.4 Hz, 3H), 1.25–1.41 (m, 1H), 1.45–1.61 (m, 1H), 2.20–2.85 (m, 14H), 3.35–3.62 (m, 4H), 8.15 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 9.92, 27.24, 43.68, 46.71, 46.75, 47.59, 48.90, 51.74, 51.99, 61.46, 69.22, 163.94.



Scheme 2 Synthesis of gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane by monoalkylation of cyclen. *Reagents and conditions*: i, Dimethylformamide dimethyl acetal, toluene, 120 °C; ii, 1) epoxide, 120 °C, 2) MeOH, H_2O, rt.; iii, NaOH, MeOH, H_2O, 70 °C; iv, $BrCH_2COOC(CH_3)_3$, Na_2CO_3, THF, H_2O, rt.; v, CF_3COOH, rt.; vi, Gd_2O_3, H_2O, 90 °C.

*1-Formyl-7-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**7b**) (Scheme 2).* Reaction of 1,4,7,10-tetraazacyclododecane (**1**) (2.58 g, 15.00 mmol) with *N*,*N*-dimethylformamide dimethyl acetal (2.05 g, 17.25 mmol) and 1,2-epoxyoctane (2.12 g, 16.50 mmol) was performed according to the general procedure. Ethanol–THF–NH_3 (25%) 1 : 3 : 1 was used as eluent. Yield: 3.45 g (70%). Mass spectrum (EI): *m/z* (relative intensity) 328 (1) [M$^+$, $C_{17}H_{36}N_4O_2$], 213 (11) [$C_{10}H_{21}N_4O$], 172 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75 (t, 6.9 Hz), 1.11–1.43 (m, 10H), 2.12–2.78 (m, 13H), 3.16–3.65 (m, 8H), 8.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.88, 22.38, 25.43, 29.28, 31.59, 34.33, 43.52, 46.87, 47.01, 47.07, 47.74, 49.68, 50.02, 52.03, 61.47, 68.11, 164.39.

*1-Formyl-7-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**7c**) (Scheme 2).* Reaction of 1,4,7,10-tetraazacyclododecane (**1**) (5.17 g, 30.00 mmol) in toluene (60 ml) with *N*,*N*-dimethylformamide dimethyl acetal (4.11 g, 34.50 mmol) and 1,2-epoxydodecane (6.08 g, 33.00 mmol) was performed according to the general procedure. Ethanol–THF–NH_3 (25%) 1 : 3 : 1 was used as eluent. Yield: 8.25 g (72%). Mass spectrum (EI): *m/z* (relative intensity) 384 (1) [M$^+$, $C_{21}H_{44}N_4O_2$], 228 (100), 213 (10) [$C_{10}H_{21}N_4O$]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 6.9 Hz, 3H), 1.22–1.44 (m, 18 H), 2.27–3.74 (m, 23H), 8.13 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 22.53, 25.55, 29.19, 29.48, 29.73, 31.77, 34.40, 43.52, 46.95, 47.10, 47.82, 49.78, 49.87, 51.99, 61.38, 68.16, 164.59.

*1-Formyl-7-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**7d**) (Scheme 2).* Reaction of 1,4,7,10-tetraazacyclododecane (**1**) (2.58 g, 15.00 mmol) in toluene (30 ml) with *N*,*N*-dimethylformamide dimethyl acetal (2.06 g, 17.25 mmol) and 1,2-epoxyhexadecane (4.69 g, 19.50 mmol) was performed according to the general procedure. Ethanol–THF–NH_3 (25%) 1 : 5 : 1 was used as eluent. Yield: 3.75 g (57%). Mass spectrum (EI): *m*/*z* (relative intensity) 439 (4) [[M-H]$^+$, $C_{25}H_{51}N_4O_2$], 284 (100), 213 (11) [$C_{10}H_{21}N_4O$]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 6.9 Hz, 3H), 1.15–1.39 (m, 26H), 2.20–2.74 (m, 14H), 3.10–3.68 (m, 6H), 8.04 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.93, 22.47, 25.53, 29.15, 29.43, 29.47, 29.66, 31.71, 34.41, 43.68, 46.88, 47.20, 47.89, 49.66, 50.30, 52.28, 61.76, 68.18, 164.23.

General procedure for the synthesis of 1-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (8) (Scheme 2). 1-Formyl-7-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (**7**) (1 equiv.) was dissolved in methanol–water (3 : 1) and sodium hydroxide (10 equiv.) was added. The mixture was stirred overnight (16 h) at 70 °C and evaporated *in vacuo*. The residue was dissolved in water and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give 1-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecanes (**8**) as colourless oils.

*1-(2-Hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**8a**) (Scheme 2).* Reaction between 1-formyl-7-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**7a**) (1.36 g, 5 mmol) and sodium hydroxide (2.00 g, 50 mmol) in methanol–water (12 ml) was performed according to the general procedure. Yield: 1.02 g (84%). Mass spectrum (EI): *m*/*z* (relative intensity) 245 (7) [[M + H]$^+$, $C_{12}H_{29}N_4O$], 200 (10), 188 (12), 159 (23), 157 (35), 116 (100). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, 7.6 Hz, 3H), 1.18–1.39 (m, 2H), 2.25–3.57 (m, 18H), 4.25 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 10.04, 27.57, 45.88, 46.36, 47.48, 52.33, 61.48, 69.93.

*1-(2-Hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**8b**) (Scheme 2).* Reaction between 1-formyl-7-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**7b**) (3.00 g, 9.13 mmol) and sodium hydroxide (3.65 g, 91.30 mmol) in methanol–water (12 ml) was performed according to the general procedure. Yield: 2.61 g (95%). Mass spectrum (EI): *m*/*z* (relative intensity) 301 (5) [[M + H]$^+$, $C_{16}H_{37}N_4O$], 256 (6), 244 (10), 215 (28), 213 (25), 172 (100), 116 (35). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, 6.9 Hz, 3H), 1.19–1.37 (m, 10H), 2.25–3.62 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 22.49, 25.63, 29.37, 31.71, 34.72, 45.75, 46.59, 47.33, 52.63, 61.74, 68.75.

*1-(2-Hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**8c**) (Scheme 2).* Reaction between 1-formyl-7-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**7c**) (6.25 g, 16.25 mmol) and sodium hydroxide (6.50 g, 162.50 mmol) in methanol–water (28 ml) was performed according to the general procedure. Yield: 5.17 g (89%). Mass spectrum (EI): *m*/*z* (relative intensity) 357 (7) [[M + H]$^+$, $C_{20}H_{45}N_4O$], 312 (10), 300 (12), 271 (23), 269 (35), 228 (100), 116 (55). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, 6.9 Hz, 3H), 1.17–1.39 (m, 18H), 2.20–3.62 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.99, 22.54, 25.68, 29.20, 29.50, 29.61, 29.64, 29.72, 31.78, 34.75, 45.70, 46.58, 47.28, 52.66, 61.71, 68.79.

*1-(2-Hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**8d**) (Scheme 2).* Reaction between 1-formyl-7-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**7d**) (1.98 g, 4.5 mmol) and sodium hydroxide (1.80 g, 45 mmol) in methanol–water (12 ml) was performed according to the general procedure. Yield: 1.60 g (78%). Mass spectrum (EI): *m*/*z* (relative intensity) 411 (7) [[M-H]$^+$, $C_{24}H_{51}N_4O$], 368 (19), 356 (27), 327 (39), 325 (35), 284 (100), 116 (49). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.78 (t, 6.9 Hz, 3H), 1.15–1.26 (m, 25H), 2.13–3.59 (m, 16H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.99, 22.54, 25.67, 29.22, 29.54, 29.70, 31.77, 34.74, 45.58, 46.51, 47.18, 52.50, 61.61, 68.70.

General procedure for the synthesis of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (9) (Scheme 2). *tert*-Butyl bromoacetate (4 equiv.) was added to 1-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (**8**) (1 equiv.) and sodium carbonate (4 equiv.) in a mixture of THF–water (30 : 1) over 30 min. After stirring overnight, solid material was collected by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and extracted with aqueous sodium carbonate (5%). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo.* The residue was submitted to flash chromatography on silica (CH_2Cl_2–methanol 9 : 1) to give yellow oils that solidified upon standing.

*1,4,7-Tris(tert-butoxycarbonylmethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**9a**) (Scheme 2).* Reaction of *tert*-butyl bromoacetate (2.89 g, 14.8 mmol), 1-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**8a**) (0.90 g, 3.7 mmol) and sodium carbonate (1.57 g, 14.8 mmol) in THF–water (31 ml) was performed according to the general procedure. Yield: 1.19 g (55%). Mass spectrum (EI): *m*/*z* (relative intensity) 586 (5) [M$^+$, $C_{30}H_{58}N_4O_7$], 485 (100) [$C_{25}H_{49}N_4O_5$], 416 (40) [$C_{20}H_{39}N_4O_5$]. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, 6.9 Hz, 3H), 1.27–1.36 (m, 2H), 1.45 (s, 18H), 1.49 (s, 9H), 1.98–3.70 (m, 24H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.91, 28.01, 29.15, 31.55, 36.43, 48.40, 48.71, 49.64, 50.44, 52.10, 52.29, 52.45, 55.48, 56.30, 59.40, 68.08, 81.92, 82.22, 82.29, 172.40, 172.51, 172.56.

*1,4,7-Tris(tert-butoxycarbonylmethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**9b**) (Scheme 2).* Reaction of *tert*-butyl bromoacetate (5.62 g, 28.8 mmol), 1-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**8b**) (2.16 g, 7.2 mmol) and sodium carbonate (3.05 g, 28.8 mmol) in THF–water (62 ml) was performed according to the general procedure. Yield: 1.70 g (37%). Mass spectrum (EI): *m*/*z* (relative intensity) 642 (5) [M$^+$, $C_{34}H_{66}N_4O_7$], 541 (100) [$C_{29}H_{57}N_4O_5$], 472 (58) [$C_{24}H_{47}N_4O_5$]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 6.9 Hz, 3H), 1.24–1.33 (m, 8H), 1.41 (s, 18H), 1.46 (s, 9H), 2.04–3.82 (m, 24H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.91, 22.40, 25.04, 27.74, 27.76, 28.01, 29.15, 31.55, 36.43, 48.40, 48.71, 49.64, 50.44, 52.10, 52.29, 52.45, 55.48, 56.30, 59.40, 68.08, 81.92, 82.22, 82.29, 172.40, 172.51, 172.56.

*1,4,7-Tris(tert-butoxycarbonylmethyl)-10-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**9c**) (Scheme 2).* Reaction of *tert*-butyl bromoacetate (4.92 g, 25.2 mmol), 1-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**8c**) (2.25 g, 6.3 mmol) and sodium carbonate (2.67 g, 25.2 mmol) in THF–water (62 ml) was performed according to the general procedure. Yield: 2.86 g (65%). Mass spectrum (EI): *m*/*z* (relative intensity) 698 (7) [M$^+$, $C_{38}H_{74}N_4O_7$], 597 (100) [$C_{33}H_{65}N_4O_5$], 528 (69) [$C_{28}H_{55}N_4O_5$]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 6.9 Hz, 3H), 1.23–1.34 (m, 16H), 1.42 (s, 18H), 1.47 (s, 9H), 2.04–3.72 (m, 25H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.97, 22.53, 25.14, 27.68, 27.77, 27.79, 28.04, 29.17, 29.41, 29.46, 29.54, 31.74, 36.48, 48.44, 48.78, 49.68, 50.48, 52.13, 52.48, 55.52, 56.34, 59.45, 68.12, 81.93, 82.23, 82.29, 172.44, 172.54, 172.59.

*1,4,7-Tris(tert-butoxycarbonylmethyl)-10-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**9d**) (Scheme 2).* Reaction of *tert*-butyl bromoacetate (4.60 g, 23.6 mmol), 1-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**8d**) (2.44 g, 5.9 mmol) and sodium carbonate (2.50 g, 23.6 mmol) in THF–water (93 ml) was performed according to the general procedure. Yield: 3.27 g (73%). Mass spectrum (EI): *m*/*z* (relative intensity) 754 (8) [M$^+$, $C_{42}H_{82}N_4O_7$], 653 (100) [$C_{37}H_{73}N_4O_5$], 584 (69) [$C_{32}H_{63}N_4O_5$]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 6.9 Hz, 3H), 1.22–1.36 (m, 26H), 1.43 (s, 18H), 1.49 (s, 9H), 2.05–3.74 (m, 29H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.97, 22.08, 22.52, 25.12, 27.66, 27.75, 28.02, 29.19, 29.41, 29.52, 31.76, 36.47, 48.41, 48.72, 49.64, 50.37, 52.11, 52.30, 55.49, 56.30, 59.41, 68.09, 68.47, 81.91, 82.21, 82.28, 172.42, 172.54, 172.58.

General procedure for the synthesis of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (4) (Scheme 2). 1,4,7-Tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (**9**) in trifluoroacetic acid was stirred under an argon atmosphere overnight at ambient temperature before the trifluoroacetic acid was removed *in vacuo*. The residue was dissolved in water and evaporated *in vacuo*. This process was repeated three times. The residue was dissolved in water and applied to a column containing poly(4-vinylpyridine) macroreticular (Reillex 425) resin. The column was eluted with water and the fractions containing the product were combined and concentrated *in vacuo*. White crystalline materials were obtained by freeze-drying overnight.

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (HB-DO3A) (**4a**) (Scheme 2).* Reaction of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**9a**) (0.73 g, 1.25 mmol) and trifluoroacetic acid (5 ml) was performed according to the general procedure. Yield: 0.70 g (95%). Anal. Calcd. (found) for $C_{18}H_{34}N_4O_7$: C, 51.67 (51.92); H, 8.13% (8.37%).

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (HO-DO3A) (**4b**) (Scheme 2).* Reaction of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**9b**) (0.83 g, 1.3 mmol) and trifluoroacetic acid (6 ml) was performed according to the general procedure. Yield: 0.66 g (98%). Anal. Calcd. (found) for $C_{22}H_{42}N_4O_7 \cdot 2.5H_2O$: C, 50.87 (50.93); H, 9.06% (9.00%).

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (HDD-DO3A) (**4c**) (Scheme 2).* Reaction of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**9c**) (5.24 g, 7.5 mmol) and trifluoroacetic acid (30 ml) was performed according to the general procedure. Yield: 3.89 g (84%). Mass spectrum (ES^+): *m/z* 531 [$[M + H]^+$, $C_{26}H_{51}N_4O_7$]. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, 6.9 Hz, 3H), 1.26–1.44 (m, 19H), 2.50–3.41 (m, 24H), 3.72 (s, 1H), 7.74 (s (br), 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.40, 21.63, 24.55, 28.26, 28.64, 28.82, 30.89, 35.40, 49.75, 50.20, 50.66, 55.30, 55.55, 59.29, 66.23, 172.26, 172.57. Anal. Calcd. (found) for $C_{26}H_{50}N_4O_7 \cdot 5.0H_2O$: C, 50.32 (50.64); H, 9.68% (9.80%).

*1,4,7-Tris(carboxymethyl)-10-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (HHD-DO3A) (**4d**) (Scheme 2).* Reaction of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**9d**) (1.54 g, 2.0 mmol) and trifluoroacetic acid (10 ml) was performed according to the general procedure. Yield: 1.10 g (91%). Mass spectrum (ES^+): *m/z* 587 [$[M + H]^+$, $C_{30}H_{59}N_4O_7$]. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, 6.5 Hz, 3H), 1.25–1.42 (m, 28H), 2.80–3.92 (m, 24H), 3.70 (s, 2H), 3.92 (s, 1H), 10.03 (s (br), 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.33, 21.57, 24.17, 28.20, 28.54, 28.59, 28.64, 30.83, 34.92, 47.50, 48.96, 51.39, 51.68, 53.90, 54.25, 58.03, 64.73, 169.96, 171.72. Anal. Calcd. (found) for $C_{30}H_{58}N_4O_7 \cdot 1.2H_2O$: C, 59.25 (59.10); H, 9.94% (9.50%).

General procedure for the synthesis of gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (5) (Scheme 2). A suspension of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (**4**) (1.0 equiv.) and Gd_2O_3 (0.5 equiv.) in water was heated at 90 °C for the designated time. The solution was cooled to ambient temperature and to it were added cation-exchange resin (Amberlite IR 120/H^+-form) and anion-exchange resin (Amberlite IRA 67/OH^--form). After stirring for 30 min at ambient temperature, the resin was collected by filtration (Millipore GSWP 0.22 μm) and the filtrate was evaporated *in vacuo*. White crystalline materials were obtained by freeze-drying overnight.

Table 1 CMC, log *P*, and T_1 relaxivity (r_1) for the gadolinium complexes

Compound	CMC/mol l^{-1}	log P [a]	T_1 relaxivity (r_1)/ s^{-1} mM^{-1} [b]
Gd–HP-DO3A	—	−3.68 [c]	3.9 [d]
Gd–HB-DO3A (**5a**)	—	−3.5	3.9
Gd–HO-DO3A (**5b**)	>2.0E-2	−1.8	4.0
Gd–HDD-DO3A (**5c**)	2.0E-3	1.9	4.6
Gd–HHD-DO3A (**5d**)	1.0E-4	3.7	9.2

[a] P = partition coefficient between octan-1-ol and water. [b] The T_1 relaxivities (r_1) are given for concentrations below CMC at 39 °C and 0.47 Tesla. [c] Ref. 15. [d] Ref. 16.

*Gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (Gd–HB-DO3A) (**5a**) (Scheme 2).* Reaction of 1,4,7-tris(carboxymethyl)-10-(2-hydroxybutyl)-1,4,7,10-tetraazacyclododecane (**4a**) (0.63 g, 1.5 mmol) and Gd_2O_3 (0.27 g, 0.75 mmol) in water (10 ml) was performed according to the general procedure. The reaction mixture was stirred for 6 h. Yield: 0.77 g (84%). Mass spectrum (ES^+): *m/z* 574 [$[M + H]^+$, $C_{18}H_{32}N_4O_7Gd$]. Anal. Calcd. (found) for $C_{18}H_{31}N_4O_7Gd \cdot 2.0H_2O$: C, 35.47 (35.70); H, 5.75% (5.63%). ICP-AES Calcd. Gd-conc. (found): 2.0 mM (1.87 mM).

*Gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (Gd–HO-DO3A) (**5b**) (Scheme 2).* Reaction of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyoctyl)-1,4,7,10-tetraazacyclododecane (**4b**) (0.48 g, 1 mmol) and Gd_2O_3 (0.18 g, 0.5 mmol) in water (7.5 ml) was performed according to the general procedure. The reaction mixture was stirred for 6 h. Yield: 0.52 g (75%). Mass spectrum (ES^+): *m/z* 630 [$[M + H]^+$, $C_{22}H_{40}N_4O_7Gd$]. Anal. Calcd. (found) for $C_{22}H_{39}N_4O_7Gd \cdot 3.5H_2O$: C, 38.15 (38.19); H, 6.65% (6.62%). ICP-AES Calcd. Gd-conc. (found): 2.0 mM (1.79 mM).

*Gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (Gd–HDD-DO3A) (**5c**) (Scheme 2).* Reaction of 1,4,7-tris(carboxymethyl)-10-(2-hydroxydodecyl)-1,4,7,10-tetraazacyclododecane (**4c**) (2.65 g, 5 mmol) and Gd_2O_3 (0.91 g, 2.5 mmol) in water (25 ml) was performed according to the general procedure. The reaction mixture was stirred overnight. Yield: 2.75 g (75%). Mass spectrum (ES^+): *m/z* 686 [$[M + H]^+$, $C_{26}H_{48}N_4O_7Gd$]. Anal. Calcd. (found) for $C_{26}H_{47}N_4O_7Gd \cdot 2.6\ H_2O$: C, 42.63 (42.63); H, 7.13% (7.09%). ICP-AES Calcd. Gd-conc. (found): 10 mM (8.33 mM).

*Gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (Gd–HHD-DO3A) (**5d**)* (Scheme 2). Reaction of 1,4,7-tris(carboxymethyl)-10-(2-hydroxyhexadecyl)-1,4,7,10-tetraazacyclododecane (**4d**) (0.59 g, 1 mmol) and Gd_2O_3 (0.18 g, 0.5 mmol) in water (10 ml) was performed according to the general procedure. The reaction mixture was stirred overnight. Yield: 0.62 g (75%). Mass spectrum (ES^+): *m/z* 742 [$[M + H]^+$, $C_{30}H_{56}N_4O_7Gd$]. Anal. Calcd. (found) for $C_{30}H_{55}N_4O_7Gd \cdot 4.5H_2O$: C, 43.80 (44.04); H, 7.79% (7.55%). ICP-AES Calcd. Gd-conc. (found): 1 mM (0.72 mM).

Critical micelle concentration and micelle size

The critical micelle concentration (CMC) was determined by preparing five-fold geometric serial dilutions from 0.02 M of the Gd complexes Gd–HO-DO3A (**5b**), Gd–HDD-DO3A (**5c**), and Gd–HHD-DO3A (**5d**). A ring tensiometer (Krüss GmbH, Germany) was employed and the surface tension was measured for each solution at 23 °C. The CMC of each Gd complex was obtained by plotting the surface tension against the logarithm of the concentration. Additional surface tensions were determined around the CMC for each complex to obtain improved values. The CMC values are given in Table 1.

For the determination of micelle size, solutions at a concentration 50 times that of the CMC values were used. This

corresponds to 5.0×10^{-2} and 1.0×10^{-2} M of complex **5c** and **5d**, respectively. The micelle diameter was measured at 25 °C by photon correlation spectroscopy at a scattering angle of 90° (Coulter DELSA 440®, Coulter Electronics Ltd, USA).

Partition coefficients

The partition coefficients for the Gd complexes were determined by a traditional shake-flask method. A 0.02 M (2 ml) aqueous solution of the Gd complex was mixed with octan-1-ol (2 ml) in a test tube. The test tube was vortexed for 2 min before it was centrifuged (Minifuge T, Heraeus Instruments GmbH, Germany) for 30 min at 5000 rpm to ensure complete separation of the phases. Samples from each phase were analysed in triplicate by HPLC to find the respective concentrations of the complexes. Due to high water solubility of Gd–HB-DO3A (**5a**) and high octan-1-ol solubility of Gd–HHD-DO3A (**5d**) these log *P* values were measured by using water–octan-1-ol (10 ml) in the ratio 10:90 and 90:10, respectively. The partition coefficients (*P*) values were calculated from eqn. (1), where

$$P = \frac{[\text{Gd-complex}]^{\text{octan-1-ol}}}{[\text{Gd-complex}]^{\text{water}}} \quad (1)$$

$[\text{Gd-complex}]^{\text{octan-1-ol}}$ and $[\text{Gd-complex}]^{\text{water}}$ are the concentrations of the Gd complex in the octan-1-ol and water phase, respectively. The log *P* values are given in Table 1.

High performance liquid chromatography analyses

HPLC analyses of the Gd complexes were performed using a Hewlett Packard 1050 pump (Hewlett Packard GmbH, Germany) connected to a Purospher RP-18 (Merck KGaA, Germany) reversed phase column (12.5 × 0.46 cm, 5 μm). Integration of the peaks was carried out using a Hewlett Packard Chemstation and a Hewlett Packard diode UV-detector for detection (205 nm). Typical eluents consisted of 10–47.5% acetonitrile in aqueous buffer (potassium hydrogen phosphate, pH 7.0). The flow rate was 1.5 ml min^{-1}.

In vitro relaxometry

The relaxation measurements were performed at 0.47 T (Minispec PC-120b, Brüker GmbH, Germany). The T_1 relaxation rates (R_1) were obtained by the inversion recovery method at 39 °C. T_1 relaxation times were recorded for Gd–HDD-DO3A (**5c**) and Gd–HHD-DO3A (**5d**) in the concentration ranges 1.0×10^{-2}–1.0×10^{-4} and 1.0×10^{-2}–1.0×10^{-5} M, respectively. The T_1 relaxivity (r_1) of each chelate was obtained by the relationship given in eqn. (2), where R_1^{obs}, R_1^{m} are the relaxation

$$r_1 = \frac{R_1^{\text{obs}} - R_1^{\text{m}}}{C} \quad (2)$$

rates (s^{-1}) of the sample and the matrix (water), respectively, and *C* is the Gd concentration (mM). The r_1 values of each dilution were plotted against the Gd concentration to give the concentration dependent relaxivity relationship, as shown in Fig. 1. The r_1 values for Gd–HB-DO3A (**5a**) and Gd–HO-DO3A (**5b**) were obtained from a linear least squares regression analysis of the relaxation rate (R_1) *vs. C.* Analyses were performed in triplicate at each concentration. The values are given in Table 1.

Results and discussion

Synthesis

Reactions between epoxides and DO3A (**3**) have been widely used in the synthesis of 10-(2-hydroxyalkyl)-DO3A derivatives, and have been successfully employed in the case of gadolinium 1,4,7-tris(carboxymethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (Gd–HP-DO3A).[9] The reactions between DO3A and epoxides were performed as described in Scheme 1, using either water or a mixture of water and methanol as the solvent. The outcome of the reaction depended on several factors such as the epoxide, solvent and temperature. While HB-DO3A (**4a**) was isolated in moderate yields after purification on an anion-exchange column when the reaction was carried out in water, the preparation of HO-DO3A (**4b**) failed under the same conditions. To avoid the heterogeneous reaction conditions that were formed in the latter attempt, a mixture of methanol and water was used in the ratio 1:2. Together with an increase in reaction temperature from ambient temperature to 50 °C, complete disappearance of the DO3A (**3**) was reached after 72 h and compound **4b** was obtained, although in low yields. However, the preparation of HDD-DO3A (**4c**) under the same reaction conditions was unsuccessful. The methanol content was increased again until the mixture turned homogeneous. Even though product formation was observed by TLC, the reaction rate was slow and the reaction mixture still contained unreacted DO3A after 9 days. No attempts were made to isolate the product from the reaction mixture.

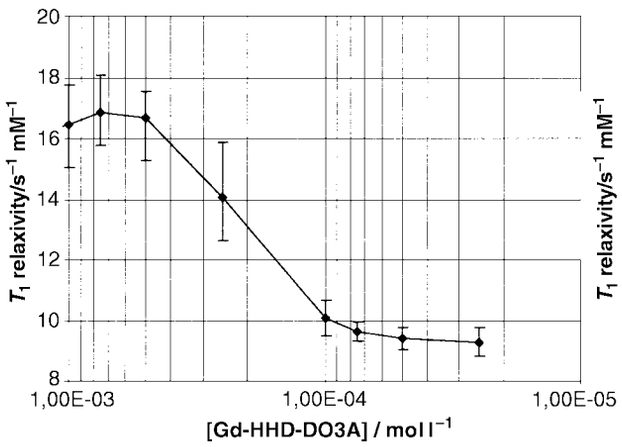


Fig. 1 Concentration dependent T_1 relaxivity (r_1) of Gd–HHD-DO3A (**5d**).

A second route to 10-(2-hydroxyalkyl)-DO3A compounds (**4**) developed by Platzek *et al.*[10] was used according to Scheme 2. Dimethylformamide dimethyl acetal was reacted with cyclen (**1**) giving 1,4,7,10-tetraazatricyclo[5.5.1.0]tridecane (**6**) in essentially quantitative yields.[11,12] The reactions between the epoxides and the protected cyclen were performed neat at 120 °C and 1,7-disubstituted cyclen derivatives (**7**) were isolated upon hydrolysis with aqueous methanol in 40–72% yield. The number of ^{13}C NMR signals obtained for these compounds was different from what is expected for the symmetric compounds. This can be explained by restricted rotation around the formamide bond that gives rise to different conformations. The formyl groups were subsequently removed by alkaline treatment in methanol–water to give the cyclen derivatives (**8**). The carboxymethylation of the free nitrogens was carried out by the use of *tert*-butyl bromoacetate in THF–water. Cleavage of the *tert*-butyl esters with trifluoroacetic acid yielded the 10-(2-hydroxyalkyl)-DO3A derivatives (**4**) in 20–39% yield from cyclen (**1**). DO3A derivatives made by both methods showed the same characteristics in NMR and MS.

The Gd complexes of **4** were prepared in high yield by the use of Gd_2O_3 in water at 90 °C. Due to zero net charge of the Gd complexes, ionic impurities were removed by stirring the reaction mixture with anion- and cation-exchange resins.

Crystalline material with high purity according to HPLC was obtained by freeze-drying.

Critical micelle concentration (CMC) and micelle size

In order to demonstrate formation of colloidal aggregates in aqueous solutions, the surface tension was measured at different concentrations. While Gd–HO-DO3A (**5b**) appeared to be a poor surfactant, Gd–HDD-DO3A (**5c**) and Gd–HHD-DO3A (**5d**) showed decreasing surface tension down to approximately 37 and 40 mN m^{-1} at 2.0×10^{-3} and 1.0×10^{-4} M, respectively. These results indicate the formation of colloidal aggregates as micelles and experiments were performed to determine the micelle size.

Micelle populations are often polydisperse, and the shape of the individual micelles varies with concentration.[13] Consequently, the measured values should only be considered as guidelines. Micellar Gd–HDD-DO3A (**5c**) was found to be in the range 6–11 nm ($n = 3$), while micellar Gd–HHD-DO3A (**5d**) was measured to be between 11–23 nm ($n = 3$). All measurements showed a broad size distribution. The difference in micelle size between the two compounds is as expected for non-ionic amphiphiles, when increasing the lipophilic chain length.

Partition coefficients

The log *P* values for the Gd complexes are given in Table 1. Partition coefficients obtained by the shake-flask method give log *P* values within 0.3 units.[14] The value of Gd–HB-DO3A (**5a**) indicates that the compound is somewhat more hydrophobic than Gd–HP-DO3A (Prohance®).[15] The value of Gd–HB-DO3A (**5a**) and Gd–HHD-DO3A (**5d**) should be considered less accurate due to the low concentrations in the octan-1-ol and the water phase, respectively.

In vitro relaxometry

The relaxivity values are given in Table 1. Gd–HB-DO3A (**5a**) and Gd–HO-DO3A (**5b**) showed expected relaxivities for water soluble Gd complexes, such as Gd–HP-DO3A.[16] Below CMC the relaxivities of Gd–HDD-DO3A (**5c**) and Gd–HHD-DO3A (**5d**) were higher than anticipated; especially for compound **5d**. This increase in relaxivity can be accounted for by two effects. 1) Increased rotational correlation time (τ_R) caused by an increase in the molecular weight of the compounds. 2) Cluster formation of the amphiphilic Gd-chelates giving a further increase in τ_R.[17] The compounds are currently being investigated to verify this explanation, and the results will be reported separately. When the concentration of Gd–HHD-DO3A (**5d**) is raised, an increase in relaxivity is observed that is consistent with the formation of micelles (Fig. 1). A similar effect was also observed for Gd–HDD-DO3A (**5c**). High relaxivity above CMC has recently been reported for another amphiphilic gadolinium chelate.[18]

Conclusion

Two methods have been employed in the syntheses of new 1,4,7-tris(carboxymethyl)-10-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane (**4**) with different chain lengths. Upon reaction with gadolinium oxide, the ligands gave neutral complexes and Gd–HDD-DO3A (**5c**) and Gd–HHD-DO3A (**5d**) were shown to have amphiphilic properties. The relaxivities were measured and showed to be concentration dependent, corresponding to micelle formation.

Further studies of the amphiphilic Gd-chelates will include liposomal formulation and the effect of different phospholipids and liposome size on relaxivity to optimise the liposome composition.

Acknowledgements

This work was financially supported by the EU in the framework of the EU-BIOMED program (MACE) and the COST D8 action. The contributions of F. K. Hansen and J. Vedde, Department of Chemistry, and G. Stensrud, School of Pharmacy, University of Oslo, are gratefully acknowledged by the authors.

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