Synthesis, Characterization, and Crystal Structure of the Gadolinium(III) Chelate of (1*R*,4*R*,7*R*)-α,α′,α″-Trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid (DO3MA)

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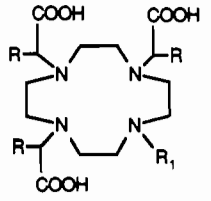
Received November 24, 1992

The tetraazatricarboxylic macrocycle, (1*R*,4*R*,7*R*)-α,α′,α″-trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3MA) (**4**) was synthesized by the simultaneous hydrogenolysis and deformylation of 10-formyl-1,4,7-tris(benzyloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (N-CHO-DO3MA-TBE) (**11**). Chelation of DO3MA with gadolinium acetate resulted in a diastereomerically pure Gd(III) chelate, Gd(DO3MA) (**4a**). X-ray structure analysis of Gd(DO3MA) crystals revealed that the three asymmetric carbons bearing α-methyl groups all have the (*R*)-configuration and that the chelate crystallizes as a dimer, [(DO3MA)Gd][(DO3MA)Gd(H_2O)$_2$], in which both the Gd atoms are *ennea*coordinate. Crystal data are as follows: [2($GdC_{17}H_{29}N_4O_6$)·2H_2O]·4H_2O, a = 17.471(6) Å, b = 25.495(6) Å, c = 10.146(3) Å, V = 4520(4) Å^3, $P2_12_12_1$, Z = 4, R = 0.035, R_w = 0.044. The relaxivity of Gd(DO3MA) was found to be 4.4 ± 0.1 mM^{-1} s^{-1} at 20 MHz and 40 °C. The measured stability constant of Gd(DO3MA) was log K_{eq} = 25.3, which makes Gd(DO3MA) the most stable heptadentate chelate known for Gd(III).

Introduction

Paramagnetic metal chelates catalyze the relaxation of water protons in living tissue, thus affecting the regional signal intensity of magnetic resonance images.[1,2] Recent research has focused on the design of linear and cyclic amino carboxylate ligands specifically for the gadolinium(III) cation because Gd(III) provides the shortest longitudinal relaxation time of coordinated water.[3] The work has led to successful clinical applications of Gd(DTPA)$^{2-}$, Gd(DOTA)$^-$, Gd(HP-DO3A), and Gd(DTPA-BMA) as contrast media.[4-8]

The results of our endeavor to develop nonionic gadolinium chelates built on DO3A (**1**) were recently communicated.[6] We described the synthesis of DO3A, its derivatives and their Gd(III) chelates, and the facile functionalization of the unique nitrogen on heptadentate DO3A, rendering R-DO3A (**3**) ligands potentially octadentate. The present work was stimulated by Brittain and Desreux's report on the luminescence and NMR studies of lanthanide complexes of DOTMA (**5**).[9] The authors reported that the Eu(III) and Tb(III) chelates of DOTMA were more rigid than those of DOTA (**2**), with the conformation of the ethylenediamine groups of the macrocyclic ring undergoing very slow inversion between 0 and 100 °C, demonstrating that the introduction of the α-methyl groups increased the rigidity of the amino carboxylate macrocyclic framework. Our goal was to determine the effect of additional rigidity on the stability, lability and relaxivity of an α-methyl analog of DO3A, DO3MA (**4**). In



DO3A (R = H, R_1 = H) (**1**)
DOTA (R = H, R_1 = CH_2COOH) (**2**)
HP-DO3A (R = H, R_1 = $CH_2CH(CH_3)OH$) (**3**)
DO3MA (R = CH_3, R_1 = H) (**4**)
DOTMA (R = CH_3, R_1 = $CH(CH_3)COOH$) (**5**)

this paper we report the synthesis of DO3MA, Gd(DO3MA), Y(DO3MA), and DOTMA. We also report determination of the thermodynamic stability constant, acid-assisted dissociation rate, relaxivity, and crystal structure of Gd(DO3MA) and the thermodynamic stability constant of Y(DO3MA).

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra, mass spectra, and microanalytical data were provided by the Analytical Department within the Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ. In the case of microanalyses, Karl Fisher (KF) analyses by either desorption or dissolution and residue on ignition (ROI) analyses were also performed to determine the water content and the amount of residual substances, respectively. Reagents were obtained from Aldrich or Sigma. ^{1}H- and ^{13}C-NMR spectra were determined on a Jeol 270-MHz GSX FT NMR spectrometer. HPLC grade solvents were obtained from Aldrich, Fisher Scientific, EM Science, or Mallinckrodt, and used without further purification unless otherwise noted. *N*-Formylcyclen[6]

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(1) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901.
(2) Tweedle, M. F. In *Lanthanide Probes in Life, Chemical, and Earth Sciences*; Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 5.
(3) Pople, J. A.; Schneider, W. G.; Bernstein, H. J. *High-Resolution Nuclear Magnetic* Resonance; McGraw-Hill: New York, 1959.
(4) Weinman, H.-J.; Brasch, R. C.; Press, W.-R.; Wesbey, G. E. *Am. J. Roengentol.* **1984**, *142*, 619.
(5) Magerstadt, M.; Gansow, O.; Brechbiel, M. W.; Colcher, D.; Baltzer, L.; Knop, R. H.; Girton, M. E.; Naegele, M. *Magn. Reson. Med.* **1986**, *3*, 808.
(6) Dischino, D. D.; Delaney, E. J.; Emswiler, J. E.; Gaughan, G. T.; Prasad, J. S.; Srivastava, S. K.; Tweedle, M. F. *Inorg. Chem.* **1991**, *30*, 1265.
(7) (a) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* 1990, *8*, 467. (b) Quay, S. C. U.S. Patent No. 4,687,659, 1987.
(8) Abbreviated names for the compounds are DTPA = diethylenetriaminepentaacetic acid, DTPA-BMA = *N*,*N*″-bis(methylcarbamoylmethyl)-*N*,*N*′,*N*″-tris(carboxymethyl)diethylenetriamine, DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, HP-DO3A = 1-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, DO3MA = α,α′,α″-trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, DOTMA = α,α′,α″,α‴-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, N-CHO-DO3MA-TBE = 1,4,7-tris(benzyloxycarbonyl)methyl-10-formyl-1,4,7,10-tetraazacyclododecane, NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid, and NOTMA = α,α′,α″-trimethyl-1,4,7-triazacyclononane-1,4,7-triacetic acid.

(9) Brittain, H.; Desreux, J. F. *Inorg. Chem.* **1984**, *23*, 4459.

0020-1669/93/1332-2912$04.00/0

was kindly provided by the Chemical Process Technology Department of the Bristol-Myers Squibb Pharmaceutical Research Institute, Chemical Division, New Brunswick, NJ. Gadolinium oxide (99.99%) and gadolinium acetate hydrate (99.9%) were obtained from Molycorp, Inc., White Plains, NY, and Aldrich, Milwaukee, WI, respectively. Analytical grade ion exchange resins, AG50W-X8 (100–200 mesh, proton form) and AG1-X8 (100–200 mesh, chloride form) were obtained from Bio-Rad Laboratories, Richmond, CA. DEAE Sephadex A-25 resin was obtained from Pharmacia LKB Biotechnology Inc., Piscataway, NJ. MCI GEL CHP20P resin (75–150 μm) was purchased from Mitsubishi Kasei Co. All resins were regenerated prior to use following the manufacturer's recommended procedures. Distilled–deionized water was obtained from a Millipore Super Q purification system (10 MΩ cm) and was used throughout to minimize trace-metal contamination of the ligands and chelates.

To monitor the progress of reactions and to evaluate the purity of both ligand and chelate products analytical HPLC methods were extensively employed. HPLC analyses were performed on a Shimadzu liquid chromatograph, Model LC-6A, equipped with a Shimadzu UV spectrophotometric detector or a Shimadzu CR 601 chromatopac data processor, a Rainin gradient HPLC system using the Dynamax HPLC Method Manager program combined with an ABI Analytical Kratos Division Spectroflow 757 absorbance detector and a Hitachi F1000 fluorescence spectrophotometer, and an Altex Model 110A HPLC pump equipped with a Kratos Spectroflow 757 absorbance detector. HPLC columns were obtained from Hamilton (PRP-x100, 4.1 × 250 mm), Machery-Nagel (C_{18} Nucleosil, 4.0 × 250 mm), or Rainin Instrument Co. (Polymer Laboratories, PLRP-S, 4.6 × 250 mm). A radiometer PHM82 Standard pH Meter with a combination electrode and Radiometer CDM83 conductivity meter equipped with a CDC314 standard flow-through cell were used to monitor fractions from ion exchange column chromatography.

L-Benzyl Lactate (8). The literature procedure described by Escher and Bünning[10] was used to prepare L-benzyl lactate. L-Lactic acid (98% from Sigma, 10 g, 111 mmol) dissolved in ethyl acetate (30 mL) containing triethylamine (11.35 g, 112 mmol) was treated with benzyl chloride (14.05 g, 111 mmol)[11] in ethyl acetate (10 mL) at ambient temperature for 1.5 h. The resulting solution was kept at reflux for 4.7 h. The 4.7-h aliquot, which was analyzed by a Hamilton PRP-x100 HPLC column, showed complete consumption of benzyl chloride. The mixture was filtered to remove the triethylammonium salt, and the filtrate was then concentrated to dryness. To the residue was added ether (75 mL) and sodium bicarbonate (10 g) dissolved in water (150 mL). The separated ether layer was extracted with saturated brine (50 mL) and water (50 mL). The organic layer was separated, and dried with sodium sulfate. Removal of the solvent gave the analytically-pure product, L-benzyl lactate **8** (14.5 g, 80.6 mmol), in 73% yield. HPLC: retention time, 3.6 min (purity 97.1% integrated intensity); RPR-x100 4.6 × 250 mm; eluent, 60% acetonitrile in 20 mM sodium phosphate (pH 7.0); flow rate, 1 mL/min; UV, 254 nm. IR (neat): 3450, 3035, 2985, 2940, 1740 (ester), 1215, 1129. ^{1}H-NMR ($CDCl_3$): δ 1.54 (3H, doublet, methyl); 2.92 (1H, singlet, OH); 4.40 (1H, quartet, methine); 5.53 (2H, singlet, methylene); 7.44-7.52 (5H, multiplet, aromatic protons). MS (CI/NH_3): m/z 198 [(M + NH_4^+)$^+$, base peak]; 181 [(M+H)$^+$]; 108 [(M^+ – benzyl)]. Anal. Calcd for $C_{10}H_{12}O_3 \cdot 0.07H_2O$: C, 66.20; H, 6.74. Found: C, 66.35; H, 6.77; H_2O, 0.68% (dissolution Karl Fisher).

L-Benzyl 2-Triflyloxypropionate (9). A literature procedure describing (*S*)-ethyl 2-triflyloxypropionate[12] was used to prepare the triflate **9**. A solution of L-benzyl lactate (**8**) (68.3 g, 354 mmol) and pyridine (30.1 mL, 372 mmol) in methylene chloride (300 mL) at 0 °C was treated with triflic anhydride (100 g, 354 mmol) in methylene chloride (50 mL) for 1 h. After removal of the pyridinium triflate salt by filtration, the crude product was purified by silica gel (63.5 g) chromatography to obtain L-benzyl 2-(triflyloxy)propionate (**9**) (2.44 g, 31%). TLC: R_f 0.65 in dichloromethane and hexanes (1:1). IR (neat): 1763, 1420, 1213, 1146, 953. ^{1}H-NMR ($CDCl_3$): 1.74 (3H, doublet, methyl); 5.30 (2H, singlet, methylene); 5.31 (1H, quartet, methine); 7.40–7.45 (5H, multiplet, aromatic protons). MS (CI/NH_3): m/z 330 [(M + NH_4)$^+$, base peak].

1,4,7-Tris(benzyloxycarbonyl)methyl-10-formyl-1,4,7,10-tetraazacyclododecane (N-CHO-DO3MA-TBE (11). 1-Formyl-1,4,7,10-tetraazacyclododecane (**10**) (*N*-formylcyclen, 12.8 g, 64 mmol) was reacted with the triflate **9** (60 g, 192 mmol) in acetonitrile in the presence of diisopropylethylamine for 2.5 h. The crude product obtained after removal of the solvent was extracted with water (250 mL) and chloroform (2 × 150 mL). The organic layers were combined and dried over magnesium sulfate. Removal of the solvent afforded an orange residue. This crude product was purified by silica gel column chromatography to obtain the tribenzyl ester N-CHO-DO3MA-TBE (**11**) (23.7 g) in 63% yield. TLC: R_f 0.34 in EtOAc and hexanes (1:1). HPLC: retention time, 12.4 min; PRP-X100 4.6 × 250 mm; eluent, 75% acetonitrile in 10 mM sodium phosphate buffer, pH 7.0; flow rate, 1.2 mL/min; UV, 254 nm. IR (neat): 2981, 2940, 2840, 1730, 1670, 1456, 1374, 1142 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.2–1.8 (9H, multiplet, methyls); 2.3–4.3 (19H, multiplet, ring methylenes and methines); 5.0–5.2 (6H, multiplet, $COOCH_2C_6H_5$); 7.3 (15H, multiplet, aromatic protons); 7.9–8.1 (1H, multiplet, N-CHO). ^{13}C-NMR ($CDCl_3$): δ 14.94, 15.06, 15.16 (methyls); 42.03, 47.32, 48.76, 49.37, 49.56, 50.13, 50.99, 52.66 (ring methylenes); 55.58, 58.76, 60.02 (methines); 65.83, 65.94, 66.05 ($COOCH_2C_6H_5$); 128.07, 128.15, 128.25, 128.36, 128.44, 128.48 (C_{2-6}); 135.68, 135.77, 135.80 (C_1); 162.71 (CHO); 173.02, 173.12, 173.17 (COO). MS: m/z 687 [(M + H)$^+$, base peak]. Anal. Calcd for $C_{39}H_{50}N_4O_7 \cdot 0.05H_2O$: C, 68.12; H, 7.34; N, 8.15. Found: C, 67.84; H, 7.40; N, 7.81, H_2O, 0.12.

α,α′,α″-Trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid, Monotriethylammonium Salt (12). The tribenzyl ester N-CHO-DO3MA-TBE (**11**) (21.6 g, 36.5 mmol) was hydrogenated overnight in 30% aqueous methanol containing 2 M HCl (129 mL of methanol and 56 mL of 2 M HCl) in the presence of Pd/carbon (10% Pd, 4.32 g). The catalyst was filtered off, and evaporation of the filtrate gave an off-white solid material. The crude product was purified by AG50W-X8, followed by DEAE Sephadex A-25 ion-exchange chromatography. Water and 1.0 M ammonium hydroxide were used as eluents in the former, and 5–500 mM triethylammonium bicarbonate solutions were employed in the latter. Removal of the triethylammonium bicarbonate and water *in vacuo* gave the ligand DO3MA as the monotriethylammonium salt **11**. HPLC: method 1; retention time, 6.7 min (purity 99.7 %); PLRP-S 4.6 × 250 mm; eluent, 5% acetonitrile in 50 mM sodium phosphate (pH 4.8); flow rate, 1.0 mL; UV, 290 nm. Copper chelation.[6] HPLC: method 2; retention time, 4.4 min (purity, 99.5%); PRP-x100 4.6 × 250 mm; eluent, 2% acetonitrile in 50 mM sodium phosphate buffer (pH 7.0); flow rate, 1.0 mL; UV, 220 nm. IR (KBr): 3429, 2978, 1593, 1458, 1396 cm^{-1}. ^{1}H-NMR (D_2O): 1.14 (21H, triplet, methyl of triethylamine); 1.35 (9H, doublet, methyl of propionate); 2.5–3.8 (65H, multiplet, methylenes of the ring and triethyl group [3.07 (quartet, methylenes of triethyl)]); 4.40 (3H, quartet, methine). ^{13}C-NMR (D_2O): 8.02 (methyl of triethyl); 10.68 (methyl of propionate); 43.23, 45.74, 47.66 (ring methylenes); 46.39 (methylene of triethyl); 59.82 (methine) 172.94, 179.12 (carboxylates). MS (FAB): m/z 389 [(M + H)$^+$, base peak]; 387 [(M – H)$^-$]. Anal. Calcd for $C_{23}H_{47}N_5O_6$ [DO3MA·triethylamine]·0.83 H_2O: C, 54.75; H, 9.72; N, 13.88. Found: C, 54.65; H, 9.72; N, 14.01; H_2O, 2.96; ROI, 0.07.

(1*R*,4*R*,7*R*)-α,α′,α″-Trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid, DO3MA (4). The DO3MA monotriethylammonium salt **12** was loaded onto an AG1-X8 anion-exchange column (formate form). The column was eluted with formic acid. Removal of water and formic acid gave a quantitative yield of DO3MA (**4**). HPLC: method 1; retention time, 6.7 min (purity 99.7%); PLRP-S 4.6 × 250 mm; eluent, 5% acetonitrile in 50 mM sodium phosphate (pH 4.8); flow rate, 1.0 mL; UV, 290 nm; copper chelation.[6] HPLC: method 2; retention time, 4.4 min (purity, 99.5%); PRP-x100 4.6 × 250 mm; eluent, 2% AcCN in 50 mM sodium phosphate buffer (pH 7.0); flow rate, 1.0 mL; UV, 220 nm. IR (KBr); 3435, 2984, 1717, 1630, and 1389 cm^{-1}. ^{1}H-NMR (D_2O): 1.21 (d, 6H, α and α″ methyls); 1.37 (d, 3H, α′ methyl), 2.5–4.2 (19H, m, ring methylenes and methines). MS (FAB): m/z 389 [(M + H)$^+$, base peak]; 387 [(M – H)$^-$, base peak]. Anal. Calcd for $C_{17}H_{32}N_4O_6 \cdot 0.36H_2O$: C, 51.70; H, 8.35; N, 14.19. Found: C, 51.77; H, 8.48: N, 14.07; H_2O, 1.64 (desorption KF); ROI, 0.00.

(1*R*,4*R*,7*R*)-α,α′,α″-Trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid, Monogadolinium Salt, Gd(DO3MA) (4a). Method 1. The free acid DO3MA (**4**) (416 mg, 1.23 mmol) was treated with gadolinium oxide (Molycorp, 99.99%, 245 mg, 0.676 mmol) in water (6 mL) at 80 °C for 18 h. The pH of the final solution was 8.0. This slightly turbid solution was filtered through an Acrodisc (LC 13 PVDF, 0.2 μm) to remove unreacted gadolinium oxide. Evaporation of the water from the filtrate gave the analytically pure monogadolinium salt of Gd(DO3MA) (660 mg) in 99% yield. The product **4a** was recrystallized from 2-propanol (15 mL). HPLC: retention time, 9.9 min; C_{18} Nucleosil 4.6 × 250 mm;

(10) Escher, R.; Bünning, P. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 277.
(11) One equivalent of benzyl chloride was used instead of 0.9 equiv as given in the ref. 10.
(12) Effenberger, F.; Burkard, U.; Willfahrt, J. *Liebigs Ann. Chem.* **1986**, 314.

eluent, 8% acetonitrile in 50 mM tris-acetate and 10 mM EDTA buffer (pH 7.0); flow rate, 1.0 mL; spectrofluorophotometer, 280-nm excitation, 320-nm emission. No free ligand DO3MA (**4**) was detected (<0.1 mol %) by the following HPLC method: PRP-x100 4.6 × 250 mm; 10% acetonitrile in 50 mM sodium phosphate buffer (pH 7.0); flow rate, 1 mL/min; UV, 330 nm. (cf. retention time of $Cu(DO3MA)^-$ of 4.2 min.) IR (KBr): 3439, 1625, 1381. MS (FAB): m/z 544 [$(M + H)^+$, ^{158}Gd]. Anal. Calcd for $C_{17}H_{29}N_4GdO_6 \cdot 1.72H_2O \cdot 0.30(CH_3)_2CHOH$: C, 36.34; H, 5.93; N, 9.47. Found: C, 35.95; H, 6.14; N, 9.03: H_2O, 5.41; $(CH_3)_2CHOH$, 3.05.[13]

Method 2. The DO3MA triethylammonium salt (**12**) (10.51 g, 21.5 mmol) was reacted with gadolinium acetate, $Gd(OAc)_3 \cdot 4H_2O$ (10.16 g, 25 mmol), at 60 °C in water (100 mL). The reaction mixture was maintained at pH 5.0 by the addition of 1.0 N NaOH and stirred for 3.25 h. The resulting solution was loaded onto a CHP20P resin column and the desired product eluted with 10% ethanol in water. Removal of the aqueous ethanol from the fractions containing the chelate gave Gd(DO3MA) (**4a**) as a white solid (8.45 g, 15.6 mmol) in 72% yield. Results of HPLC and IR analyses on the chelate were in agreement with those for the product described in method 1. Anal. Calcd for $C_{17}H_{29}N_4GdO_6 \cdot 1.15H_2O$: C, 36.24; H, 5.60; N, 9.94. Found: C, 36.25; H, 5.71; N, 9.88: H_2O, 3.65.

Recrystallization by slow evaporation of aqueous DMF (1:1, v/v) solutions of **4a** produced colorless prisms suitable for X-ray analysis. Anal. Calcd for $[Gd_2(H_2O)_2C_{34}H_{58}N_8O_{12}] \cdot 4H_2O$: C, 34.22; H, 5.91; N, 9.39; H_2O, 6.04 (for the four waters not bound to gadolinium). Found: C, 34.28; H, 5.90; N, 9.36: H_2O, 6.44 (desorption Karl Fisher).

(1*R*,4*R*,7*R*)-α,α′,α″-Trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid, Monoyttrium Salt, Y(DO3MA) (4b). The free acid DO3MA (**4**) (65 mg, 0.17 mmol) was treated with yttrium oxide (69 mg, 0.28 mmol) in water (10 mL) at 80 °C. After 24 h, the final solution was adjusted to pH 8.0 and the heterogeneous mixture was filtered through an Acrodisc (LC 13 PVDF, 0.2 μm) to remove unreacted yttrium oxide. Removal of the water from the filtrate gave the analytically pure yttrium(III) chelate Y(DO3MA) (**4b**) (50 mg) in 63% yield. MS (FAB): m/z 474 [$(M + H)^+$]. Anal. Calcd for $C_{17}H_{29}N_4O_6Y \cdot 1.63H_2O$: C, 39.12; H, 6.61; N, 10.73. Found: C, 38.93; H, 6.46; N, 10.50; H_2O, 5.82 (desorption KF).

(1*R*,4*R*,7*R*,10*R*)-α,α′,α″,α‴-Tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid, Tetrabenzyl Ester, DOTMA-TBE (14). L-Benzyl 2-triflyloxypropionate (**9**) (28.1 g, 90 mmol) in acetonitrile (25 mL) was added to cyclen (**13**) (3.86g, 22.4 mmol) in acetonitrile (25 mL) in the presence of pulverized potassium carbonate at ambient temperature for 1 h. The reaction mixture was stirred for 16 h and filtered to remove the potassium carbonate, and the filtrate was concentrated to dryness under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 300 g) using ethyl acetate and hexanes (1:1, v/v) to afford the desired tetraester DOTMA-TBE **14** (6.4 g) in 35% yield. IR (neat): 3032, 2978, 2938, 2830, 1728, 1134 cm^{-1}. ^1H-NMR ($CDCl_3$): δ 1.34 (12H, doublet, methyls); 2.65, 2.92 (16H, dd, ring methylenes); 3.63 (4H, q, methines); 5.22 (8H, s, CH_2Ph); 7.4–7.5 (20H, m, aromatic hydrogens). ^{13}C-NMR ($CDCl_3$): δ 15.08 (methyls); 49.97 (ring methylenes); 57.81 (methines); 65.71 (CH_2Ph); 127.91, 128.02, 128.10, 128.41 (C_{2-6}); 136.05 (C_1); 173.75 (COO). MS (FAB): m/z 821 [$(M + H)^+$, base peak]. Anal. Calcd for $C_{48}H_{60}N_4O_8 \cdot 0.60H_2O$: C, 69.31; H, 7.55, N, 6.54. Found: C, 69.69; H, 7.55; N, 6.54; H_2O, 1.29 (dissolution KF).

(1*R*,4*R*,7*R*,10*R*)-α,α′,α″,α‴-Tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid, DOTMA (5).[9,14] The tetrabenzyl ester DOTMA-TBE (**14**) (5.8 g, 7.07 mmol) was dissolved in ethyl acetate (60 mL), methanol (90 mL), and water (15 mL) and hydrogenated (35 psi) in the presence of Pd/C (1.72 g, 0.8 mmol Pd) at ambient temperature. Most of the product **5** was found in the Pd/carbon sludge. About 1 L of hot water was needed to completely extract the ligand **5** from the carbon sludge. The combined aqueous solution was loaded onto a DEAE Sephadex column and then eluted with triethylammonium bicarbonate buffer. Removal of the buffer from the fractions containing the product yielded DOTMA (**5**) as a white solid in 56% yield. HPLC: method 1; retention time, 7.1 min (purity, 99.9%); PLRP-S 4.6 × 250 mm; eluent, 10% acetonitrile in 50 mM sodium phosphate (pH 4.8); flow rate, 1.0 mL; UV, 290 nm. Copper chelation.[6] HPLC: method 2; retention time, 7.1 min (purity, 99.6%); PRP-x100 4.6 × 250 mm; eluent, 8% acetonitrile in 50 mM sodium phosphate buffer (pH 7.0); flow rate, 1.0 mL; UV, 220 nm. IR (KBr): 3435, 1744, 1642, 1383, 1348 cm^{-1}. ^1H-NMR (D_2O): 1.21 (12H, doublet, methyl); 2.4–3.6 (16H, multiplet, ring methylenes); 4.0 (4H, quartet, methine). MS (FAB): m/z 461 [$(M + 1)^+$, base peak]; 483 [$(M + Na)^+$, base peak]; 459 [$(M - H)^-$. Anal. Calcd for $C_{20}H_{36}N_4O_8 \cdot 0.04H_2O$: C, 52.09; H, 7.88; N, 12.15. Found: C, 51.82; H, 8.04; N, 12.07: H_2O, 0.14 (dissolution KF).

Table I. Crystallographic Data for Gd(DO3MA) (**4a**)

formula	$[Gd_2(H_2O)_2C_{34}H_{58}N_8O_{12}) \cdot 4H_2O]$
a, Å	17.471(6)
b, Å	25.495(6)
c, Å	10.146(3)
V, Å^3	4520(4)
Z	4
fw	1193.5
space group; syst	$P2_12_12_1$ (No. 19); orthorhombic
T, °C	22
λ source, Å	Mo Kα, 0.71069 Å
D_{obs}, g cm^{-3}	1.734
D_{calc}, g cm^{-3}	1.754
μ, cm^{-1}	30.0
transm factors	0.92–1.21
R; $R_{enantiomer}$[a]	0.035, 0.042
R_w; $R_{w\ enantiomer}$	0.041, 0.050

[a] R is defined as $\sum||F_o| - |F_c||/\sum|F_o|$. $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$. $w^{-1} = \epsilon^2 + (0.04\ I_o)^2$ where ϵ = statistical counting error.

Crystal Structure Analysis of Gd(DO3MA). Colorless prisms were obtained by slow evaporation of aqueous dimethylformamide solutions of the complex. Intensity data were measured on an Enraf-Nonius CAD4S diffractometer with the θ–2θ variable scan technique and were corrected for Lorentz–polarization factors and for absorption by the DIFABS method. Background counts were measured at the extremes of the scan for half the time of the scan. Two standard reflections were monitored for decay; no significant decrease in intensity was observed during data collection. The structure was solved by heavy-atom techniques and refined on the basis of 3701 "observed" reflections with $I \geq 3\sigma(I)$. Although some hydrogen positions were evident in difference Fourier maps, no reliable positions were observed for the hydrogens on N and O atoms. Hydrogens on carbon were introduced in idealized positions and their scattering was taken into account in the terminal stages of refinement. All calculations utilized the SDP program package with minor local modifications.[15] The positions and anisotropic temperature factors of all non-hydrogen atoms were refined, as well as the occupancy factors for all six water molecules. All water sites were fully occupied except lattice water O95 which had an occupancy factor of 0.6. The final difference map contained no significant features. Unit cell data are given in Table I. Atomic fractional coordinates are given in Table II.

Stability Constants and Relaxivity Determinations. The stock solution of $GdCl_3$ was standardized by EDTA titration using xylenol orange as the indicator. The concentration of the DO3MA stock solution was determined by either complexometric titration or acid–base titration. A stock solution of Gd(DO3MA) was prepared in 0.1 M bis(2-hydroxyethyl)-iminotris(hydroxymethyl)methane) (Bistris) buffer (pH 7.0) for relaxivity measurements. Several solutions needed for relaxivity measurements were prepared by serial dilutions of the stock solution of Gd(DO3MA).

All spectrophotometric measurements were made with an HP-8452 diode-array spectrophotometer interfaced with an HP-310 data station. Conditional stability constants were determined by a spectrophotometric method using Arsenazo-III as an indicator.[16] The conditional stability constant of Gd(DO3MA) at pH 4.0, from spectrophotometry, and the ligand protonation constants of DO3MA, determined potentiometrically,[16a,17] were measured at 25 ± 0.1 °C in the presence of tetramethylammonium chloride (μ = 0.1 M). The spin-lattice relaxation time (T_1) of Gd(DO3MA) was determined at 20 MHz and 40 °C with

(13) The identification of 2-propanol was done by TGA(thermogravimetric analysis)/FT-IR method. The proposed content (3.05%) of 2-propanol was obtained by subtracting the measured Karl Fisher (5.41%) from the total weight loss as determined by TGA/IR (8.52%).

(14) Only relative configurations of the four chiral centers are given in ref 9.

(15) Structure Determination Package (SDP), Enraf-Nonius, Bohemia, NY. Scattering factors, including f and f' in the SDP software were taken from: *International Tables for Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV, Tables 2.2A and 2.3.

(16) (a) Cachris, W. P.; Nickle, S. K.; Sherry, A. D. *Inorg. Chem.* **1987**, *26*, 958. (b) Budesinsky, B. *Talanta* **1968**, *15*, 1063.

(17) Kumar, K.; Chang, C. A.; Francesconi, L. C.; Dischino, D.; Malley, M.; Gougoutas, J. Z.; Tweedle, M. F. Submitted for publication.

Table II. Atomic Fractional Coordinates of the Non-Hydrogen Atoms of GdDO3MA (**4a**)

atom	*x*	*y*	*z*
Gd1	0.62261(3)	0.23195(2)	0.34079(5)
N1	0.7110(5)	0.2695(4)	0.1505(9)
N2	0.6969(5)	0.3187(3)	0.4230(11)
N3	0.7153(5)	0.2111(4)	0.5477(9)
N4	0.7346(5)	0.1710(4)	0.2800(10)
O1	0.5683(4)	0.3013(3)	0.2227(8)
O2	0.5578(6)	0.3559(3)	0.0545(8)
O3	0.5689(4)	0.2753(3)	0.5237(8)
O4	0.5330(7)	0.3474(4)	0.6282(11)
O5	0.5997(4)	0.1497(3)	0.4472(7)
O6	0.6213(5)	0.0911(3)	0.6027(7)
O9	0.4795(4)	0.2179(3)	0.3451(9)
C1	0.7654(9)	0.3075(6)	0.2101(16)
C2	0.7348(8)	0.3429(5)	0.3020(15)
C3	0.7537(8)	0.3056(5)	0.5207(17)
C4	0.7350(8)	0.2634(5)	0.6141(13)
C5	0.7866(7)	0.1854(6)	0.5063(14)
C6	0.7774(7)	0.1473(5)	0.4011(15)
C7	0.7929(8)	0.1890(4)	0.1873(14)
C8	0.7565(7)	0.2236(6)	0.0928(13)
C9	0.5916(6)	0.3203(4)	0.1125(11)
C10	0.6634(7)	0.2946(4)	0.0507(11)
C11	0.6348(7)	0.3537(4)	0.4712(12)
C12	0.5745(7)	0.3245(5)	0.5504(13)
C13	0.6272(6)	0.1368(4)	0.5599(11)
C14	0.6671(6)	0.1804(4)	0.6386(11)
C15	0.7013(10)	0.3305(6)	−0.048(13)
C16	0.6619(8)	0.4017(5)	0.5422(13)
C17	0.7072(7)	0.1580(5)	0.7629(12)
Gd20	0.54749(3)	0.05485(2)	0.41920(5)
N21	0.5769(5)	−0.0207(3)	0.2451(9)
N22	0.4214(5)	0.0128(3)	0.3283(9)
N23	0.4677(5)	0.0090(3)	0.6128(9)
N24	0.6211(6)	−0.0235(3)	0.5198(9)
O21	0.6577(4)	0.0651(3)	0.2958(8)
O22	0.7243(5)	0.0573(4)	0.1115(9)
O23	0.4913(4)	0.0980(3)	0.2405(7)
O24	0.3850(6)	0.1404(4)	0.1932(14)
O25	0.4554(4)	0.1066(3)	0.5320(7)
O26	0.4376(6)	0.1401(3)	0.7325(8)
C21	0.5071(6)	−0.0511(4)	0.2130(12)
C22	0.4382(7)	−0.0166(4)	0.2061(13)
C23	0.3854(7)	−0.0224(4)	0.4288(13)
C24	0.3899(6)	−0.0021(4)	0.5674(12)
C25	0.5046(8)	−0.0392(5)	0.6589(14)
C26	0.5915(7)	−0.0356(4)	0.6557(13)
C27	0.6198(7)	−0.0706(4)	0.4394(13)
C28	0.6365(7)	−0.0575(4)	0.2992(12)
C29	0.6684(6)	0.0468(4)	0.1789(13)
C30	0.6079(6)	0.0081(5)	0.1293(10)
C31	0.4175(7)	0.1020(5)	0.2363(14)
C32	0.3712(6)	0.0589(4)	0.2939(11)
C33	0.4515(7)	0.1030(4)	0.6587(11)
C34	0.4676(7)	0.0492(4)	0.7179(10)
C35	0.6359(9)	−0.0258(5)	0.0170(14)
C36	0.3001(7)	0.0464(5)	0.2156(13)
C37	0.4156(10)	0.0392(5)	0.8354(14)
O99	0.4770(7)	0.3065(5)	0.8554(12)
O98	0.5257(7)	0.2043(4)	0.9085(11)
O97	0.4288(5)	0.2332(3)	0.6000(9)
O96	0.5816(4)	0.1787(3)	0.1513(8)
O95	0.4013(8)	0.3057(7)	0.1873(21)

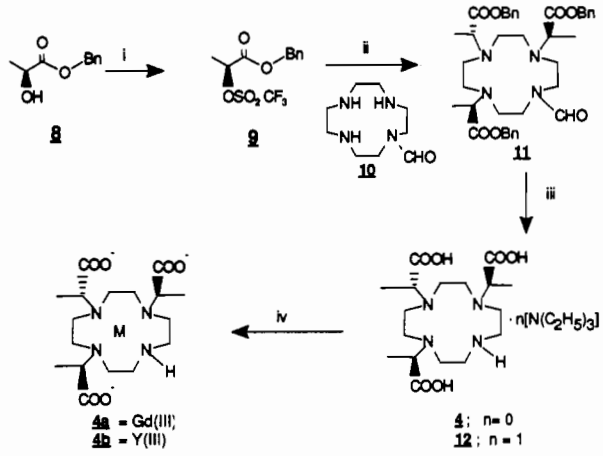
an IBM PC/20 relaxometer using Gd(HP-DO3A) as a control standard ($^{20}r_1$ = 3.7 ± 0.1 mM^{-1} s^{-1}).

Results and Discussion

Synthesis of DO3MA, Gd(DO3MA), and Y(DO3MA). It is reasonable to expect that the various diastereomers of DO3MA, arising from its three asymmetric centers, might exhibit different coordination chemistry with Gd(III). Cummins et al.,[18] in fact, recently reported a modest stereoselectivity in the chelation of

(18) Cummins, C. H.; Rutter, Jr., E. W.; Fordyce, W. A. *Bioconjugate Chem.* 1991, *2*, 180.

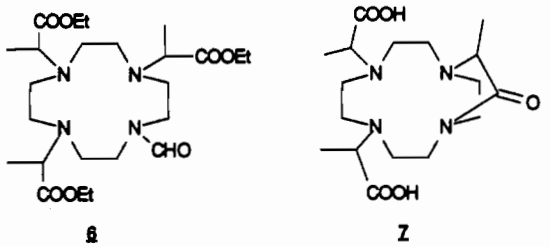
Scheme I



i. triflic anhydride, 31%
ii. Hünig base in CH_3CN, RT, 63%
iii. H_2/Pd in aq. HCl in MeOH, 78%
iv. Gd_2O_3 or $Gd(OAc)_3$, 73%; Y_2O_3, 63%

1-(4-aminobenzyl)–DTPA with Y(III) and indicated that chelation reactions with other lanthanide ions yielded comparable results. In view of this, we decided at the outset of the synthesis of DO3MA to prepare the chiral product in the stereoisomerically homogeneous form.

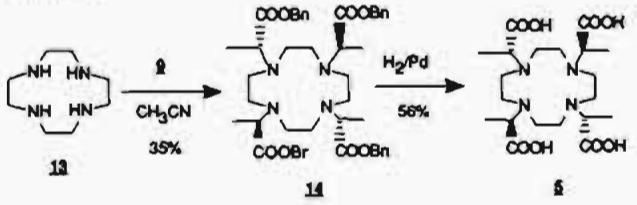
Initially, we attempted the direct alkylation of *N*-formylcyclen (**10**), a monoprotected tetraazamacrocycle, with (*S*)-2-chloropropionic acid in a basic aqueous solution at 85–90 °C. In this preparation of DO3MA, we followed the literature procedure for DOTMA[9] with a slight modification; *N*-formylcyclen (**10**) was reacted with 3.0–3.5 equiv of the alkylating agent rather than 4.1. Progress of the alkylation reaction was monitored by reverse-phase PLRP/HPLC analysis using a copper chelation method.[6] The HPLC monitoring indicated that less than 20% of the expected product, DO3MA, was formed in the reaction mixture. (±)-2-Bromopropionic acid was also employed to investigate the alkylation chemistry, but the outcome was not significantly different. In order to facilitate the alkylation reaction of *N*-formylcyclen (**10**) and to avoid possible extensive racemization under the forcing conditions aformentioned,[12] the more reactive L-ethyl 2-(triflyloxy)propionate was used to alkylate *N*-formylcyclen (**10**) to obtain 10-formyl-1,4,7-tris(ethyloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (N-CHO-DO3MA-TEE) (**6**). However, acidic and basic hydrolyses of the triethyl ester **6** yielded significant amounts of a lactam (**7**) (presumed, based on a FAB mass spectral analysis) along with the expected product, DO3MA. The lactam **7** could not be readily separated from the desired product **4** by DEAE Sephadex ion-exchange chromatography. In order to prevent the formation of the lactam



6 **7**

7, we needed a carboxy protecting group that could be readily removed under mild, neutral conditions. Since the benzyl protecting group would fit this requirement, we prepared L-benzyl 2-(triflyloxy)propionate (**9**) and employed it for the alkylation of **10**. Scheme I delineates the results of this approach to DO3MA, Gd(DO3MA), and Y(DO3MA). HPLC analysis of the 4.7-h reaction mixture leading to L-benzyl lactate (**8**) showed the formation of a ca. 1:3 ratio of hydroxy- to carboxy-benzylated

Scheme II



products, which were easily separated and purified. Treatment of **8** with triflic anhydride followed by flash column chromatography gave a 55% yield of the corresponding triflate **9**. Treatment of **9** with *N*-formylcyclen (**10**) in acetonitrile at ambient temperature, in the presence of Hünig's base, afforded the tribenzyl ester of *N*-formyl DO3MA (N-CHO-DO3MA-TBE) (**11**), which was purified by silica gel column chromatography. Simultaneous deformylation and debenzylation of **11** were achieved in aqueous methanolic HCl in the presence of palladium catalyst at ambient temperature. Purification of the crude product by DEAE Sephadex ion exchange chromatography with elution by aqueous triethylammonium bicarbonate buffer gave the monotriethylammonium salt **12** of DO3MA. The pure acid form of DO3MA was obtained, in quantitative yield, by passing the salt through an AG1 (formate form) resin column, the overall yield being 34% from *N*-formylcylen (**10**). Treatment of either the triethylammonium salt (**12**) of DO3MA with gadolinium acetate or the free acid of DO3MA with gadolinium oxide resulted in pure Gd(DO3MA) in 73% yield. Complexation of yttrium oxide with DO3MA gave Y(DO3MA) in 63% yield. X-ray structure analysis of crystallized Gd(DO3MA) revealed that the three asymmetric carbons bearing α-methyl groups all have (*R*)-configurations. In addition the Gd product was found to be optically active in solution (ORD spectra in supplementary material). These facts, together with the synthetic methods chosen, suggest that significant racemization did not occur during the synthesis of Gd(DO3MA).

Synthesis of DOTMA. Since the use of L-benzyl 2-(triflyloxy)-propionate (**9**) gave the efficacious synthesis of DO3MA and Gd(DO3MA) as presented in Scheme I, we applied this triflate-mediated approach to the synthesis of DOTMA *via* the corresponding tetrabenzyl ester intermediate **14**. In our hands, several attempts to alkylate cyclen (**13**) with 4 equiv or more of 2-chloro- or 2-bromopropionic acid did not give the reported results.[9] As shown in Scheme II the tetrabenzyl ester DOTMA-TBE (**14**) was obtained in 35% yield by the reaction of cylen (**13**) with 4.0 equiv of the triflate **9**. Catalytic hydrogenolysis of **14** followed by purification using the DEAE Sephadex ion exchange methodology gave the expected DOTMA in 56% yield. The proton NMR spectrum of DOTMA in D_2O clearly displayed the doublet and quartet spin coupling patterns of the methyl and methine groups. Due to the limited solubility of DOTMA in the free acid form in D_2O, no ^{13}C-NMR data could be obtained. This observed low solubility in water explains why DOTMA was recovered from the carbon sludge as described in the Experimental Section.

X-ray Crystal Structure of Gd(DO3MA). In the solid state, two crystallographically-independent Gd(DO3MA) complexes and two water molecules are joined to form a "dimeric" unit (Figure 1). For each independent Gd(DO3MA) complex the following points are observed (Figure 2). (a) The tetraazacyclododecane macrocycle adopts a distorted quadrangular [3333] conformation. (b) An oxygen atom from each of the three carboxyl arms and the four essentially coplanar nitrogens are coordinated to the embedded Gd atom (Gd–O distance = 2.35(2) Å. All distances and angles are averaged over the two complexes and figures in parentheses are average deviations). As in the structure of In(DO3A),[19] the three (tert)N–Gd distances (2.66(2) Å) are longer than the (sec)N (4 or 24)–Gd distance (2.58(1) Å). (c) In addition to these seven "internal" ligating

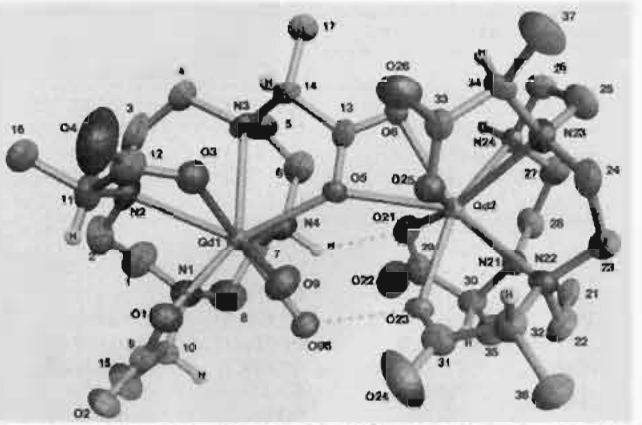


Figure 1. Dimeric structure of Gd(DO3MA). Only two (O9 and O96) of the six water molecules in the asymmetric unit are shown. Anisotropic thermal parameters are represented by 50% probability ellipsoids. Most hydrogens have been omitted; the eight hydrogens on the Cα and secondary nitrogen atoms have arbitrary radii. Probable hydrogen bonds are indicated by dashed lines between the two complexes.

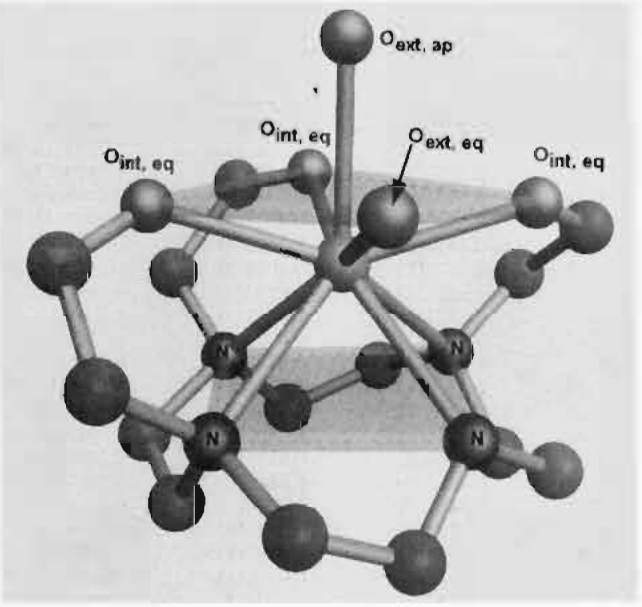


Figure 2. Ball and stick representation of the bonding to the embedded metal. The Gd1 complex is shown here. The Gd2 complex has essentially the same structure except for an enantiomeric arrangement of the eight methylene carbon atoms of the macrocycle below the plane of the nitrogens. External, internal, equatorial, and apical oxygen atoms have subscripts ext, int, eq, and ap, respectively.

atoms of the ligand, two other "external" oxygens are also coordinated to the metal, which accordingly is *ennea*coordinate. One of the "external" oxygen atoms (Gd–O (6 or 96) distance = 2.451(5) Å) lies approximately in the (equatorial) plane of the three coordinating carboxyl oxygens while the other "external" oxygen atom occupies an apical position (Gd–O (5 or 9) (apical) distance = 2.56 (4) Å. The Gd atom lies 1.62(1) Å above the least-squares plane of the nitrogens, and 0.83(7) Å below the least squares plane of the four "equatorial" oxygen atoms. The apical oxygen lies 1.69(7) Å above this equatorial oxygen plane while the Gd–O (apical) bond is within 14° of its normal. The plane of the nitrogens (rms deviation = 0.02 Å) and the plane of the equatorial oxygens (rms deviation = 0.09 Å) are nearly parallel (dihedral angle = 4°). (d) The carboxyl side arms are rotated about the N-Cα bond such that the α methyl groups are anti to the Gd atom. Given the homogeneous R configuration of the Cα chiral centers, the Gd–N–Cα–C torsional angles are

(19) Riesen, A.; Kaden, T. A.; Ritter, W.; Mäcke, H. R. *J. Chem. Soc., Chem. Commun.* 1989, 460.

Table III. Protonation Constants of the Ligands DO3A, DOTA, and DO3MA in the Presence of Tetramethylammonium Chloride (μ = 0.1 M) at 25 °C

ligand	log K_1	log K_2	log K_3	log K_4
DO3A	11.59[a]	9.24[a]	4.34[a]	3.84[a]
DOTA	11.73[a]	9.40[a]	4.50[a]	4.19[a]
DO3MA	13.38, 13.46[b]	9.15	5.30	4.07
DO3MA	10.82[c]	8.42[c]	4.98[c]	

[a] Taken from ref 17. [b] By spectrophotometric titration. [c] At 25 °C with sodium chloride (μ = 0.1 M).

therefore consistently positive (+42°), and the N–Cα–C–O angles are consistently negative (–25(9)°).

The two independent Gd(DO3MA) complexes differ in two respects. (a) Although all of the carboxyl arms are rotated in the same sense, the quadrangular conformations of the tetraazacyclododecane macrocycle are enantiomeric with average NCCN, CCNC, and CNCC torsional angles of 56(2), –160(3), and 81(7)° in one complex and –58(1), 162, and –74(5)° in the other. The two complexes therefore have diasteromeric conformations.[2b] (b) The two "external" oxygen ligands (one apical and one equatorial) are water molecules in one complex (O9 and O96), while in the other complex the two "external" oxygens (O5 and O6) are donated by a bridging carboxyl group of the other complex. This bridging carboxyl is therefore a tridentate ligand in which one of its oxygens (O5) is apical with respect to one complex and equatorial with respect to the other.

The bridging carboxyl group[20] as well as two hydrogen bonds (O–O distances = 2.75, 2.96 Å) involving the two "external" water ligands and an NH- -O hydrogen bond (N–O distance = 3.016 Å) join the two complexes to form a "dimeric" unit. Several other hydrogen bonds involving the four lattice waters further link the two complexes.

Physicochemical Characteristics of DO3MA and Gd(DO3MA). The protonation constants of the heptadentate ligand, DO3MA, are presented in Table III along with data for DO3A and DOTA.[21] The data are consistent with the protonation of the ring nitrogens and carboxylate oxygens.[17] The validity of the first protonation constant of DO3MA was confirmed by a spectrophotometric titration, because it is outside of the accurate range of the glass electrode. The first nitrogen of DO3MA was considerably more basic than those of DO3A and DOTA, presumably due to electronic and steric effects[22] of the α-methyl groups on the propionic acid arms of DO3MA. Small alkyl substitutions alpha to the nitrogens are known to raise the first pK_a of aminocarboxylate ligands, for example R-EDTA, by up to an order of magnitude.[23] The first protonation constant of DO3MA was very sensitive to the presence of sodium ion, indicating that DO3MA complexes the alkali metal cation, as do DO3A and DOTA.[17,24] The thermodynamic stability constant and relaxivity of Gd(DO3MA) are given in Table IV, compared with Gd(DO3A), Gd(HP-DO3A), and Gd(DOTA)$^-$. Outstanding in Table IV is the fact that Gd(DO3MA) has a stability constant (log K_{eq}) of 25.3, which is nearly as high as that of Gd(DOTA)$^-$, contrary to expectations based on the difference in coordination number between these two ligands. An explanation may be traced to the relative basicities of the two ligands: the sums of the pK_a values for the neutral ligands are 27.8 and 29.8, respectively. This enhanced stability due to α-methyl groups is consistent with the findings of a recent communication dealing with indium and gallium complexes of the six coordinate macrocyclic carboxylate ligands, NOTA and NOTMA.[25] The stability of Gd(DO3MA) was studied at physiologic pH, because free Gd(III) aqua ion and free ligands are poorly tolerated in vivo.[1,2] No release of Gd(III) was observed by HPLC (<0.002 mol %) upon storing Gd(DO3MA) as a 0.5 M solution at pH 7.4 for 3 months. However, in 0.1 M HCl (μ = 1.0 M NaCl) and at 25 °C an instantaneous 45% fluorescence intensity decrease was observed, followed by a slow time dependent decrease in intensity. The final fluorescence intensity of the mixture corresponded to the intensity of Gd(III) aqua ion. We attributed the instantaneous fluorescence intensity decrease to the protonation of Gd(DO3MA) and the slow fluorescence intensity decrease to its acid-assisted dissociation. A protonation constant (K_{H_1} = 175±25 M^{-1}) of Gd(DO3MA) was calculated from the instantaneous fluorescence intensity decrease with [H$^+$]. The half-life of Gd(DO3MA) under these conditions was 1.2 h. Figure 3 shows the observed pseudo-first-order rate constants for acid-assisted dissociation of Gd(DO3MA)(H$^+$), saturating as a function of acid concentration, and possessing a positive intercept. Dissociation of the mono-protonated complex is proposed to occur by two paths: (1) direct dissociation (k_d = 3.7 × 10^{-5} s^{-1}) and (2) a proton-assisted dissociation (K_{H_2} = (5 ± 2) M^{-1} and k_2 = (5.2 ± 0.5) × 10^{-4} s^{-1}). The complex is 10-fold more inert than Gd(DO3A) in 0.1 M HCl.[26] Because the

Table IV. Stability Constants and Relaxivities of Gd(III) and Y(III) Chelates

chelate	log K_{eq}[a], M^{-1}	ligand coord no.	relaxivity,[b] mM^{-1} s^{-1}
Gd(DO3A)	21.1[c]	7	4.8 ± 0.1[d]
Gd(HP-DO3A)	23.8[c]	8	3.7 ± 0.1[d]
Gd(DOTA)	25.8[c]	8	3.5 ± 0.1[d]
Gd(DO3MA)	25.3	7	4.4 ± 0.1[e]
Y(DO3A)	21.1[c]	7	
Y(DO3MA)	25.2	7	

[a] At 25 °C with tetramethylammonium chloride (μ = 0.1 M). Accuracy is approximately ±1 log unit. [b] 20 MHz and 40 °C. [c] Taken from ref 17. [d] Taken from ref 27. [e] Reference 28.

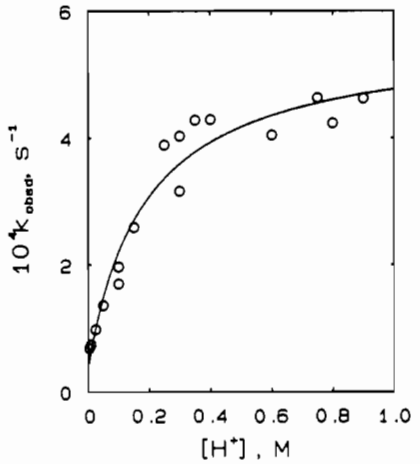


Figure 3. Plot of pseudo-first-order rate constants as a function of [H$^+$] for acid-assisted dissociation of Gd(DO3MA)(H) at 25 °C and sodium chloride (μ = 1.0 M). The solid line is calculated from the resolved values of the rate and equilibrium constants, k_d = 3.7 × 10^{-5} s^{-1}, k_2 = (5.2 ± 0.5) × 10^{-4} s^{-1}, and K_{H_2} = 5 ± 2 M^{-1}.

(20) Gries, H.; Frenzel, U.; Niedballa, U.; Platzek, J.; Press, W.-R.; Radüchel, B.; Weinmann, H.-J. Polyhydroxylated Macrocyclic Contrast Agents for MRI. Presented at the 10th Meeting of Society of Magnetic Resonance in Medicine (SMRM); Aug 10–16, 1991, San Francisco, CA. The authors reported that a dimeric crystal structure of the gadolinium chelate of 10-[(1-hydroxymethyl-2,3-dihydroxy)propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid in which the gadolinium ions are connected via carboxylate groups. Both the Gd atoms are *enneacoordinate* with no inner-sphere water molecule.

(21) Martell, A. E.; Motekaitis, R. J. In *The Determination and the Use of Stability Constants*; VCH Publishers; New York, 1988.

(22) Wagenaar, A.; Engberts, J. B. F. N. *J. Org. Chem.* **1988**, *53*, 768.

(23) Martell, A. E.; Smith, R. M. In *Critical Stability Constants*; Plenum: New York, 1989; Vol. 6 (second supplement), pp 96–105.

(24) Aime, S.; Anelli, P. L.; Botta, M.; Fedeli, F.; Grandi, M.; Paoli, P.; Uggeri, F. *Inorg. Chem.* **1992**, *31*, 2422.

(25) (a) Allen, D. R.; Brücher, E.; Sherry, A. D. Presented at the 28th International Conference on Coordination Chemistry; Gera, Germany; Aug 13–19, 1990. (b) Matthews, R. C.; Parker, D.; Ferguson, G.; Kaitner, B.; Harrison, A.; Royle, L. *Polyhedron* **1991**, *10*, 1951.

complexes are both fully protonated under these conditions, we attribute the difference in inertia to additional rigidity of Gd(DO3MA) imparted by the α-methyl groups. It is known that α-methyl substituents accelerate ring forming reactions.[22] The coordinated Gd(III) ion and, probably, the proton involved in the first pK_a of the neutral ligand are part of five membered ring systems as depicted in the following structures.

We propose that the methyl groups impart additional stability to these ring systems, accounting for the high degree of basicity, thermodynamic stability, and kinetic inertia of DO3MA. The T1 relaxivity of Gd(DO3MA) was found to be 4.4 ± 0.1 mM^{-1} s^{-1} while Gd(DO3A) was 4.8 ± 0.1. For small monomeric chelates with similar charge types, the primary determinant of relaxivity is the hydration number. Gd(DO3A) and Gd(DO3MA) have hydration numbers of 1.8 ± 0.1 and 1.4 ± 0.1 inner-sphere coordinated water molecules, respectively.[27,28] The low hydration number of Gd(DO3MA) again appears to be consistent with the additional structural rigidity imparted by the buttressing effect of the three α-methyl substituents.

Acknowledgment. We are grateful to colleagues in the Bristol-Myers Squibb Pharmaceutical Research Institute (BMS-PRI), Princeton, NJ, for providing spectral and analytical data. We thank Drs. E. Delaney and F. J. Okuniewicz at the BMS-PRI Chemical Division in New Brunswick, NJ, for the kind provision of 1-formyl-1,4,7,10-tetraazacyclododecane. We also thank Dr. Douglas D. Dischino for a sample of 1-benzylcyclen, Dr. Xun Zhang and Ms. Maria Fernandez for the hydration number and relaxivity data, and Dr. H.G. Brittain for an ORD.

Supplementary Material Available: Tables giving some additional details of data collection and refinement, parameters for the hydrogen atoms, listing of bond distances and angles, and thermal parameters for the crystal structure of **4a**, a figure showing a stereoview of **4a**, and figures showing optical rotatory dispersions (14 pages). Ordering information is given on any current masthead page.

(26) Kumar, K.; Chang, C. A.; Tweedle, M. F. Submitted for publication.

(27) Zhang, X.; Chang, C. A.; Brittain, H. G.; Garrison, J. M.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* (in press), 1992, 31, 5597.

(28) Unpublished result determined as described in ref 27.