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Synthesis of Nonionic Gadolinium Chelates Useful as Contrast Agents for Magnetic Resonance Imaging. 1,4,7-Tris(carboxymethyl)-10-substituted-1,4,7,10-tetraazacyclododecanes and Their Corresponding Gadolinium Chelates†

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The synthesis of a new and synthetically useful ligand, 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, DO3A (**2**), was achieved through a variety of synthetic approaches.[1] These routes included (1) the partial carboxymethylation of unprotected cyclen, 1,4,7,10-tetraazacyclododecane (**1**), with chloroacetic acid followed by ion-exchange chromatography (Scheme I), (2) reductive debenzylation (Pd/C, H_2) of 1,4,7-tris(carboxymethyl)-10-(phenylmethyl)-1,4,7,10-tetraazacyclododecane, Bz-DO3A (**7**) (Scheme II), and (3) carboxymethylation of 1-formyl-1,4,7,10-tetraazacyclododecane (**9**) with chloroacetic acid (or *tert*-butyl bromoacetate) followed by removal of the protecting group(s) (Scheme III). Method III was found to be the most efficient. The novel formyl cyclen was prepared by the partial hydrolysis of the tricyclic cyclen derivative, 1,4,7,10-tetraazatricyclo[5.5.1.0]-tridecane.[2] The heptadentate ligand, DO3A, is a versatile intermediate, being easily derivatized to produce potentially octadentate ligands and bifunctional chelating agents. A variety of octadentate ligands and their gadolinium(III) chelates were synthesized. Many of these gadolinium chelates are neutral, stable, and highly water soluble (>0.5 M), properties desirable in clinically useful MRI contrast media.

Introduction

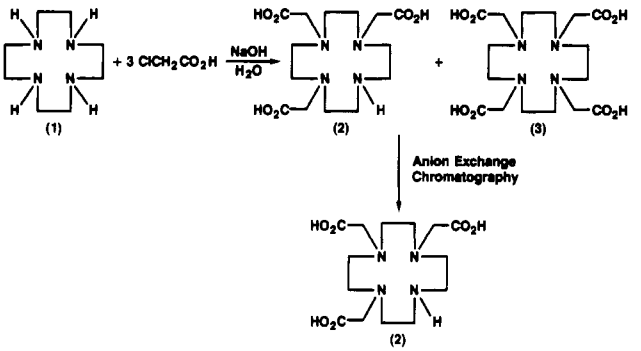
A medically useful chelating ligand must form inert complexes with metals while at the same time conferring upon the complex desirable chemical, physical, and/or biological properties. Our primary interest was to develop Gd(III) complexes as paramagnetic contrast agents for magnetic resonance imaging (MRI). The minimal requirements in this application are that the complexes be highly water soluble (≥0.5 M), have high relaxivity (usually achieved by having an inner-sphere coordinated water molecule in rapid exchange with the bulk water), and be extremely inert to loss of Gd^{3+} ion, which is very poorly tolerated in vivo as the free ion.[3] The existing prototypes when this work began (1983) were Gd(III) complexes of the well-known ligands, diethylenetriaminepentaacetic acid (DTPA)[4] and 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA).[5] Although $Gd(DTPA)^{2-}$ and $Gd(DOTA)^{-}$ form water-soluble, stable gadolinium chelates (thus satisfying the minimal requirements for use as paramagnetic contrast agents), they both carry a formal overall charge that makes the concentrated injectable solutions (0.5–1.0 M) hyperosmolar with respect to blood and most body fluids. These ionic complexes also contain no unique site that is readily accessible for further synthetic derivatization. The objective of this program was to prepare a new family of stable, highly water soluble nonionic gadolinium chelates. To achieve our goals, we synthesized a new ligand, 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, DO3A (**2**). The core ligand, DO3A, is heptadentate, providing four macrocyclic nitrogen atoms and three carboxylates. This novel ligand is easily derivatized on the unique nitrogen on the macrocyclic ring, providing potentially octadentate ligands, which, upon reaction with gadolinium, form neutral Gd(III) complexes that are highly water soluble, inert to dissociation and substitution for Gd(III), and effective as proton relaxation catalysts.

In this paper we report the first detailed syntheses of DO3A,[1] substituted R–DO3A, and their Gd(III) complexes. Of the three routes to DO3A that were developed (see Scheme I), the preferred one was through the previously unknown 1-formyl-1,4,7,10-tetraazacyclododecane (**9**) formed by the partial hydrolysis of the known 1,4,7,10-tetraazatricyclo[5.5.1.0]tridecane (**8**).[2]

Experimental Section

Reagents and solvents were obtained from either Aldrich Chemical Co. or Fisher Scientific and used as received unless specifically noted.

Scheme I. Synthesis of 1,4,7-Tris(carboxymethyl)-1,4,7,10-Tetraazacyclododecane, DO3A



1,4,7,10-Tetraazacyclododecane (**1**) was purchased from Parish Chemical Co., Orem, UT. Alternatively, 1,4,7,10-tetraazacyclododecane may be prepared by several literature methods.[6-8] Gadolinium oxide (99.99%) was obtained from Molycorp, Inc., White Plains, NY. Analytical grade AG 1-X8 anion-exchange resin (200–400 mesh, formate form), Dowex 50-X2 strong cation-exchange resin (400 mesh, H^+ form), and Chelex-100 (200–400 mesh, Na^+ form) were obtained from Bio-Rad Laboratories, Richmond, CA. Amberlite IRA 900C (OH^- form) was obtained from Schweizerhall, Inc., South Plainfield, NJ. Poly-4-vinylpyridine (PVP) resin was obtained from the Reilly Tar and Chemical Co., Indianapolis, IN. All resins were regenerated following manufacturer's recommended procedures prior to use. Distilled–deionized water was obtained from a Millipore Super Q purification system (10 MΩ cm) and used to minimize trace-metal contamination of the ligands and complexes.

Elemental analyses, mass spectra, infrared spectra, and NMR spectra were obtained from the respective departments within the Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

Analytical Methods. HPLC methods were used to evaluate the purity of both the ligand and chelate preparations. HPLC columns were ob-

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(1) Tweedle, M. F.; Gaughan, G.; Hagan, J. J. U.S. Patent No. 4,885,363, 1989; Eur. Pat. Appl. EP 232-751-A, 1986.
(2) Atkins, T. J. U.S. Patent No. 4,085,106, April 18, 1978.
(3) Magerstadt, M.; Gansow, O. A.; Brechbiel, M. W.; Colcher, D.; Baltzer, L.; Knop, R. H.; Girton, M. E.; Naegele, M. *Magn. Reson. Med.* **1986**, *3*, 808.
(4) Weinmann, H. J.; Brasch, R. C.; Press, W. R.; Wesby, G. E. *AJR, Am. J. Roentgenol.* **1984**, *142*, 619.
(5) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319.
(6) Richman, E. J.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268.
(7) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Org. Synth.* **1978**, *58*, 86.
(8) Chavez, F.; Sherry, A. D. *J. Org. Chem.* **1989**, *54*, 2990.

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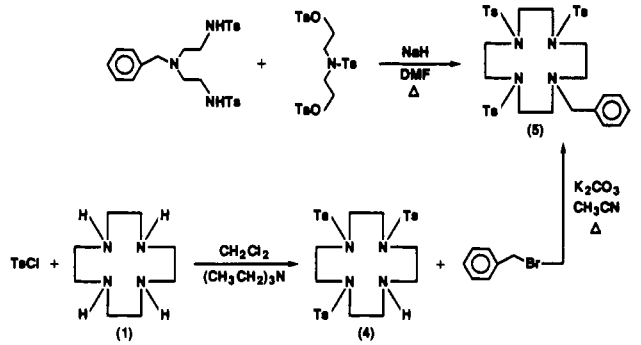
tained from either Hamilton, Macherey-Nagel, or Rainin Instrument Co. The ligand assay involves adding an excess of Cu^{2+}, as 10 mmol of copper(II) acetate, to a solution of the ligand (<10 mmol) and then analyzing the resulting copper complexes on a Hamilton PRP X-100 anion-exchange column. A typical eluent consists of 95% buffer (1.25 mM Tris acetate, 2.5 mM Na_2EDTA, pH 7.0) and 5% CH_3OH. (Minor changes in the percent of methanol may be used depending upon the exact chemical structure of the ligand under study.) The flow rate of the column was 1.0 mL/min. A Kratos 757 Spectroflow absorbance detector (290 nm) was used to monitor the elution of the copper complexes in the ligand assay. The gadolinium chelates were monitored on a Nucleosil 5C 18 reversed-phase column.[9] A typical eluent consists of 98% buffer (50 mM Tris acetate, 10 mM Na_2EDTA, pH 7.0) and 2% CH_3CN. The flow rate of the column was 1.0 mL/min. A Perkin-Elmer 650 S fluorescence detector (excitation 280 nm, emission 316 nm) was used to monitor the elution of the gadolinium chelates.

Synthesis. 1,4,7-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (2) (Scheme I). A solution of 69.0 g (0.187 mol) of 1,4,7,10-tetraazacyclododecane bis(sulfuric acid) salt in 310 mL of H_2O was brought to pH 9.0 with 70 mL of 6 N KOH. To this solution was added 35.3 g (0.374 mol) of chloroacetic acid, and the pH was adjusted to 9.0 with 6 N KOH. The temperature was increased to 55–60 °C and the pH maintained at 8.5–9.0 with the addition of 6 N KOH as necessary. After 1.5 h, an additional 16.0 g (0.169 mol) of chloroacetic acid was added, and the pH was readjusted. The reaction was continued for 16 h at 50 °C, and the pH was maintained between 8.5 and 9.0. The reaction mixture was then cooled, the pH was brought to 3 with concentrated HCl, and the solution was diluted with 300 mL of CH_3OH. The mixture was filtered and the filtrate evaporated. The solid was dissolved in 4 L of H_2O, the solution was divided into equal fractions, and then each was passed down a cation-exchange column (2-L column bed, Dowex 50 X2-400, H^+ form). The column was washed well with deionized water and the ligand brought off the column with 3 L of 0.5 M NH_4OH. The solvent was removed by rotary evaporation (50 °C, 20 mmHg) and yielded the ammonium salt of the ligand. The ammonium salt was then dissolved in 6 L of deionized H_2O, the sample was divided into two equal fractions, and each was passed down a column of anion-exchange resin (2 L, Dowex AG1-X8, HCO_2^- form). The column was washed well with deionized water and the ligand eluted off the column with 3 L of 0.5 M HCO_2H. The solvent was removed by a rotary evaporator to yield a white solid. The solid was dissolved in 200 mL of deionized H_2O and concentrated in vacuo. This process was repeated four times in an effort to remove residual HCO_2H. The solid was triturated with CH_3OH to yield 17.0 g (26.2%) of 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (**2**) as a colorless solid. Mass spectrum (FAB): *m/e* 345 $[(M - H)^-]$ and 347 $[(M + H)^+]$. ^{13}C NMR (D_2O): δ 176.9, 171.0, 57.0, 55.7, 52.7, 50.3, 49.3, 43.6. Anal. Calcd (found) for $C_{14}H_{26}N_4O_6$: C, 48.54 (48.29); H, 7.57 (7.75); N, 16.18 (16.03).

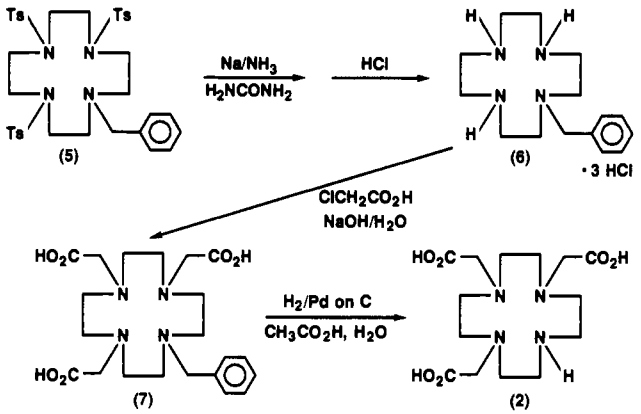
1,4,7-Tris[(4-methylphenyl)sulfonyl]-1,4,7,10-tetraazacyclododecane (4). To 100 g (0.58 mol) of 1,4,7,10-tetraazacyclododecane (**1**) dissolved in 1.85 L of CH_2Cl_2 containing 586.9 g (5.80 mol, 10 equiv) of triethylamine at 0 °C was added 331.75 g (1.74 mol, 3 equiv) of *p*-toluenesulfonyl chloride dropwise over 1.5 h. After 1.5 h, the reaction vessel was removed from the ice bath and the reaction was allowed to warm to ambient temperature. After 20 h, the organic layer was extracted with 1 L of H_2O, the layers were separated, and the organic layer was dried with $MgSO_4$. The solvent was then removed by a rotary evaporator, the residue was dissolved in a minimum amount of CH_3OH, and the solvent was removed on a rotary evaporator to yield 337.1 g (91.6%) of crude **4** as a white solid. The crude product was then recrystallized from $CHCl_3$/hexane to yield 293.0 g (79.6%) of **4**; mp 198–199 °C dec. Mass spectrum (CI): *m/e* 635^+ $[(M + H)^+]$. ^{13}C NMR ($CDCl_3$): δ 143.46, 143.38, 135.89, 135.11, 129.70, 127.60, 127.28, 50.74, 50.62, 49.91, 49.85, 21.42. Anal. Calcd (found) for $C_{29}H_{38}N_4O_6S_3$: C, 54.87 (54.44); H, 6.03 (5.77); N, 8.83 (8.76); S, 15.15 (14.88).

1-(Phenylmethyl)-4,7,10-tris-[(4-methylphenyl)sulfonyl]-1,4,7,10-tetraazacyclododecane (5) (Scheme II). To 41.3 g (0.065 mol) of **4** dissolved in 700 mL of warm CH_3CN was added 13.9 g (0.081 mol) of benzyl bromide and 17.9 g (0.13 mol) of K_2CO_3. The mixture was allowed to stir at reflux for 5 h. After 5 h, the mixture was filtered, and the filtrate was stored at 0 °C for 14 h. After 14 h, the solid was filtered to obtained 41 g of crude **5**. The crude product was recrystallized from $CHCl_3/CH_3OH$ (1:1) and then purified via flash chromatography (silica gel, $CHCl_3$ followed by 5% EtOAc/$CHCl_3$) to yield 36.1 g (77%) of **5** as a crystalline solid; mp 213–214 °C. Mass spectrum (FAB): *m/e* 725^+ $[(M + H)^+]$, 723^- $[(M - H)^-]$. ^{13}C NMR ($CDCl_3$) δ 143.49, 136.34, 135.64, 134.72, 129.92, 129.71, 128.26, 127.58, 127.44, 59.55, 55.14, 51.67, 50.92, 48.63, 21.54, 21.49. Anal. Calcd (found) for $C_{36}H_{44}N_4O_6S_3$: C, 59.64 (59.62); H, 6.12 (6.13); N, 7.73 (7.63); S, 13.27 (12.97). TLC (silica gel, 5% EtOAc/$CHCl_3$): R_f of **5** 0.5, R_f of **4** 0.0.

Scheme II. Synthesis of 1,4,7-Tris(carboxymethyl)-1,4,7,10-Tetraazacyclododecane, DO3A



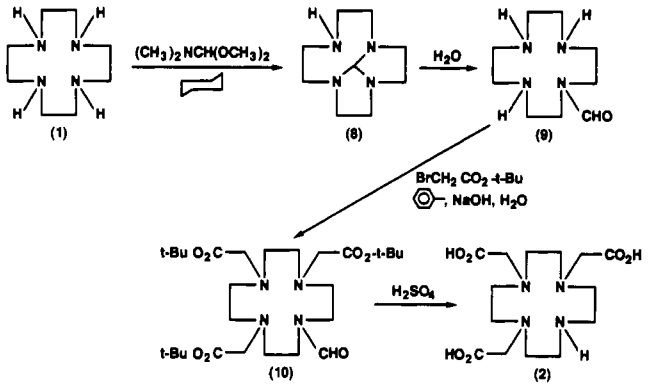
1-(Phenylmethyl)-1,4,7,10-tetraazacyclododecane Trihydrochloride (6). Into a 2-L three-neck round-bottom flask containing 14.5 g (0.020 mol) of **5** and 3.5 g (0.058 mol) of urea was condensed approximately 1 L of liquid NH_3. The flask was maintained in a dry ice/acetone bath while 6.0 g (0.26 mol) of sodium metal was added to the reaction mixture under a N_2 atmosphere. The reaction mixture was stirred with a blackened Teflon stirring bar. (A blackened Teflon stirring bar can be obtained by treating a white Teflon stirring bar with a solution of Na/NH_3. Alternatively, a glass stirring bar can be used instead of the blackened Teflon stirring bar.) The royal blue color of the reaction mixture (solvated electrons) was maintained for over 1 h, at which time sufficient NH_4Cl was added to the reaction mixture to quench the reaction. The liquid ammonia was allowed to evaporate overnight. The residue was dissolved in 300 mL of H_2O and concentrated to 150 mL on a rotary evaporator. The pH of the solution was made basic (>12) by the addition of 3 N NaOH. The basic solution was extracted with 800 mL of CH_2Cl_2 and concentrated on a rotary evaporator to 250 mL, and then the organic layer was extracted with 600 mL of 1 N HCl. The acidic solution was concentrated on a rotary evaporator to yield 2.6 g of the crude hydrochloride salt of **6** as a beige solid. The solid was then triturated with 100 mL of cold CH_3OH and the product collected by filtration. The white solid was then dried under vacuum for 16 h at 75 °C to yield 2.0 g (26.4%) of **6** as a trihydrochloride salt. Mass spectrum (CI): *m/e* 263 $[(M + H)^+]$. ^1H NMR (D_2O): δ 3.2 (m, 16 H), 4.1 (s, 2 H), 7.3 (s, 5 H). ^{13}C NMR (D_2O): δ 131.11, 130.84, 129.41, 129.10, 58.65, 49.34, 43.39, 42.99, 42.50. Anal. Calcd (found) for $C_{15}H_{26}N_4 \cdot 3.05HCl \cdot 0.31H_2O$: C, 47.51 (47.64); H, 7.89 (8.21); N, 14.77 (14.46); Cl, 28.51 (28.86); H_2O (1.49).



1,4,7,10-Tetraazacyclododecane-1-carboxaldehyde (9). To 246 g (1.35 mol) of 1,4,7,10-tetraazatricyclo[5.5.1.0]tridecane (**8**)[2] cooled to 4 °C was added 1 L of 50% aqueous ethanol that had been chilled to −20 °C. The mixture was allowed to slowly warm to room temperature and then stirred under N_2 for 24 h. The reaction mixture was then concentrated in vacuo and dissolved in 1 L of CH_3CN, and then the solution was concentrated in vacuo. This process was repeated three times to remove traces of H_2O. The residue was dried under vacuum at room temperature overnight to yield 270 g (100%) of **9** as a hygroscopic white solid. ^{13}C NMR (D_2O): δ 167.9, 50.8, 48.5, 47.8, 46.8, 46.7, 45.6, 45.4, 44.6. Anal. Calcd (found) for $C_9H_{20}N_4O \cdot 1.0H_2O$: C, 49.52 (49.38); H, 10.16 (10.06); N, 25.67 (25.46); H_2O (8.25).

(9) Hagan, J. J.; Taylor, S. C.; Tweedle, M. F. *Anal. Chem.* **1988**, *60*, 514.

Scheme III. Synthesis of 1,4,7-Tris(carboxymethyl)-1,4,7,10-Tetraazacyclododecane, DO3A



10-Formyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, Tris-(1,1-dimethylethyl) Ester (10). To 140.0 g (0.70 mol) of **9** dissolved in 560 mL of DMF with agitation under N_2 and cooled to 2.5 °C was added 546.2 g (2.8 mol) of *tert*-butyl bromoacetate over 13 min. The temperature of the reaction mixture was maintained at 30 °C for 35 min, during which time a thick orange slurry formed. Anhydrous sodium carbonate (296 g, 2.8 mol) dissolved in 2.8 L of H_2O was then added over 8 min. The resulting mixture was stirred for 30 min, after which 575 mL of toluene was added and the mixture allowed to stir for an additional 3.5 h. The reaction mixture was transferred with 250 mL of toluene to a separatory funnel, and the layers were separated. The toluene layer was extracted three times with 400 mL of 1 M Na_2CO_3, once with 1 L of 0.8 M HCl, and then with 500 mL of H_2O. The HCl and H_2O layers were combined and back-extracted with 500 mL of toluene. The aqueous layer was combined with 1.5 L of CH_2Cl_2 in an Erlenmeyer flask, and the pH of the solution was made basic (9.4) with the addition of 130 g of anhydrous Na_2CO_3. The neutralized mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted twice with 250 mL of CH_2Cl_2, and all of the CH_2Cl_2 layers were then combined and back-extracted twice with 250 mL of H_2O. The organic layer was then concentrated in vacuo at 30 °C to 400 mL. This solution was then used without additional purification in the preparation of **2**. Alternatively, **10** has also been isolated in pure form by chromatography of the crude product on silica gel. The desired compound was eluted from the column with 4% CH_3OH/CH_2Cl_2. The fractions containing the desired material were combined and concentrated in vacuo at room temperature to yield **10** as a viscous oil. ^{13}C NMR ($CDCl_3$): δ 170.9, 170.6, 163.5, 80.9, 80.8, 80.6, 57.8, 56.6, 54.7, 53.1, 52.5, 52.3, 51.9, 51.7, 50.9, 47.7, 43.2, 28.1. Anal. Calcd (found) for $C_{27}H_{50}N_4O_7 \cdot 0.93H_2O$: C, 57.96 (58.07); H, 9.34 (9.17); N, 10.01 (9.94); H_2O (3.0).

1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid (2) (Scheme III). The methylene chloride concentrate of **10**, described above, was slowly added over 1 h to 67 mL of concentrated H_2SO_4 dissolved in 1 L of H_2O. The aqueous solution was heated to 55–60 °C, and a vigorous nitrogen sparge was maintained throughout the addition of the methylene chloride concentrate. After 5 h, the reaction mixture was concentrated in vacuo to a thick residue and 50–75 mL of H_2O was added. The substances were then mixed thoroughly and reevaporated (to remove residual formic acid). The material was then dissolved in 400 mL of H_2O and applied to a 4-kg column of Reillex 425 PVP resin. The column was eluted with H_2O, and the fractions containing the desired compound were combined and concentrated in vacuo. The solution may optionally be lyophilized to provide 180 g (69%) of **2**. Anal. Calcd (found) for $C_{14}H_{26}N_4O_6 \cdot 1.42H_2O$: C, 45.19 (45.46); H, 7.82 (7.72); N, 15.06 (15.05); H_2O (6.89).

[1,4,7-Tris(carboxymethyl)-1,4,7,10-Tetraazacyclododecanato]gadolinium (11). To a solution of 106.52 g (0.3 mol) of **2** in 600 mL of H_2O was added 67.6 g (0.186 mol) of Gd_2O_3, and the suspension was refluxed for 16 h with stirring. The solution was then cooled to room temperature and filtered through a 0.2-μm filter. The filtrate was concentrated to dryness under reduced pressure to yield 153.45 g (99.6%) of **8** as an off-white solid. Mass spectrum (FAB): *m/e* 499$^+$–504$^+$ [(M + H)$^+$]. Anal. Calcd (found) for $C_{14}H_{23}N_4O_6Gd \cdot 4.20H_2O$: C, 29.17 (28.75); H, 5.49 (5.59); N, 9.72 (9.39); H_2O (13.14).

1,4,7-Tris(carboxymethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (12). To 194.0 g (0.56 mol) of **2** dissolved in 450 mL of deionized H_2O was added sufficient 5 N NaOH to increase the pH of the solution to 12.0–12.5. The temperature of the solution was maintained below 30 °C during the addition of the sodium hydroxide. To the solution was added 65 g (1.12 mol) of propylene oxide, and the solution was allowed to stir at ambient temperature for 6 h. The progress of the reaction was monitored by HPLC. After 6 h, the excess propylene oxide was removed from the reaction mixture by vacuum distillation at 30 °C. The solution was applied to a column containing 20 L of Amberlite IRA 900C resin (hydroxide form). The column was washed with three bed volumes of deionized water and the product eluted from the column by using one bed volume of 1 M H_2SO_4. The eluate was transferred directly to a column containing 28 L of Reillex 425 PVP resin. The column was eluted with H_2O and the eluate collected. The fractions containing the product were combined, concentrated to dryness, and then dissolved in a minimum amount of hot CH_3OH (1.5 L). Upon cooling to room temperature, the product precipitated from the solution. The thick slurry was diluted with EtOAc (2 L) and the solid filtered under suction to yield 218.4 g (96%) of **12** as a white amorphous solid. Mass spectrum (FAB): *m/e* 405$^+$ [(M + H)$^+$], 403$^-$ [(M – H)$^-$]. Anal. Calcd (found) for $C_{17}H_{32}N_4O_7 \cdot 0.06H_2O$: C, 50.34 (50.55); H, 7.98 (8.04); N, 13.81 (13.72); H_2O (0.28).

[1,4,7-Tris(carboxymethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecanato]gadolinium (13). To 20.22 g (0.050 mol, uncorrected for H_2O) of **12** dissolved in 100 mL of deionized H_2O was added 9.54 g (0.0263 mol) of Gd_2O_3. The suspension was allowed to stir at 95 °C for 20 h. The suspension was then filtered through a 0.2-μm filter. The clear solution was evaporated on a rotary evaporator to yield a glassy solid. The solid was crystallized from 50% CH_3OH/CH_3COCH_3, filtered, and dried in a vacuum oven (75 °C) for 48 h, to yield 15.8 g (56%) of **13** as a white solid; mp >225 °C. Mass spectrum (FAB): *m/e* 557$^+$–562$^+$ [(M + H)$^+$]. Infrared spectrum (KBr, cm^{-1}): 3429, 2978, 2861, 1708, 1613, 1385, 1325, 1085. Anal. Calcd (found) for $C_{17}H_{29}N_4O_7Gd \cdot 0.31H_2O$: C, 36.19 (36.48); H, 5.29 (5.66); N, 9.93 (9.88); H_2O (0.98).

1,4,7-Tris(carboxymethyl)-10-(2,3-dihydroxypropyl)-1,4,7,10-tetraazacyclododecane (14). To 11.97 g of **2** (0.033 mol, adjusted for 3.7% H_2O) in 300 mL of H_2O was added 5 N NaOH to increase the pH of the solution to 9.7. To this was added 3.77 mL (4.21 g, 0.057 mol, 1.7 equiv) of glycidol and the solution allowed to stir at room temperature. After 22 h, an additional 1.1 mL (1.23 g, 0.017 mol, 0.51 equiv) of glycidol was added and the reaction allowed to continue for an additional 72 h. The reaction mixture was then purified by anion-exchange chromatography as described for compound **12** to yield 10.5 g (68%) of **14** as a white solid. Mass spectrum (FAB): *m/e* 421 [(M + H)$^+$]. Anal. Calcd (found) for $C_{17}H_{32}N_4O_8 \cdot 2.85H_2O$: C, 43.28 (43.26); H, 8.05 (8.03); N, 11.88 (11.59); H_2O (10.88).

[1,4,7-Tris(carboxymethyl)-10-(2,3-dihydroxypropyl)-1,4,7,10-tetraazacyclododecanato]gadolinium (15). To 9.5 g (0.020 mol, corrected for 10.88% H_2O) of **14** dissolved in 200 mL of H_2O was added 4.30 g (0.0118 mol) of Gd_2O_3 and the suspension allowed to stir at 80 °C for 14 h. After 14 h, the cloudy solution was cooled to room temperature and then filtered through a 0.2-μm filter. The solution was adjusted to pH 9.7 with concentrated NH_4OH and applied to a 2.5 × 40 cm column of Chelex-100 (NH_4^+ form). The column was eluted with H_2O and the eluate collected and rotary evaporated to yield a white solid. The solid was subsequently purified via preparative HPLC (Rainin Dynamax C_{18} 4.5 × 45 cm column, 2% CH_3CN, 15 mL/min) to yield 7.91 g (43%) of **15** as a white solid. Mass spectrum (FAB): *m/e* 576$^+$ [(M + H)$^+$], 574$^-$ [(M – H)$^-$]. Anal. Calcd (found) for $C_{17}H_{29}N_4O_8Gd \cdot 4.11H_2O$: C, 31.47 (31.64); H, 5.78 (5.76); N, 8.64 (8.62); H_2O (11.43).

1,4,7-Tris(carbamoylmethyl)-10-(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (16). The pH of a solution of 1.40 g (3.84 mmol, assuming 5% H_2O) of **2** in 4 mL of H_2O was adjusted to 9.5 with 40% aqueous benzyltrimethylammonium hydroxide. To the resulting solution was added 412 mg (4.4 mmol, 1.15 equiv) of 2-chloroacetamide. The temperature was increased to 80 °C, and base was added as necessary to maintain the pH at 9.5–10. After 3 h, the solution was cooled to room temperature and the pH of the solution lowered to 3 with concentrated HCl. The resulting solution was evaporated under reduced pressure to a colorless sludge. The mixture was taken up in about 25 mL of CH_3OH, and the solution was reevaporated. The thick residue was triturated with a 1:1 mixture of acetone and ethanol to provide a granular solid and colorless solution. The solid was collected by filtration, washed with acetone/ethanol followed by acetone and finally ether, and then dried in a vacuum oven at 50 °C for 2 h to yield 1.54 g (3.23 mmol, 84%) of **16** as a colorless powder. The product was twice recrystallized from ethanol/water. Mass spectrum (FAB): *m/e* 404$^+$ [(M + H)$^+$], 402$^-$ [(M – H)$^-$]. Anal. Calcd (found) for $C_{16}H_{31}N_5O_7Cl_2 \cdot 0.27H_2O$: C, 39.94 (39.96); H, 6.61 (6.74); N, 14.55 (14.33).

[1,4,7-Tris(carboxymethyl)-10-(carbamoylmethyl)-1,4,7,10-tetraazacyclododecanto]gadolinium (17). A mixture of 87 mg (0.21 mmol) of **16** and 40 mg (0.22 mmol of Gd^{3+}, 1.05 equiv) of Gd_2O_3 in 0.8 mL of H_2O was heated to 80 °C for 3 h. After cooling to room temperature, the

slightly cloudy solution was filtered through a 0.22-μm filter. The water was removed under reduced pressure. The residue was crystallized from a mixture of H_2O/EtOH/CH_3CN (1:2:4) to yield 104 mg (83%) of **17** as a colorless solid. Anal. Calcd (found) for $C_{16}H_{26}N_5O_7Gd\cdot 2.03H_2O$: C, 32.33 (32.59); H, 5.10 (5.10); N, 11.78 (11.62); H_2O (6.18).

{1,4,7-Tris(carboxymethyl)-10-[(*N*-methylcarbamoyl)methyl]-1,4,7,10-tetraazacyclododecanato}gadolinium (19). To 7.08 g (0.019 mol, adjusted for 8.1% H_2O) of **2** dissolved in 68 mL of H_2O was added sufficient 5 M KOH to increase the pH of the solution to 9.5. To this solution was added 4.4 g (0.040 mol) of *N*-methyl-2-chloroacetamide. The pH of the solution was maintained at 9.5 and the solution allowed to stir at 50 °C. After 23 h, the solution was cooled to ambient temperature, the pH of the solution was adjusted to 3.0 with concentrated HCl, and the solution was applied to a 4.7 × 25 cm column of Dowex 50-X2 (strong cation-exchange resin, H^+ form). The column was rinsed with 4 L of H_2O and then with 1 L of 0.5 M NH_4OH. The eluate was collected and concentrated on a rotary evaporator. The residue was dissolved in 20 mL of CH_3OH and then precipitated by the addition of 100 mL of acetone to yield 3.9 g of the ammonium salt of 1,4,7-tris-(carboxymethyl)-10-((*N*-methylcarbamoyl)methyl)-1,4,7,10-tetraazacyclododecane (**18**).

To a solution of 3.5 g (approximately 8.3 mmol) of **18** dissolved in 33 mL of H_2O was added 1.58 g (0.0044 mol) of Gd_2O_3. The pH of the solution was adjusted to 4.0 with glacial acetic acid and the solution allowed to stir at 100 °C. After 2 h, the solution was cooled to ambient temperature and filtered through a 0.2-μm filter. The pH of the filtrate was adjusted to 8.5 with NH_4OH, and the solution was applied to a 2.5 × 20 cm column of Chelex-100 (NH_4^+ form). The column was eluted with water, and the eluate was collected and concentrated on a rotary evaporator. The residue was then dissolved in 10 mL of H_2O, and the solution was purified via preparative HPLC (Rainin Dynamax C_{18} on silica, 4.25 × 45 cm column, 100% H_2O, 10 mL/min) to yield 3.1 g (58%) of **19** as a white solid. Mass spectrum (FAB): *m/e* 571$^+$ [(M + H)$^+$], 569$^-$ [(M − H)$^-$]. Anal. Calcd (found) for $C_{17}H_{28}N_5O_7Gd\cdot 4.08H_2O$: C, 31.69 (31.67); H, 5.50 (5.44); N, 10.87 (10.48); H_2O (11.47).

{1,4,7-Tris(carboxymethyl)-10-[*N*-(2-hydroxyethyl)carbamoyl]-1,4,7,10-tetraazacyclododecanato}gadolinium (21). To 5.0 g (13.3 mmol, corrected for 8.1% H_2O) of **2** dissolved in 50 mL of H_2O was added sufficient 5 M KOH to increase the pH of the solution to 9.5. To this solution was added 3.98 g (28.9 mmol, 2.17 equiv) of *N*-(2-hydroxyethyl)-2-chloroacetamide in 10 mL of H_2O. The pH of the solution was adjusted to 9.5 and allowed to stir at 80 °C. After 24 h, the solution was cooled to 21 °C and adjusted to pH 3.5 with concentrated HCl. The solution was diluted with 200 mL of H_2O and applied to a 4.5 × 20 cm column of Dowex 50-X2 (strong cation-exchange resin, H^+ form). The column was eluted with 2 L of H_2O and then with 800 mL of 0.5 M NH_4OH. The ammonium hydroxide eluate was concentrated on a rotary evaporator to yield 6.53 g of the crude ammonium salt of 1,4,7-tris-(carboxymethyl)-10-[*N*-(2-hydroxyethyl)carbamoyl]-1,4,7,10-tetraazacyclododecane (**20**).

To 5.0 g (approximately 11 mmol) of the crude ammonium salt of **20** in 60 mL of H_2O was added 2.04 g (0.0056 mol) of Gd_2O_3. The pH of the solution was adjusted to 4.0 with glacial acetic acid and allowed to stir at 100 °C. After 5 h, the suspension was filtered through a 0.2-μm filter. The pH of the filtrate was adjusted to 9 with concentrated NH_4OH and the solution applied to a 2.5 × 25 cm column of Chelex-100 (NH_4^+ form). The column was eluted with H_2O and the eluate collected and concentrated under reduced pressure to a glassy solid. The solid was then dissolved in 10 mL of H_2O, and the sample was then purified via preparative HPLC (Rainin Dynamax C_{18} on silica 4.25 × 45 cm column, 100% H_2O, 10 mL/min) to yield 3.5 g (43%) of **21** as an off-white solid. Mass spectrum (FAB): *m/e* 603$^+$ [(M + H)$^+$], 601$^-$ [(M − H)$^-$]. Anal. Calcd (found) for $C_{18}H_{30}N_5O_8Gd\cdot 0.66H_2O$: C, 35.23 (35.02); H, 5.14 (5.60); N, 11.42 (11.56); H_2O (1.94).

1-Methyl-4,7,10-tris[(4-methylphenyl)sulfonyl]-1,4,7,10-tetraazacyclododecane (22). To 63.5 g (0.10 mol) of **4** dissolved in 400 mL of dry DMF was added 55.3 g (0.40 mol) of K_2CO_3 and 14.2 g (0.10 mol) of CH_3I, and the mixture was allowed to stir at ambient temperature. After 16 h, the mixture was diluted with 2 L of H_2O and extracted with 0.5 L of CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. The concentrate was then partially purified via flash chromatography (silica gel, 10% EtOH/CH_2Cl_2) to yeidl 55 g of crude **22**. A 25-g sample of crude **22** was then purified via flash chromatography (silica gel, 5% EtOAc/CH_2Cl_2) to yield 16.1 g (54%) of **22** as a white solid; mp 196–200 °C dec. Mass spectrum (CI): *m/e* 649$^+$ [(M + H)$^+$]. ^{13}C NMR ($CDCl_3$): δ 143.40, 143.17, 136.58, 134.94, 129.64, 127.48, 127.25, 59.29, 51.57, 49.73, 48.49, 42.24, 21.42, 21.36. Anal. Calcd (found) for $C_{30}H_{40}N_4O_6S_3$: C, 55.53 (55.51); H, 6.21 (6.17); N, 8.64 (8.66).

1-Methyl-1,4,7,10-tetraazacyclododecane (23). To 16.0 g (0.024 mol) of **22** was added 125 mL of H_2SO_4 and the mixture allowed to stir at 100 °C under N_2. After 48 h, the solution was cooled to 20 °C and then slowly poured over 300 g of ice. The solution was then made strongly basic (pH >13) by the careful addition of 6 N NaOH. The basic solution was then extracted with $CHCl_3$ (200 mL, 4×) and the extract was dried over $MgSO_4$ and concentrated on a rotary evaporator. The residue was partially purified via sublimation and then crystallized from cyclohexane to yield 0.95 g (20%) of **23**; mp 70–71 °C. Mass spectrum (CI): *m/e* 187$^+$ [(M + H)$^+$]. ^{13}C NMR ($CDCl_3$): δ 54.22, 47.28, 46.78, 45.25, 43.79. Anal. Calcd (found) for $C_9H_{22}N_4$: C, 58.02 (58.19); H, 11.90 (12.20); N, 30.08 (30.15).

1,4,7-Tris(carboxymethyl)-10-methyl-1,4,7,10-tetraazacyclododecane (24). To 1.52 g (0.0815 mol) of **23** dissolved in 16 mL of H_2O was added 2.33 g (0.0247 mol) of chloroacetic acid. The pH of the solution was increased to 9.5 by the addition of 5 N NaOH, and the reaction was allowed to stir at 50 °C for 28 h. The pH of the solution was maintained at 9.5–10.0 by the addition of 5 N NaOH. After 28 h, the solution was cooled to 20 °C, and the pH of the solution was lowered to 3.0 by the addition of concentrated HCl. The compound was then purified as described for compound **12** to yield 1.4 g (48%) of **24** as a white solid; mp >225 °C. Mass spectrum (FAB): *m/e* 361$^+$ [(M + H)$^+$]. ^{13}C NMR (D_2O): δ 175.91, 169.87, 55.94, 54.99, 52.75, 51.35, 48.73, 48.45, 42.50. Anal. Calcd (found) for $C_{15}H_{29}N_4O_6\cdot 0.21H_2O$: C, 49.46 (49.24); H, 7.87 (7.86); N, 15.38 (15.04); H_2O (1.06).

[1,4,7-Tris(carboxymethyl)-10-methyl-1,4,7,10-tetraazacyclododecanato]gadolinium (25). To 2.5 g (0.0069 mol) of **24** dissolved in 14 mL of H_2O was added 1.38 g (0.0038 mol) of Gd_2O_3 and the suspension heated to 85 °C and allowed to stir for 16 h. After 16 h, the suspension was cooled to 20 °C and then filtered through a 0.2-μm filter. The solution was then applied to a 5 × 45 cm column of Chelex-100 (NH_4^+ form). The column was eluted with H_2O at a flow rate of 15 mL/min. The eluate was collected and concentrated on a rotary evaporator to yield a white solid, which was subsequently purified via preparative HPLC (Rainin Dynamax C_{18} on Silica 4.25 × 45 cm column, 98% H_2O, 2% CH_3CN, 20 mL/min) to yield 2.6 g of **25** as a white solid. Anal. Calcd (found) for $C_{15}H_{26}N_4O_6Gd\cdot 0.64H_2O$: C, 34.17 (33.94); H, 5.22 (5.16); N, 10.63 (10.26); H_2O (2.19).

Results and Discussion

We have evaluated a variety of synthetic pathways to the core ligand 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, DO3A (**2**). The most direct synthesis of **2** involves the alkylation of the commercially available 1,4,7,10-tetraazacyclododecane (**1**) with chloroacetic acid followed by ion-exchange chromatography (Scheme I). As expected, this method did not provide a high yield of the trisalkylated material even when only 3 equiv of chloroacetic acid were used. The major side product in this case was the tetrasubstituted macrocycle DOTA (**3**), which was removed during ion-exchange chromatography.

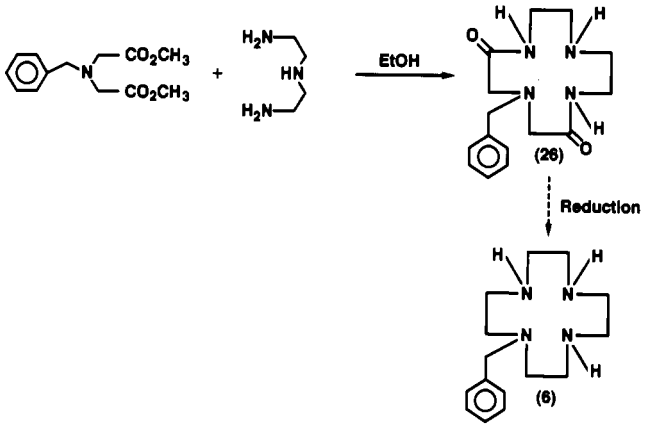
In an effort to improve the yield of DO3A, we prepared substituted 1,4,7,10-tetraazacyclododecanes, in which the protecting group, either benzyl or formyl, could be readily removed after reaction of the protected macrocycle with chloroacetic acid. We have found that the hydrolysis of 1,4,7,10-tetraazatricyclo-[5.5.1.0]tridecane (**8**) yields the corresponding formyl tetraaza-macrocycle (**9**) in a near-quantitative yield.[1] (Analogous chemistry on the selective N-protection of medium-ring triamines yielding formyl cyclic triamines was reported by Weisman et al. in 1987.[10])

The synthesis of 1,4,7,10-tetraazacyclododecane-1-carboxy-aldehyde (**9**) has permitted us to develop a more efficient synthesis of DO3A from 1,4,7,10-tetraazacyclododecane (**1**). The reaction of **9** with *tert*-butyl bromoacetate followed by simultaneous acid removal of both the *tert*-butyl and formyl protecting groups has permitted the synthesis of **2** in gram to kilogram quantities. The relative ease of formation of the key intermediates in the latter approach coupled with the high yields associated with each step make this method the most efficient one for the synthesis of DO3A (**2**).

We also evaluated the synthesis of 1-(phenylmethyl)-1,4,7,10-tetraazacyclododecane (**6**) via different synthetic approaches. Our first approach was to prepare the benzyl diamide **26** which could then be reduced with diborane to yield **6**. Com-

(10) Weisman, G. R.; Vachon, D. J.; Johnson, V. B.; Gronbeck, D. G. *J. Chem. Soc., Chem. Commun.* **1987**, 886.

Scheme IV. Proposed Route to the Synthesis of 1-(Phenylmethyl)-1,4,7,10-Tetraazacyclododecane (**6**)

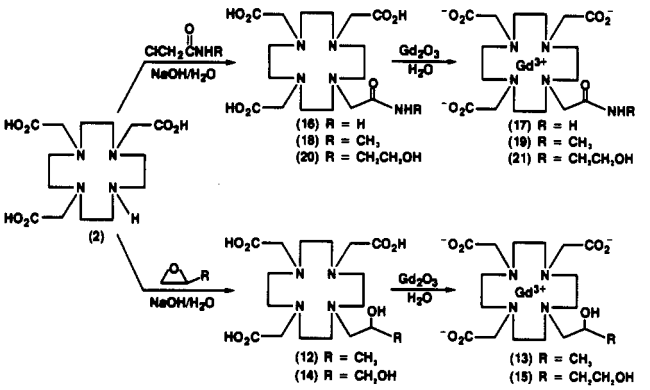


pound **26** was prepared, but in low yield (12%), via the high dilution condensation of dimethyl *N*-benzyliminodiacetate with diethylenetriamine. This poor yield discouraged further development on this approach to **6** (Scheme IV).

Another approach to prepare **6** was through a multistep synthesis in which the final step required the detosylation of 1-(phenylmethyl)-4,7,10-tritosyl-1,4,7,10-tetraazacyclododecane (**5**) (Scheme II). Two routes were studied for the preparation of 1-(phenylmethyl)-4,7,10-tritosyl-1,4,7,10-tetraazacyclododecane (**5**). The first route was based on the procedure of Richman and Atkins[6] in which the sodium salt of 4-benzyl-1,7-ditosyl-1,4,7-diethylenetriamine was reacted with the tritosylate of diethanolamine in DMF. Although the desired product was obtained, this process required the synthesis of a variety of precursors and the overall yield was only moderate. The second route was based on the direct alkylation of 1,4,7-tritosyl-1,4,7,10-tetraazacyclododecane (**4**) with benzyl bromide to yield **5**. In 1984 Ciampolini et al.[11] reported a 17% yield of **4** via the reaction of the disodium salt of 1,4,7-tritosyldiethylenetriamine and bis(chloroethyl)amine in DMF. We have found that **4** can be prepared directly and in high yield (80%) from commercially available 1,4,7,10-tetraazacyclododecane (**1**) and *p*-toluenesulfonyl chloride in CH_2Cl_2 and triethylamine at 0 °C. Detosylation of **5** in Na/NH_3 yielded **6** but in only a low yield (26%). Others have reported the detosylation of macrocyclic compounds containing both the benzyl and tosyl groups by treatment with HBr/glacial acetic acid/phenol.[12]

The subsequent reaction of **6** with chloroacetic acid in $NaOH/H_2O$ yielded 1,4,7-tris(carboxymethyl)-10-(phenylmethyl)-1,4,7,10-tetraazacyclododecane (**7**). Removal of the benzyl group by catalytic hydrogenation over Pd/C yeidled our core ligand, DO3A (**2**).

Scheme V. Derivatization of DO3A and Synthesis of the Corresponding Gadolinium Chelates



Derivatization of DO3A (**2**) was readily accomplished by reacting **2** with various alkylating agents in aqueous NaOH (or KOH or benzyltrimethylammonium hydroxide) (Scheme V). The ligand can be purified at this stage through anion-exchange chromatography, or the crude ligand can be subsequently reacted with gadolinium oxide and the gadolinium chelates purified via preparative HPLC or crystallization.

The gadolinium chelates reported in this paper have been thoroughly evaluated as potential candidates for use as contrast agents in magnetic resonance imaging. The results of these biological studies as well as physical–chemical measurements will be reported separately.[13-16]

Conclusions

We have prepared a new and synthetically useful ligand, 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (**2**). The most efficient synthesis of our core ligand **2** involved the synthesis of a novel intermediate **9** prepared by the hydrolysis of the orthoamide **8**. DO3A (**2**) is easily derivativized providing potentially octadentate ligands. These ligands can be reacted with gadolinium oxide to form stable, neutral, water-soluble chelates useful as contrast agents in magnetic resonance imaging.[17]

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(11) Ciampolini, M.; Micheloni, M.; Nardi, N.; Vizza, F.; Buttafava, A.; Fabbrizzi, L.; Perotti, A. *J. Chem. Soc., Chem. Commun.* **1984**, 998.
(12) Spirolet, M.-R.; Rebizant, J.; Desreux, J. F.; Loncin, M.-F. *Inorg. Chem.* **1984**, *23*, 359.
(13) Wedeking, P. W.; Tweedle, M. F. *Nucl. Med. Biol.* **1988**, *15*, 395.
(14) Tweedle, M. F.; Hagan, J. J.; Kumar, K.; Mantha, S.; Chang, C. A. To be submitted for publication. Data report in: *Abstracts of the 6th Annual Meeting of The Society of Magnetic Resonance in Medicine*; 1987; Vol. 2, p 477.
(15) Chang, C. A.; Francesconi, L. C.; Dischino, D. D.; Malley, M.; Gougoutas, J.; Tweedle, M. F. To be submitted for publication.
(16) Tweedle, M. F. Nonionic Macrocyclic Gadolinium(III) Complexes. In *Contrast and Contrast Agents in Magnetic Resonance Imaging*, European Workshop on Magnetic Resonance Imaging, State University, Mons, Belgium, B 7000, 1989; Rink, P. A., Ed. (work in progress).
(17) Compound **13**, [1,4,7-tris(carboxymethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecanto]gadolinium, Gd(HP-DO3A), Gadoteridol, ProHance, is currently in clinical trials as a contrast agent in magnetic resonance imaging.