Synthesis of DTPA Analogues Derived from Piperidine and Azepane: Potential Contrast Enhancement Agents for Magnetic Resonance Imaging

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Two DTPA derivatives (PIP-DTPA and AZEP-DTPA) as potential contrast enhancement agents in MRI are synthesized. The T1 and T2 relaxivities of their corresponding Gd(III) complexes are reported. At clinically relevant field strengths, the relaxivities of the complexes are comparable to that of the contrast agent, Gd(DTPA) which is in clinical use. The serum stability of the ^{153}Gd-labeled complexes is assessed by measuring the release of ^{153}Gd from the ligands. The radiolabeled Gd chelates are found to be kinetically stable in human serum for up to at least 14 days without any measurable loss of radioactivity.

Introduction

DTPA (diethylenetriaminepentaacetic acid) and its derivatives have been used in MRI,[1] radiotherapy,[2] and radiodiagnostics.[3] In particular, its gadolinium complex, Gd(DTPA), has been successful as a contrast agent in MRI and is currently in clinical use along with several derivatives.[1] Although Gd(III) is an optimal paramagnetic metal for MRI due to high electronic spin and high relaxation rate, its toxicity produces severe side effects. Thus, it is required that Gd(III) complexes be stable in vivo and excreted completely after administration. Research has been focused on the synthesis and physical characteristics of new Gd(III) complexes to minimize toxicity while maintaining high relaxation enhancement.[4]

In general, conformational constraint of chelating agents, i.e., inclusion of a rigid structure to control the geometry of the metal binding donor groups may have a significant effect on the stability of the formed metal complexes.[5] For example, we have reported that DTPA derivatives having a rigid cyclohexyl ring have shown considerable improvement of in vivo stability.[6] We have also found that the stereochemistry of cyclohexyl substituted DTPA derivatives can provide significant increases on the in vivo stability of their metal complexes.[6e] High lipophilicity is known to result in greater hepatobiliary clearance. Some DTPA chelates with suitable liphophilicity are found to provide significantly enhanced hepatobiliary clearance as compared to the parent DTPA.[7]

In our continuing effort to develop Gd(III) contrast agents for MRI,[8] DTPA chelates (AZEP-DTPA, **1**, and PIP-DTPA, **2**, vide infra) based on either a piperidine ring or an azepane ring were designed and synthesized. The liphophilicity and rigidity of these chelates are greater than those of the parent DTPA. Substitution of either the piperidine ring or an azepane ring with an amino group of the DPTA is expected to potentially increase complex stability and hepatobiliary clearance of the system in terms of liphophilicity and rigidity.

Herein, we report the synthesis of two DTPA derivatives and relaxometric studies of the corresponding Gd

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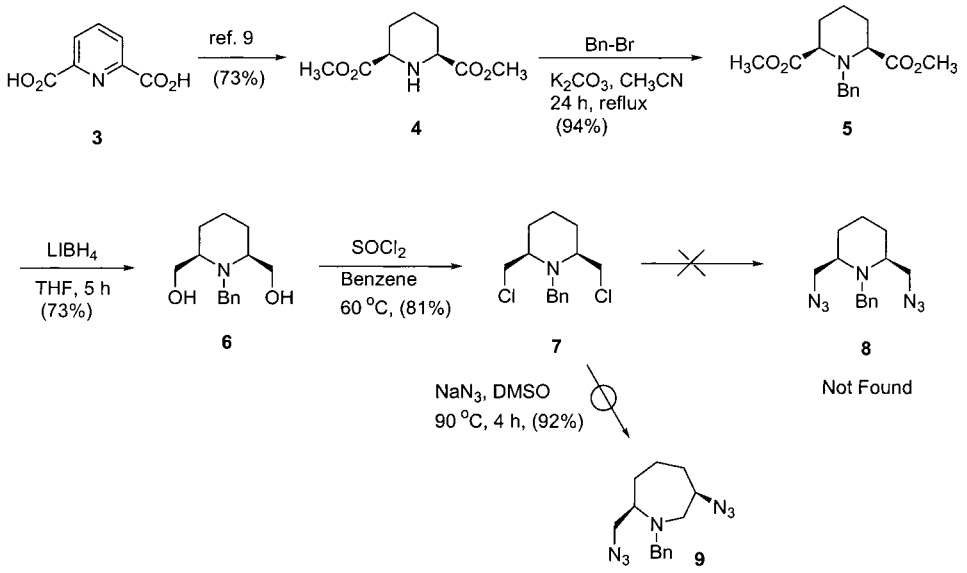
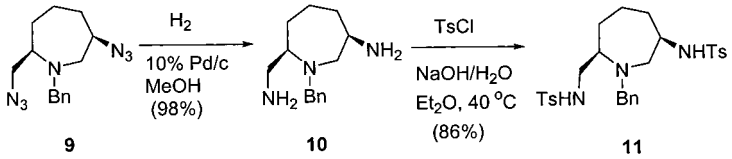
Scheme 1



Scheme 2



complexes. The serum stability of the ^{153}Gd-labeled complexes was investigated for an understanding of the in vitro dissociation of the complexes.

Result and Discussion

Synthesis of DTPA Derivatives 1 and 2. The azepane-backboned DTPA **1** was in fact prepared by a fortuitous discovery of a rearrangement leading to diazide **9** while in route to prepare triamine **18**. The synthetic route for diazide **9** is summarized in Scheme 1. Commercially available 2,6-pyridinedicarboxylic acid **3** was used as starting material and converted into *cis*-2,6-bis-(methoxycarbonyl)piperidine (**4**) in 73% yield by following the reported procedure of Chênevert and Dickman.[9] Reaction of **4** with benzyl bromide in the presence of K_2CO_3 produced compound **5** in 94% yield. The base-promoted reaction of **5** with $LiBH_4$ in THF produced the N-benzylated diol **6**[10] in 73% yield. The subsequent reaction of **6** with $SOCl_2$ provided **7**,[11] which was reacted with NaN_3 in DMSO. However, the latter reaction provided only the ring-expanded diazide **9** rather than the expected compound **8**. As an initial effort to solve the structure of diazide **9**, mass spectra, ^{1}H, and ^{13}C NMR spectra were analyzed. The NMR spectra reveal loss of molecular symmetry, which was expected for desired diazide **8**. In its ^{13}C NMR spectrum, diazide **9** displays four peaks between δ = 127.3−138.3 ppm as expected from the aromatic unit and eight peaks between δ = 22.0−61.2 ppm, two of which are the additional peaks based on symmetry. A triplet signal at δ 34.8 ppm strongly indicates that the piperidine moiety in the starting material **7** has disappeared and the diazide **9** does not possess the plane of symmetry as in **7**. The ^{1}H NMR spectrum of **9** shows a multiplet at δ 3.4 ppm for the C-6 methine proton. In addition, a doublet at 3.31 ppm for the methylene group at the side chain and a multiplet at δ 2.89 ppm for the C-7 methylene proton and the C-2 methine proton were observed, supporting the structure of the ring-expanded **9**. The HRMS produced a molecular ion peak at *m*/*z* 286, which corresponds to the expected diazide **8,** but also supports the structure of **9** (see the Experimental Section). Ultimately, viscous diazide **9** was converted to tosylate **11** to confirm structure and stereochemistry via X-ray analysis (Scheme 2). Thus, diazide **9** was hydrogenated and subsequently reacted with TsCl to afford compound **11**. The structure and stereochemistry of the derivative **11** was confirmed via application of X-ray diffraction methods.[12]

It seems likely that ring expansion leading to diazide **9** proceeds via an aziridinum intermediate, which further undergoes regioselective nucleophilic attack of azide anion at the methine carbon.[12] In fact, several reports on synthesis of a few interesting piperidine or azepane derivatives via similar ring expansion were made in the literature.[13] However, these reports showed that the ring-expanded products were routinely obtained along with the expected products, which were then inseparable by chromatographic techniques. The result of rearrangement shown herein, to the best of our knowledge, is a

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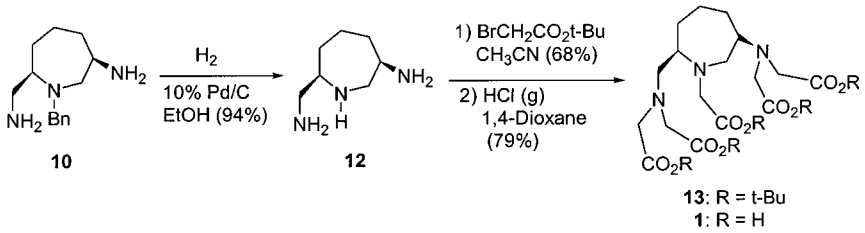
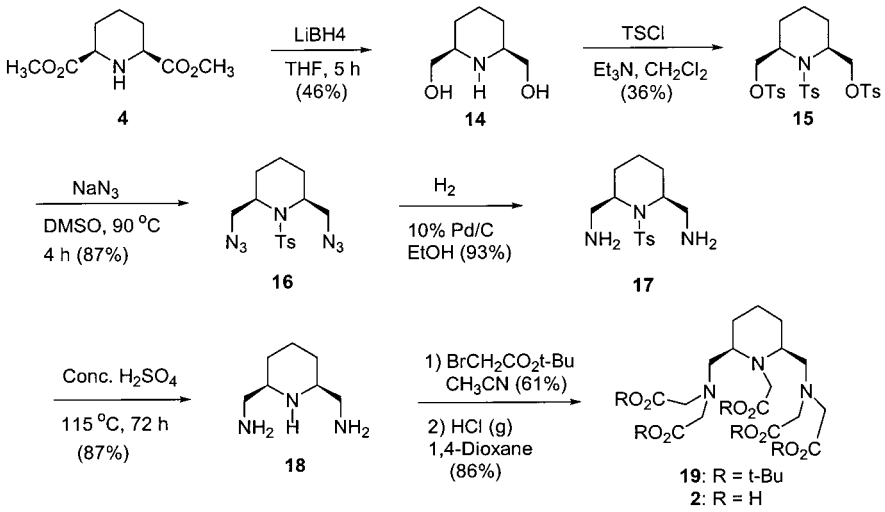
Scheme 3



Scheme 4



unique example affording only the ring-expanded product with exclusive regioselectivity. Furthermore, this rearrangement is of particular value in that the rearranged product was obtained only as diastereomerically pure cis isomer and in high yield (92%). This example might provide a useful entry for the synthesis of precursor molecules of azepane or piperidine-backboned natural products[14] such as (−)-balanol,[14a] a fungal metabolite having potent protein kinase C inhibitory properties.

Our continuing interest in the synthesis of chelating agents prompted us to prepare the DTPA derivative **1** (AZEP-DTPA) from the rearranged diazide **9**, which contains a DTPA moiety. The procedure used to prepare **1** is shown in Scheme 3. Thus, triamine **12** was obtained by de-N-benzylation of compound **10**, and subsequent alkylation with *tert*-butyl bromoacetate provided **13** in 68% yield. The penta-acid **1** as a HCl salt was obtained after deprotection of the *tert*-butyl ester (HCl(g)/dioxane).

The synthetic route for DTPA derivative **2** (PIP-DTPA)-having a piperdine backbone[15] is outlined in Scheme 4. The starting material, *cis*-2,6-bis(methoxycarbonyl)piperidine (**4**),[9] was directly reduced to 2,6-dimethanolpiperidine (**14**)[16] under similar reaction conditions used for the preparation of **6**. To eliminate the possibility of rearrangement, the free amine of **14** was protected with a tosyl group. Thus, **14** was treated with TsCl to afford compound **15**, which on reaction with sodium azide provided the expected diazide **16** in 87% yield. Interestingly, the tosyl-protected compound **15** did not afford any rearranged diazide contrary to **7** protected by a benzyl group (see the Experimental Section).

Reductive hydrogenation of diazide **16** afforded **17** in 93% yield and subsequent deprotection of the tosyl group with concentrated sulfuric acid provided 2,6-bis(aminomethyl)piperidine (**18**) in high yield. The triamine **18** was alkylated with *tert*-butyl bromoacetate to afford **19** in 61% yield. The *tert*-butyl group in **19** was removed (HCl(g)/dioxane) to provide pentaacid **2** in 86% yield.

Serum Stability. The serum stability of the Gd(III) complexes was assessed by measuring the release of ^{153}-Gd from the chelates (**1** and **2**). Serum stability of the ^{153}Gd-labeled complexes was evaluated for up to 14 days. The results thereby obtained are shown in Figure 1. The two Gd(III) complexes are found to be stable in serum at least for 14 days without any noticeable loss of radioactivity. Previously, we reported that, in serum, Gd-(DTPA) lost 20% of the radioactivity after 5 days (Figure 1).[8a] Although serum stability is not necessarily an absolute indicator of in vivo stability, it is noteworthy that the two novel DTPA derivatives studied herein display superior stability in serum as compared to the parent DTPA when coordinated to Gd(III).

Relaxivity. T1 and T2 NMRD profiles for the Gd(III) complexes are depicted in Figures 2 and 3, respectively. For comparison, the T1 and T2 relaxivities of the commercially available Gd(DTPA) (Magnevist) are measured. Qualitatively and quantitatively, the T1 and T2 NMRD profiles for the Gd(III) complexes Gd(PIP-DTPA) and Gd(AZEP-DTPA) are similar to those of the clinically used MR contrast agent GdDTPA (Magnevist) (Figures 2 and 3) and to those of other previously reported Gd acylic and macrocyclic complexes where the Gd(III) is

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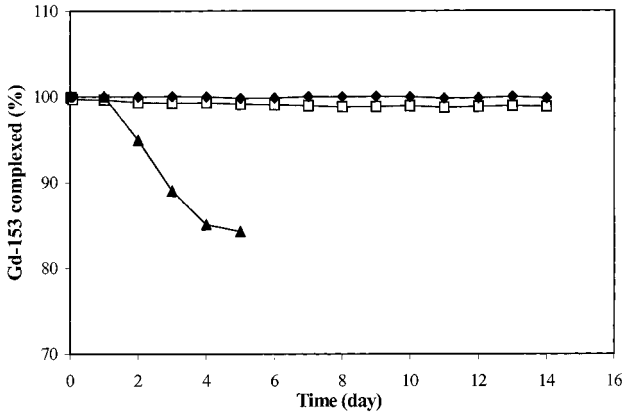

Figure 1. Serum stability of Gd(AZEP-DTPA) (□), Gd(PIP-DTPA) (♦), and Gd(DTPA) (▲)[a] at pH 7 and 37 °C. [a]Previous result in ref 8a.

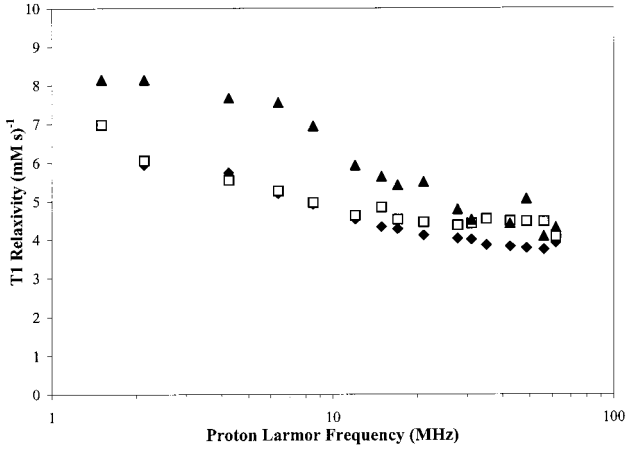

Figure 2. Plots of T1 relaxivity for Gd(AZEP-DTPA) (□), Gd-(PIP-DTPA) (♦), and Gd(DTPA) (▲) at pH 7 and 23 °C.

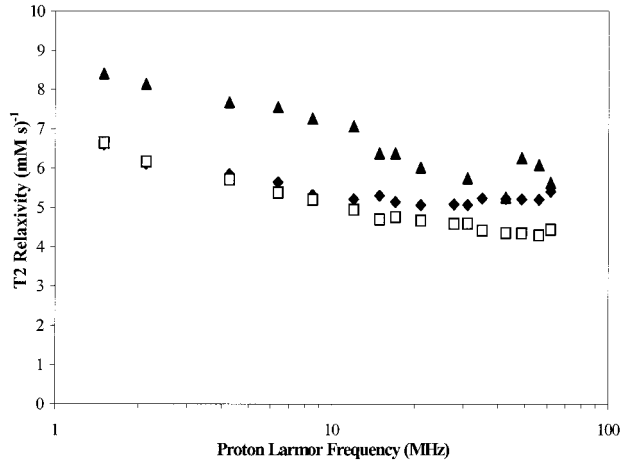

Figure 3. Plots of T2 relaxivity for Gd(AZEP-DTPA) (□), Gd-(PIP-DTPA) (♦), and Gd(DTPA) (▲) at pH 7 and 23 °C.

coordinated to the eight donor atoms of the ligands with one vacant coordination site available to bind a water molecule.[1a] The relaxation rates for the Gd(III) chelates are dominated by the inner-sphere dipolar coupling between the coordinated water molecule and the paramagnetic Gd(III). The measured relaxivities arise from the exchange between the coordinated water molecule and the surrounding water molecules in solution. An increase in the number of coordinated water molecules to the Gd(III), which could have occurred with dissociation of the Gd(III) from the chelate in solution, would have resulted in a significant increase in the measured relaxivities. No indication of Gd dissociation is evident from the NMRD profiles, which is in agreement with the serum stability studies.

Conclusion

The azepane- and piperidine-backboned DTPA derivatives (**1** and **2**) for gadolinium contrast agents in MRI have been synthesized. The azepane-backboned DTPA **1** was prepared by a fortuitous discovery of a rearrangement leading to diazide **9**, which was isolated as a single regioselective isomer and in high yield (92%). The result of the rearrangement might be applied to synthesis of precursor molecules for azepane or piperidine-backboned natural products. The T1 and T2 relaxivities and the serum stability of the corresponding Gd(III) complexes are reported. Serum stability studies show that the novel Gd(III)-labeled complexes are stable in serum for up to at least 14 days without any measurable loss of radioactivity, a very promising result in that the clinically used Gd(DTPA) loses 20% of ^{153}Gd after 5 days. The relaxivites of two Gd(III) complexes are comparable to that of the contrast agent, Gd(DTPA) which is in clinical use. The similar relaxivity to the clinically used MR contrast agent, Gd(DTPA) (Magnevist) and the exceptional serum stability indicate that the chelates studied herein may definitely be effective in vivo MR contrast agents. Further toxicological evaluations such as biodistribution and blood clearance studies of these promising MR contrast agents will be disclosed in the due course.

Additionally, these chelates might be modified for the covalent attachment to biomolecules (bifunctional chelating agents) or may be derivatized for noncovalent interaction with in-vivo serum proteins for use in MR angiography and perfusion. Studies on chelate modifications are currently being pursued.

Experimental Section

General Methods. ^{1}H, ^{13}C, and APT NMR spectra were obtained using a Varian Gemini 300 instrument, and chemical shifts are reported in ppm on the δ scale relative to TMS, TSP, or solvent. Proton chemical shifts are annotated as follows: ppm (multiplicity, integral, coupling constant (Hz)). Fast atom bombardment mass spectra (FAB-MS) were obtained on an Extrel 4000 in the positive ion detection mode. Chromatograms (SE-HPLC) were obtained on a Dionex isocratic system with a Waters 717 autosampler, a Gilson 112 UV detector and an in-line IN/US γ-Ram Model 2 radiodetector. Elemental microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Gd(DTPA) was used as its *N*-methylglucamine salt, also known as gadopentate dimeglumine (Magnevist), and was obtained from Berlex Laboratories, Wayne, NJ. The ^{153}Gd was obtained from Isotope Products, Valencia, CA. Phosphate buffered saline (PBS), 0.1 M of pH 7.4 consisted of 0.08 M Na_2HPO_4, 0.02 M KH_2PO_4, 0.01 M KCl, and 0.14 M NaCl.

Caution: *^{153}Gd ($t_{1/2}$ = 241.6 days) is a γ-emitting radionuclide. Appropriate shielding and handling protocols should be in place when using this isotope. Caution: Perchlorate salts can be explosive and should be handled with care.*

***N*-Benzyl-*cis*-2,6-bis(methoxycarbonyl)piperidine (5).** To a solution of **4** (6 g, 29.8 mmol) and K_2CO_3 (20.6 g, 149 mmol) in CH_3CN (100 mL) under argon was added benzyl bromide (5.1 g, 29.8 mmol), and the resulting mixture was refluxed for 48 h. The reaction mixture was allowed to cool gradually to ambient temperature and then filtered and the filtrate concentrated in vacuo. The residue was purified via

column chromatography on silica gel eluting with 15% EtOAc−hexane. Pure **5** (8.2 g, 94%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.22−1.41 (m, 2 H), 1.70−1.95 (m, 4 H), 3.20−3.28 (m, 2 H), 3.60 (s, 6 H), 3.87 (s, 2 H), 7.24−7.38 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 20.4 (t), 28.7 (t), 51.4 (d), 59.0 (t), 62.0 (q), 127.2 (d), 128.0 (d), 128.4 (d), 137.4 (s), 173.4 (s). Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26. Found: C, 66.01; H, 7.42.

General Procedure for Reduction of Carbamate Diesters 4 and 5. A solution of **4** or **5** (28 mmol) in anhydrous THF (150 mL) under N_2 was cooled to 0 °C via application of an external ice−water bath. To this cooled solution was added $LiBH_4$ (2.44 g, 111 mmol) portionwise with stirring. The resulting mixture was stirred at 0 °C for 1 h. The reaction mixture was allowed to warm gradually to ambient temperature with stirring during 12 h. The reaction mixture was poured into a stirred mixture of EtOAC (300 mL), 5% $NaHCO_3$ solution (100 mL), and $NaHCO_3$ (10 g) and stirred for 30 min. After saturation of the aqueous phase with NaCl, the resulting mixture was extracted with EtOAc (4 × 150 mL). The combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo.

***N*-Benzyl-*cis*-2,6-bis(hydroxymethyl)piperidine (6).** The residue was purified via column chromatography on silica gel by eluting with 50% EtOAc−hexane. Pure **6** (4.8 g, 73%) was thereby obtained as a colorless viscous oil. The IR, ^{1}H NMR, and ^{13}C NMR spectra of the material thereby obtained were essentially identical to data reported previously for authentic **6**.[10]

***cis*-2,6-Bis(hydroxymethyl)piperidine (14).** The residue was purified via column chromatography on silica gel by eluting with 50% MeOH−CH_2Cl_2. Pure **14** (1.87 g, 46%) was thereby obtained as a colorless viscous oil. The IR, ^{1}H NMR, and ^{13}C NMR spectra of the material thereby obtained were essentially identical to data reported previously for authentic **14**.[16]

***N*-Benzyl-*cis*-2,6-bis(chloromethyl)piperidine (7).** A solution of **6** (2.8 g, 11.9 mmol) in dry benzene (30 mL) was saturated with HCl(g) at 0 °C. After addition of thionyl chloride (5 mL), the mixture was heated at 60 °C for 3 h. The cooled reaction mixture was concentrated and neutralized with 5% Na_2CO_3 solution. The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL), the combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo to afford crude **7** (2.62 g, 81%): ^{1}H NMR ($CDCl_3$) δ 1.45−2.03 (m, 6 H), 3.00−3.12 (m, 2 H), 3.38 (t, J_{AB} = 11.3 Hz, 2 H), 3.63 (d, J_{AB} = 9.4 Hz, 2 H), 3.98 (s, 2 H), 7.34−7.50 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 18.2 (t), 26.5 (t), 45.7 (t), 56.4 (d), 61.2 (t), 126.6 (d), 127.0 (d), 128.0 (d), 140.0 (s). The crude product was used directly in the next step. The IR, ^{1}H NMR, and ^{13}C NMR spectra of **7** as a HCl salt were essentially identical to data reported previously.[10]

General Procedure for the Reaction with Sodium Azide of 7 and 15. A mixture of **7** or **15** (3.3 mmol) and NaN_3 (7.6 mmol) in DMSO (5 mL) was heated to 90 °C for 4 h. The resulting mixture was poured into ice−water and extracted with Et_2O (2 × 30 mL). The combined organic layers were washed with H_2O (3 × 15 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo.

***cis*-6-Azido-2-azidomethyl-1-benzylazepane (9).** The crude product could be used for the next step or could be purified via column chromatography on basic alumina eluting with 10% EtOAc−hexane. Pure **9** was thereby obtained as a colorless viscous oil (866 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 1.38−1.76 (m, 3 H), 1.84−2.15 (m, 3 H), 2.89−3.18 (m, 3 H), 3.31 (d, 2 H), 3.4−3.53 (m, 1 H), 3.98 (A*B*, J_{AB} = 15.5 Hz, 1 H), 4.11 (A*B*, J_{AB} = 15.5 Hz, 1 H), 7.38−7.51 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 22.0 (t), 31.8 (t), 34.8 (t), 51.6 (t), 54.3 (t), 59.2 (t), 59.3 (d), 61.2 (d), 127.3 (d), 128.4 (d), 128.6 (d), 139.3 (s); IR (film) ν 3066, 3032, 2943, 2862, 2094, 1735, 1453, 1265, 739, 701 cm^{-1}; MS (CI/NH_3) *m*/*z* 286 [M_r + H]. Anal. Calcd for $C_{14}H_{19}N_7$: C, 58.93; H, 6.71. Found: C, 59.30; H, 6.96.

***N*-Tosyl-*cis*-2,6-bis(azidomethyl)piperidine (16).** The crude product could be used directly for thenext step or purified via column chromatography on basic alumina eluting with 30% CH_2Cl_2−hexane. Pure **16** (1.0 g, 87.0%) was thereby obtained as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.35−1.80 (m, 6 H), 2.53 (s, 3 H). 3.42−3.49 (m, 2 H), 3.59−3.65 (m, 2 H), 4.10−4.23 (m, 2 H), 7.41 (A*B*, J_{AB} = 10.3 Hz, 2 H), 7,81 (A*B*, J_{AB} = 10.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.5 (t), 21.4 (q), 24.3 (t), 51.0 (d), 54.3 (t), 126.6 (d), 129.8 (d), 137.3 (s), 145.6 (s). Anal. Calcd for $C_{14}H_{19}N_7SO_2$: C, 48.13; H, 5.48. Found: C, 48.42; H, 5.63.

General Procedure for Reductions of Diazides 9 and 16. To a solution of **9** or **16** (3.85 mmol) in CH_3OH (20 mL) was added 10% Pd/C catalyst (100 mg). The resulting mixture was subjected to hydrogenolysis by agitation with excess H_2-(g) at 25 psi in a Parr hydrogenator appartus at ambient temperature for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo.

***cis*-7-Aminomethyl-1-benzylazepan-3-ylamine (10).** The residue was purified via column chromatography on neutral alumina eluting with 15% CH_3OH−EtOAc. Pure **10** (830 mg, 98%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.20−2.15 (m, 8 H), 2,60−3.25 (m, 8 H), 3.95 (A*B*, J_{AB} = 15.2 Hz, 1 H), 4.10 (A*B*, J_{AB} = 15.2 Hz, 1 H), 7.35−7.52 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 22.6 (t), 32.0 (t), 39.4 (t), 45.8 (t), 48.4 (d), 55.9 (t), 59.5 (t), 65.0 (d), 126.8 (d), 128.2 (d), 128.5 (d), 140.5 (s). Anal. Calcd for $C_{14}H_{23}N_3(H_2O)_{0.5}$: C, 70.69; H, 9.96. Found: C, 70.45, H, 9.90.

***N*-Tosyl-*cis*-2,6-bis(aminomethyl)piperidine (17).** The residue was purified via column chromatography on neutral alumina eluting with 15% CH_3OH−EtOAc. Pure **17** (630 mg, 93%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.10−1.62 (m, 6 H), 2.28 (s, 4 H), 2.44 (s, 3 H), 2.80−3.10 (m, 4 H), 4.00−4.14 (m, 2 H), 7.13 (A*B*, J_{AB} = 7.8 Hz, 2 H), 7.25 (A*B*, J_{AB} = 7.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.9 (t), 21.1 (q), 24.7 (t), 45.0 (t), 54.2 (d), 126.2 (d), 129.5 (d), 138.1 (s), 142.8 (s). Anal. Calcd for $C_{14}H_{23}N_3SO_2(H_2O)_{0.5}$: C, 54.88; H, 7.89. Found: C, 54.52; H, 7.82.

***cis*-*N*-(1-Benzyl-6-toluenesulfonylaminoazepan-2-ylmethyl)toluenesulfonamide (11).** To a solution of **10** (260 mg, 1.11 mmol) and sodium hydroxide (140 mg, 3.5 mmol) in H_2O (2 mL) was added dropwise a solution of TsCl (637 mg, 3.34 mmol) in diethyl ether (5 mL). The reaction mixture was stirred for 3 h at ambient temperature, at which time the reaction mixture was diluted with diethyl ether (20 mL) and washed with H_2O (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on silica gel by eluting with 30% EtOAc−hexane. Pure **11** (518 mg, 86%) was thereby obtained as a colorless solid and recrystallized from CH_2Cl_2/hexane: ^{1}H NMR ($CDCl_3$) δ 1.50−1.80 (m, 6 H), 2.45 (s, 3 H), 2.53 (s, 3 H), 2.78−3.37 (m, 6 H), 3.56 (A*B*, J_{AB} = 12.4 Hz, 1 H), 3.75 (A*B*, J_{AB} = 12.4 Hz, 2 H), 5.34 (d, J = 8.3 Hz, 2 H), 7.16−7.46 (m, 11 H), 8.75 (A*B*, J_{AB} = 10.3 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 19.5 (t), 21.36 (q), 21.41 (q), 30.0 (t), 36.6 (t), 45.3 (t), 51.0 (d), 52.7 (t), 58.4 (t), 61.4 (d), 126.8 (d), 126.9 (d), 127.3 (d), 128.7 (d), 128.8 (d), 129.4 (d), 129.6 (d), 136.7 (s), 137.3 (s), 139.3 (s), 142.8 (s), 143.2 (s). Anal. Calcd for $C_{28}H_{35}N_3S_2O_4$: C, 62.08; H, 6.51. Found: C, 62.00, H, 6.70.

***cis*-7-Aminomethylazepan-3-ylamine (12).** To a solution of **10** (900 mg, 3.9 mmol) in CH_3OH (10 mL) was added 10% Pd/C catalyst (150 mg). The resulting mixture was subjected to hydrogenated by agitation with excess H_2(g) at 60 psi in a Parr hydrogenation apparatus at ambient temperature for 72 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified via column chromatography on neutral alumina eluting with 30% CH_3OH−EtOAc. Pure **12** (528 mg, 94%) was thereby obtained as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.21−1.53 (m, 4 H), 1.67−2.04 (m, 4 H), 2.44−2.90 (m, 6 H), 3.31−3.38 (m, 3 H); ^{13}C NMR (CD_3OD) δ 23.4 (t), 35.4 (t), 38.2 (t), 48.2 (t), 53.8 (t), 54.0 (d), 61.8 (t); HRMS (positive ion FAB) calcd for $C_7H_{15}N_3$ M_r+ *m*/*z* 144.1501, found M_r+ *m*/*z* 144.1490.

General Procedure for Alkylation of Triamines 12 and 18. To a suspension of **12** or **18** (2.1 mmol) and K_2CO_3 (1.45 g, 10.5 mmol) in CH_3CN (25 mL) under argon was added dropwise *tert*-butyl bromoacetate (4.08 g, 20.95 mmol), and the

resulting mixture was heated at 65 °C for 24 h. The reaction mixture was allowed to cool gradually to ambient temperature and after being filtered, and the filtrate was concentrated in vacuo.

***cis*-[6-(Bis-*tert*-butoxycarbonylmethylamino)-2-[(bis-*tert*-butoxycarbonylmethylamino)methyl]azepan-1-yl]-acetic Acid *tert*-Butyl Ester (13).** The residue was purified via column chromatography on silica gel eluting with 10% EtOAc−hexane. Pure **13** (1.02 g, 68%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.29−1.97 (m, 45 H), 2.03−2.30 (m, 4 H), 2.52−2.60 (m, 2 H), 2.50−3.10 (m, 6 H), 3.35−3.75 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 23.0 (t), 27.8 (q), 27.9 (q), 27.9 (q), 32.0 (t), 33.1 (t), 52.3 (t), 53.2 (t), 56.6 (t), 57.7 (t), 58.2 (t), 60.9 (d), 61.0 (d), 80.0 (s), 80.2 (s), 80.4 (s), 170.6 (s), 171.4 (s), 171.8 (s). Anal. Calcd for $C_{37}H_{67}N_3O_{10}$: C, 62.25; H, 9.46. Found: C, 62.04; H, 9.62.

***cis*-2,6-Bis[*N*,*N*-bis(*tert*-butoxycarbonylmethyl)aminomethyl]-1-piperidineacetic Acid *tert*-Butyl Ester (19).** The residue was purified via column chromatography on silica gel eluting with 20% EtOAc−hexane. Pure **19** (913 mg, 61%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.20−1.95 (m, 45 H), 2.52−2.65 (m, 4 H), 2.76−3.07 (m, 8 H), 3.45 (s, 8 H), 3.65 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 23.2 (t), 27.8 (q), 30.0 (t), 51.3 (t), 56.2 (t), 58.9 (t), 59.0 (d), 79.7 (s), 80.3 (s), 170.2 (s), 171.9 (s). Anal. Calcd for $C_{37}H_{67}N_3O_{10}$: C, 62.25; H, 9.46. Found: C, 61.87; H, 9.27.

General Procedure for Deprotection of *tert*-Butyl Ester in 13 and 19. A solution of **13** or **19** (0.21 mmol) in dry 1,4-dioxane (10 mL) in an ice−water bath was saturated with HCl for 4 h, at which time the mixture was allowed to ambient temperature and then was stirred for 18 h. The precipitate was collected and washed with ethyl ether. The collected solid was dissolved in water immediately and lyophilized.

***cis*-[2,7-Bis[biscarboxymethylamino)methyl]azepan-1-yl]acetic Acid (1).** Pure **1** (90 mg, 79%) was obtained as a salt: ^{1}H NMR (D_2O, pD 1, CD_3OD as reference) δ 1.05−1.75 (m, 3H), 1.86−2.22 (m, 3 H), 2.90−3.76 (m, 8 H), 4.02−4.18 (m, 8 H), 4.75−5.03 (m, 5 H); ^{13}C NMR (D_2O, pD 1, CD_3OD as reference) δ 27.9 (t), 30.4 (t), 45.8 (t), 52.7 (t), 53.9 (t), 54.8 (t), 55.3 (t), 58.7 (d) 63.0 (d), 65.9 (t), 171.5 (s), 171.4 (s), 179.6 (s). Anal. Calcd for $C_{17}H_{30}N_3O_{10}Cl_3(H_2O)_2$: C, 35.28; H, 5.92; N, 7.26. Found: C, 35.66; H, 6.03; N, 7.08.

***cis*-2,6-Bis[*N*,*N*-bis(carboxymethyl)aminomethyl]-1-piperidineacetic Acid (2).** Pure **2** (83 mg, 86%) was obtained as a salt: ^{1}H NMR (D_2O, pD 1, CD_3OD as reference) δ 1.15−1.57 (m, 6 H), 3.03−3.42 (m, 8 H), 3.50−3.84 (m, 13 H); ^{13}C NMR (D_2O, pD 1, CD_3OD as reference) δ 21.0 (t), 21.5 (t), 42.7 (t), 55.4 (t), 55.5 (t), 61.1 (d), 171.0 (s), 171.5 (s). Anal. Calcd for $C_{17}H_{30}N_3O_{10}Cl_3(H_2O)_2$: C, 35.28; H, 5.92; N, 7.26. Found: C, 35.36; H, 6.33; N, 7.15.

***N*-Tosyl-*cis*-2,6-bis(methyltosyl)piperidine (15).** A solution of **14** (900 mg. 6.2 mmol) in CH_2Cl_2 (3 mL) under nitrogen was cooled to 0 °C via application of an external ice−water bath. To this cooled solution were added sequentially Et_3N (1.88 g, 18.6 mmol) and a solution of TsCl (3.55 g, 18.6 mmol) in CH_2Cl_2 (4 mL). The resulting mixture was allowed to warm gradually to ambient temperature with stirring during 18 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and with saturated NaCl solution. The organic layer was dried ($MgSO_4$) and filtered and the filtrate concentrated in vacuo. The residue was purified via column chromatography on silica gel eluting with 20% EtOAc−hexane. Pure **15** (1.36 g, 36%) was thereby obtained as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.18−1.35 (m, 6 H), 2.70 (d, J = 13.2 Hz, 2 H). 2.46 (s, 3 H), 2.51 (s, 6 H), 4.01−4.18 (m, 8 H), 7.32 (A*B*, J_{AB} = 8.6 Hz, 2 H), 7.44 (A*B*, J_{AB} = 8.6 Hz, 2 H), 7.65 (A*B*, J_{AB} = 8.6 Hz, 2 H), 7.86 (A*B*, J_{AB} = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.3 (t), 21.3 (q), 21.4 (q), 24.3 (t), 49.6 (d), 70.2 (t), 126.4 (d), 127.6 (d), 129.7 (d), 129.8 (d), 132.1 (s), 136.5 (s), 143.7 (s), 145.0 (s). Anal. Calcd for $C_{28}H_{33}NS_3O_8$: C, 55.34; H, 5.47. Found: C, 55.43; H, 5.80.

***cis*-2,6-Bis(aminomethyl)piperidine (18).** *N*-Tosyl-2,6-diaminomethylpiperidine (**17**, 700 mg, 2.35 mmol) was dissolved in concentrated H_2SO_4 (10 mL) and heated to 115 °C for 72 h under argon. The resulting solution was cooled to ambient temperature and added in portions to Et_2O (150 mL) at −60 °C. The resulting precipitate was collected, washed with Et_2O (20 mL), and immediately dissolved in H_2O (25 mL). The aqueous solution was extracted with Et_2O (10 mL), concentrated to 5 mL, and neutralized with 50% NaOH. The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL), the combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo to afford **18** (292 mg, 87.0%): ^{1}H NMR ($CDCl_3$) δ 1.08−1.75 (m, 6 H), 2.21−2.62 (m, 11 H);^{13}C NMR ($CDCl_3$) δ 24.0 (t), 30.0 (t), 47.8 (t), 58.6 (d). HRMS (Positive ion FAB) calcd for $C_7H_{17}N_3$ M_r^+ *m*/*z* 144.1501, found M_r^+ *m*/*z* 144.1498.

Measurements of Serum Stability. The ^{153}Gd complexes of **1** and **2** were prepared by the addition of 350 μCi of ^{153}Gd (0.1 M HCl adjusting the pH to 4.5 with 5 M NH_4OAc) to 20 μL of 0.2 M ligand solution in 0.15 M NH_4OAC of pH 4.5. The reactions went to completion after the reaction mixture was heated at 80 °C for 18 h and were then loaded onto a column of Chelex-100 resin (1 mL volume bed, equilibrated with 0.15 M NH_4OAc). The complexes were eluted from the resin with 0.15 M NH_4OAc while the resin retained free ^{153}Gd. The pH of ^{153}Gd complex solutions was adjusted to 7 with PBS buffer, and 250 μCi of the complex was added to 1 mL of human serum which was incubated at 37 °C. A aliquot of the serum (5−10 μL) was taken at selected times (Figure 1) and analyzed by SE-HPLC. The serum stability of the ^{153}Gd complexes was assessed by measuring the release of ^{153}Gd radionuclide from the complexes to serum proteins using SE-HPLC with TSK 3000 column. The column was eluted with PBS at 1 mL/min flow rate.

Measurements of Relaxivity. NMR dispersion measurements were made on a custom-designed variable field T1-T2 analyzer (Southwest Research Institute, San Antonio, TX) at 23 °C. The magnetic field was varied from 0.02 to 1.5 T (corresponding to a proton larmor frequency of 1−64 MHz). T1 was measured by using a saturation recovery pulse sequence with 32 incremental recovery times. The relaxivities (relaxation rates per mM Gd(III) concentration) were obtained after subtracting the water contribution. The commercial Gd-(DTPA) (Magnevist) was used without further modifications. The other Gd chelates were prepared by dissolving the appropriate ligand and $GdCl_3$ in water with stirring at a 1:0.9 mole ratio with the pH adjusted to 6.5 with 1M NaOH. The total Gd concentration for each sample was determined by ICP-AAS (Desert Analytics, Tuscon, AZ).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compound **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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