Synthesis and Biological Evaluation of Novel Macrocyclic Ligands with Pendent Donor Groups as Potential Yttrium Chelators for Radioimmunotherapy with Improved Complex Formation Kinetics

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Novel 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (NOTA) based octadentate ligands [2-(4,7-biscarboxymethyl[1,4,7]triazacyclononan-1-ylethyl)carbonylmethylamino]acetic acid tetrahydrochloride (**1**) and [3-(4,7-biscarboxymethyl[1,4,7]triazacyclononan-1-yl-propyl)carbonylmethylamino]acetic acid tetrahydrochloride (**2**) with pendent donor groups as potential yttrium chelators for radioimmunotherapy (RIT) have been prepared via a convenient and high-yield cyclization route. The complexation kinetics of the novel chelates with Y(III) was investigated and compared to that of 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA), a macrocyclic chelating agent well recognized as forming very stable complexes with yttrium but also limited in usage because of slow Y(III) complex formation rates. The in vitro stability of the corresponding ^{88}Y-labeled complexes in human serum was assessed by measuring the release of ^{88}Y from the complexes over 14 days. The in vivo biodistribution of ^{86}Y-labeled **1** in mice was evaluated and compared to that of the ^{86}Y−DOTA complex. Formation of the Y complex of **1** was significantly more rapid than that of either **2** or DOTA. Serum stability of the ^{88}Y complex formed with **1** was equivalent to the DOTA complex, while the complex formed with **2** proved to be significantly unstable. The results obtained from a biodistribution study indicate that the ^{86}Y−**1** complex possesses in vivo stability comparable to the analogous DOTA complex.

Introduction

Monoclonal antibodies (mAb's) have been employed as a targeting biomolecule for the delivery of radionuclide into tumor cells in radioimmunotherapy (RIT), and numerous clinical trials have been performed to validate this modality of cancer therapy.[1] Several useful β^--emitting radionuclides including ^{131}I, ^{90}Y, ^{177}Lu, and ^{153}Sm have been employed for labeling mAb's for RIT applications.[2] A variety of macrocyclic ligands have been prepared and evaluated as suitable chelates to bind specific radionuclides.[3] Since the release of the radiometals from the chelate is a potential source of radiotoxic effects for nontumor cells and normal tissue,[4] the requirement of having a chelate that forms a kinetically inert complex with the radiometal is a critical aspect for performing successful targeted radiotherapy.

The pure β^--emitting radionuclide, ^{90}Y (E_{max} = 2.28 MeV; $T_{1/2}$ = 64.1 h) has been extensively studied in RIT because of its physical properties.[5] The macrocyclic chelating agent, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) is well-known to be the most effective chelator of Y(III) and the lanthanides,[6] and numerous bifunctional analogues suitable for protein conjugation have been reported in the literature.[7] Although the Y(III)−DOTA complex shows ideal stability both in vivo[3c] and in vitro,[3b] the extremely slow formation rate of the complex[6] is a major obstacle that complicates and limits the routine use of these ligands in RIT applications. In general, DOTA conjugated to mAb's displays relatively slow and inefficient radiolabeling with Y(III) isotopes under mild conditions.[8] This is contrary to the rapid and high-yield radiolabeling (>90%) of mAb's conjugated with bifunctional derivatives of the acyclic chelating agent diethylenetriaminepentaacetic acid (DTPA).[3c,8] Low radiolabeling yields require a chromatographic purification of the product to separate unchelated Y(III), which is not always practical for RIT applications. With this background in mind, this laboratory has endeavored to prepare a suitable Y(III) chelating agent that would possess comparable complex stability of DOTA and the superior, practical complexation kinetics of DTPA. Such efforts have resulted in the synthesis and evaluation of several chelates including the CHX-DTPA[9] and 1B4M-DTPA (Scheme 1),[10] which display significantly improved complexation kinetics with Y(III) compared to DOTA.[9c] However, the corresponding radio-yttrium complexes remain somewhat less stable in serum and in vivo.[9c] Despite this shortcoming, significant clinical use of these ligands has been made because of their practical and reproducible high specific activity radiolabeling chemistry.[11]

Our continued interest in finding a suitable chelator to complex Y(III) isotopes efficiently prompted the design of the structurally novel chelators **1** and **2** (Scheme 1). The chelators **1** and **2** possess the same octadentate coordinating groups as both DOTA and

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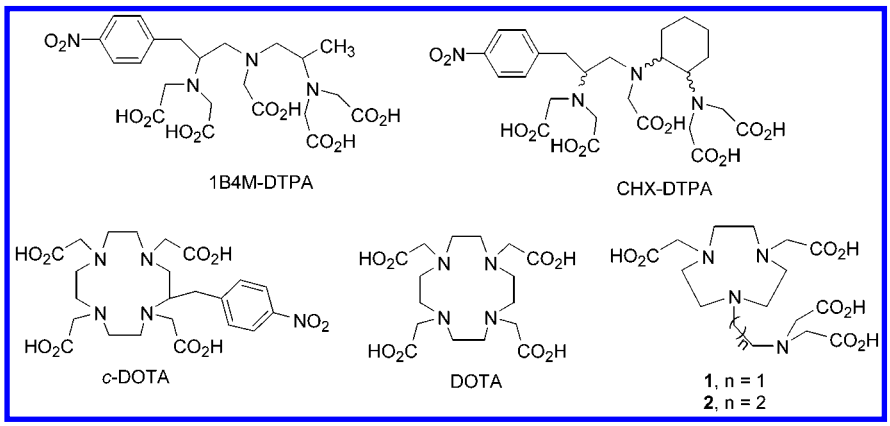
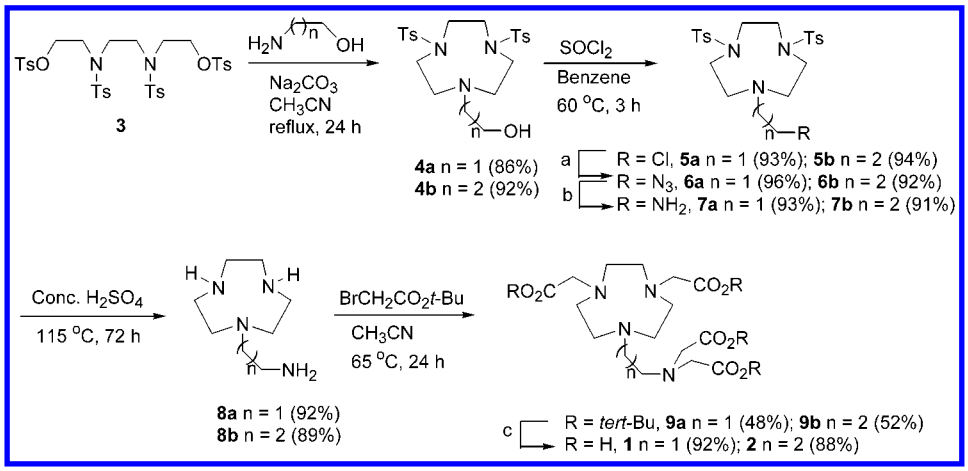
Scheme 1



Scheme 2[a]



[a] Reaction conditions: (a) NaN_3, DMSO, 90 °C, 4 h; (b) H_2, 10% Pd/C, EtOH, 3 h, 20 psi; (c) $HCl_{(g)}$.

DTPA. However, these new chelators are a combination of both macrocyclic and acyclic character. The macrocyclic component chosen is based on 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (NOTA), while the acyclic component is a pendent bis(carboxymethyl)-amino donor group that is connected by either an ethylene or a propylene bridge for **1** and **2**, respectively. The cooperative binding of the pendent donor groups coupled with the preorganization and macrocyclic effect of the NOTA substructure is expected to accelerate complexation with Y(III) isotopes while maintaining a high level of stability of the complexes.

Herein, we report the synthesis of novel octadentate ligands **1** and **2** for improved kinetics of complexation with Y(III) isotopes for RIT. The relative Y(III) complexation kinetics of ligands **1**, **2**, and DOTA was investigated using a competition reaction with arsenazo-(III).[6b] The serum stability of the ^{88}Y-labeled complexes of **1** and **2** was evaluated. The in vivo biodistribution of the ^{86}Y−**1** complex and ^{86}Y−DOTA complex was evaluated in mice.

Result and Discussion

Synthesis. For the preparation of ligands **1** and **2**, efficient cyclization using two precursor molecules is a crucial step. A variety of cyclization methods in the synthesis of macrocyclic chelating agents have been developed.[12] Representative synthetic methods for bimolecular cyclization include reaction of a diamine and diester under high dilution conditions,[12a] metal-templated cyclization,[12b] and the Richman−Atkin cyclization conditions.[12d] These cyclization methods, when employed to prepare smaller polyaza macrocyclic structures, are frequently problematic, resulting in low yields, inconvenient procedures, and difficult purification regimens due to the presence of higher homologue byproducts. Our present report introduces a convenient approach to cyclization, which employs primary amines having a pendent hydroxyl functional group as a precursor molecule. Thus, primary amines bearing a distal hydroxyl group are reacted with an *N*-tosyl-protected ditosylate to provide the 1,4,7-triazacyclononane macrocyclic ring. After cyclization, the pendent hydroxyl group is available for further chemistry to introduce additional pendent donor groups as desired. This cyclization method is a convenient, clean, and high-yield route using readily available starting materials. This strategy can be directly applied to construction of a wide range of macrocyclic rings by using modified primary amines.

The synthetic routes for the NOTA-based macrocyles **1** and **2** are shown in Scheme 2. The parent macrocyclic rings in **1** and **2** are obtained via the efficient cyclization of ethanolamine ($n = 1$) or propanolamine ($n = 2$) with *N*,*N*′-ditosylethylenediamine ditosylate **3**. Thus, ditosylate **3**[13] was reacted with ethanolamine or propanolamine in the presence of Na_2CO_3 to afford the tosyl-protected macrocycles **4a** and **4b** in 86% and 92% yields, respectively. Interestingly, reaction of ditosylate **3** with ethanolamine in the presence of K_2CO_3 as the templat-

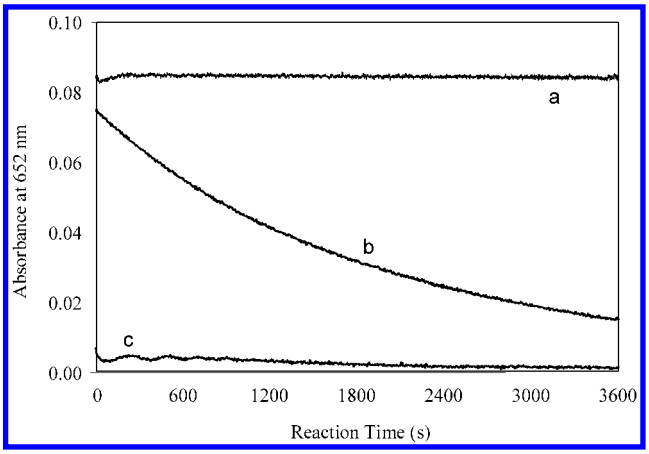


Figure 1. Plot of absorbance at 652 nm vs time of Y−**1** (c), Y−**2** (a), and Y−DOTA (b) at pH 4 and 25 °C.

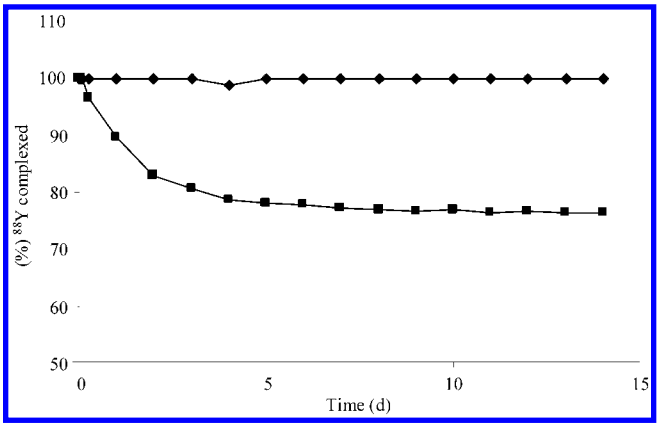


Figure 2. Serum stability of ^{88}Y−**1** (◆) and ^{88}Y−**2** (■) at pH 7 and 37 °C.

ing base failed to provide the desired macrocycle **4a**. The choice of the appropriate base for templating these reactions appears to be quite important. The subsequent reaction of **4a** and **4b** with $SOCl_2$ provided **5a** and **5b**, which were reacted with NaN_3 in DMSO to afford **6a** and **6b**, respectively. The diazides **6a** and **6b** were hydrogenated to provide the corresponding amines **7a** and **7b**. Deprotection of the tosyl groups in compounds **7a** and **7b** was effected with concentrated sulfuric acid at 115 °C for 72 h. After deprotection, alkylation of triamines **8a** and **8b** with *tert*-butyl bromoacetate provided **9a** and **9b**, respectively. Tetraacids **1** and **2** were obtained as HCl salts after deprotection of the *tert*-butyl esters with anhydrous $HCl_{(g)}$-dioxane. The synthetic route to ligands **1** and **2** reported herein is efficient and high-yielding. Beginning with the readily available starting material **3**, the straightforward synthetic method provided ligands **1** and **2** in 86% and 85% overall yields, respectively.

Kinetics Study. The complexation kinetics of novel chelates **1**, **2**, and DOTA with Y(III) was qualitatively investigated using a competing reaction with arsenazo(III) according to a modification of a previously reported procedure.[6b] The absorbance (A_{652}) for the Y(III)−arsenazo(III) complex was measured in the absence and in the presence of the chelates (**1**, **2**, and DOTA**)** over 1 h at room temperature. The absorbance (A_{652}) was 0.089 for arsenazo(III)−Y(III) in the absence of the ligand. A plot of the absorbance at 652 nm versus time is shown in Figure 1. Inspection of Figure 1 indicates that the complex formation of ligand **1** with Y(III) is quite rapid and is essentially complete very shortly after the starting point of the measurement. However, ligand **2** displays very slow complexation with Y(III) compared to **1**. Furthermore, the degree of complexation of **2** was slight and remained unchanged over the period of measurement. In the presence of ligand **2**, the absorbance of arsenazo(III)−Y(III) declined to 0.083 at 1 h (Figure 1). Complexation of Y(III) by **2** is assumed to occur to some degree, but the formed complex might undergo decomplexation because of an absence of complex stability, ultimately leading to an unfavorable equilibrium situation. This interpretation also agrees with the serum stability result of the ^{88}Y-labeled complex with **2**, which displayed a significant loss of radio-yttrium in serum. As expected,[6] DOTA displayed sluggish complexation with Y(III). The time scan measurements obviously show that **1** forms a complex with Y(III) at a much greater rate than DOTA.

Serum Stability. The stability of the corresponding ^{88}Y-labeled complexes formed with chelators **1** and **2** in human serum was assessed by measuring the release of ^{88}Y from the complexes at 37 °C over 14 days. The serum stability of ^{88}Y-labeled complexes thereby obtained is shown in Figure 2, which indicates that ^{88}Y-labeled ligand **1** having an ethylene bridge is stable in serum for up to 14 days with no measurable loss of radioactivity. However, considerable release of radioactivity was observed from the ^{88}Y chelate formed with **2**, which possesses the longer propylene bridge. The percentage of ^{88}Y released from this complex at 14 days was about 25%. The data show that the stability of the Y(III) radiolabeled complex in serum is dependent on the length of the carbon chain between the pendent donor groups and the macrocyclic ring. The observed release of radioactivity from chelate **2** might be a consequence of the formation of a six-membered chelate ring as opposed to what is generally considered to be the best arrangement of donors, i.e., five-membered chelate ring for complexation of Y(III). A number of reports have shown that DOTA possesses exceptional stability in human serum.[3b,c] Ligand **1** shows in vitro stability comparable to that of DOTA. Thus, ligand **1**, after appropriate derivatization to provide a bifunctional analogue, might serve as a viable alternative to the use of DOTA in RIT applications.

In Vivo Biodistribution. To evaluate the in vivo stability of ^{86}Y-labeled ligand **1**, which displayed excellent serum stability and kinetics, a biodistribution study was performed in mice. For the purpose of comparison, a biodistribution of ^{86}Y-labeled DOTA in mice was also performed. The results of the biodistribution studies for ^{86}Y−**1** and ^{86}Y−DOTA are shown in Figure 3. Radioactivity that accumulated in selected organs and cleared from the blood of mice was measured at five time points, 0.5, 1, 4, 8, and 24 h postinjection, of the two ^{86}Y-labeled complexes. The data in Figure 3 show that the accumulated radioactivity in the organs at 4 h postinjection is negligible for both ^{86}Y−**1** and ^{86}Y−DOTA. Both ^{86}Y−**1** and ^{86}Y−DOTA display a slightly higher radioactivity level in the kidney compared to that in other organs, no doubt associated with whole-body clearance and elimination of these complexes. At 24 h, radioactivity that accumulated in the kidney was 0.54 ± 0.13% injected dose per gram (% ID/g) and 0.84 ± 0.16% ID/g

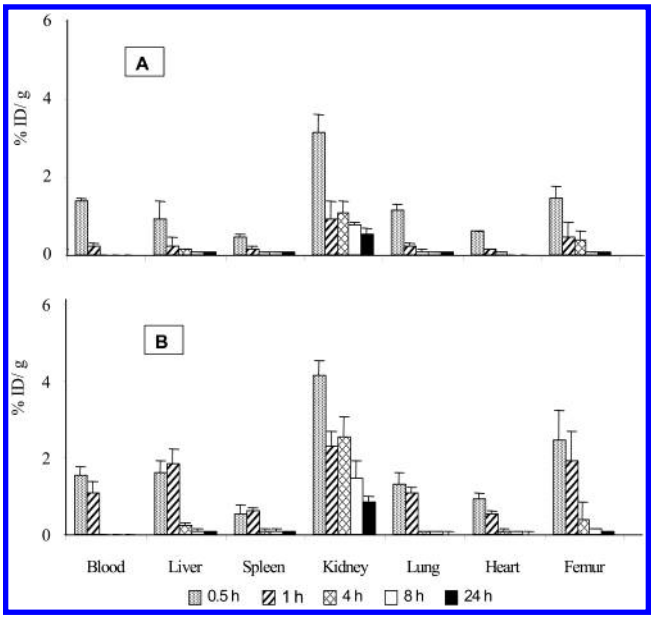


Figure 3. Biodistribution of ^{86}Y−**1** (A) and ^{86}Y−DOTA (B) in Balb/c mice following iv injection.

for ^{86}Y-**1** and ^{86}Y-DOTA, respectively. The data in Figure 3 also indicate a rapid blood clearance for ^{86}Y−**1** and ^{86}Y−DOTA. The ^{86}Y−**1** displayed low bone uptake that peaked at 1.47 ± 0.28% ID/g at 0.5 h to decline to 0.05 ± 0.01% ID/g at 24 h. This trend is a noteworthy result in that free yttrium is known to be efficiently deposited at the bone.[14] Compared to that of complex ^{86}Y−**1**, the bone accumulation of ^{86}Y−DOTA was actually slightly higher at all time points. The results of the biodistribution studies as a whole indicate that ^{86}Y−**1** displays in vivo stability comparable to that of ^{86}Y−DOTA.

Conclusions

Novel NOTA-based chelators (**1** and **2**) with pendent donor groups are prepared to provide stable complexes while also providing improved Y(III) complexation kinetics as an alternative to the use of DOTA derivatives in RIT. The syntheses of **1** and **2** are performed via a convenient and high-yield cyclization route. The relative complexation kinetics of these new chelates (**1**, **2**) and DOTA with Y(III) indicates that **1** forms a Y(III) complex most rapidly among those studied and displays a dramatically enhanced formation rate compared to DOTA. Serum stability studies indicate that the ethylene-bridged ligand **1** in serum forms a more stable complex with ^{88}Y(III) than the analogous propylene-bridged ligand **2.** There is no measurable loss of ^{88}Y(III) from the ^{88}Y-labeled chelate **1** for up to 14 days, indicating serum stability comparable to that of ^{88}Y−DOTA. However, ^{88}Y−**2**, having a longer sidearm, exhibits a substantial release of ^{88}Y from the ligand. Thus, the strategy of providing a partial NOTA platform for the metal ion to set in to increase the complex formation rate and then to stabilize the complex by introducing pendent donor groups appears to be successful. The biodistribution studies show that ^{86}Y−**1** is stable in vivo and is compared favorably with ^{86}Y−DOTA. The results of serum stability, in vivo biodistribution, and the kinetics study suggest that the novel chelator **1** reported herein might be a potential yttrium chelating agent for RIT applications. Derivatization of **1** as a bifunctional-chelating agent for conjugation with mAb's or peptides for RIT applications is in progress and will be reported in due course.

Experimental Section

General. All solvents and reagents were obtained from Aldrich and used as received unless otherwise noted. ^{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)). Elemental microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Fast atom bombardment (FAB-MS) mass spectra 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. Time scan measurements were obtained using an HP-8452A diode array spectrophotometer. The ^{88}Y was obtained from Los Alamos National Laboratory and purified as previously reported.[9c] The ^{86}Y was produced and purified as previously reported.[15] Phosphate-buffered saline (PBS) (0.1 M, 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: ^{88}Y ($t_{1/2}$ = 106.6 day) is a γ-emitting radionuclide. ^{86}Y ($t_{1/2}$ = 14.7 h) is a β^+-emitting radionuclide. Appropriate shielding and handling protocols should be in place when using these isotopes.

General Procedure for Synthesis of Macrocyclics 4a and 4b. To a solution of **3**[13] (1 mmol) and Na_2CO_3 (10 mmol) in CH_3CN (10 mL) under argon was added either ethanolamine or propanolamine (1 mmol), and the resulting mixture was heated to reflux for 24 h. The reaction mixture was allowed to cool gradually to ambient temperature and was filtered, and the filtrate was concentrated in vacuo.

2-[4,7-Bis(*p*-toluene-4-sulfonyl)[1,4,7]triazacyclononan-1-yl]ethanol (4a). The residue was purified via column chromatography on silica gel eluting with EtOAc. Pure **4a** (414 mg, 86%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 1 H), 2.40 (s, 6 H), 2,79 (t, J = 3.2 Hz, 2 H), 3.01 (t, J = 3.8 Hz, 4 H), 3.26 (t, J = 3.6 Hz, 4 H), 3.44 (t, J = 1.8 Hz, 4 H), 3.61 (t, J = 2.7 Hz, 2 H), 7.31 (d, J = 9.1 Hz, 4 H), 7.67 (d, J = 9.1 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.2 (q), 52.3 (t), 52.7 (t), 54.9 (t), 59.3 (t), 59.9 (t), 126.8 (d), 129.5 (d), 134.8 (s), 143.2 (s). Anal. Calcd for $C_{22}H_{31}N_3S_2O_5$: C, 54.86; H, 6.49. Found: C, 54.51; H, 6.64.

3-[4,7-Bis(*p*-toluene-4-sulfonyl)[1,4,7]triazacyclononan-1-yl]propan-1-ol (4b). The residue was purified via column chromatography on silica gel, eluting with EtOAc. Pure **4b** (454 mg, 92%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.78 (t, J = 3.5 Hz, 2 H), 2.13 (s, 1 H), 2.50 (s, 6 H), 2.87 (t, J = 3.3 Hz, 2 H), 3.04−3.12 (m, 4 H), 3.26−3.41 (m, 4 H), 3.50 (s, 2 H), 3.87 (t, J = 2.9 Hz, 4 H), 7.40 (d, J = 8.7 Hz, 4 H), 7.41 (d, J = 8.7 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.4 (q), 29.5 (t), 51.5 (t), 52.8 (t), 54.4 (t), 54.7 (t), 61.5 (t), 127.0 (d), 129.7 (d), 135.0 (s), 143.5 (s). Anal. Calcd for $C_{23}H_{33}N_3S_2O_5$: C, 55.74; H, 6.71. Found: C, 55.87; H, 6.84.

General Procedure for Chlorination of 5a and 5b. A solution of either **4a** or **4b** (2 mmol) in dry benzene (20 mL) was saturated with $HCl_{(g)}$ at 0 °C. After addition of thionyl chloride (40 mmol), the mixture was heated at 60 °C for 3 h. The cooled reaction mixture was concentrated and neutralized with 5% Na_2CO_3 solution (5 mL). 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.

1-(2-Chloroethyl)-bis(*p*-toluene-4-sulfonyl)[1,4,7]-triazacyclononane (5a). The residue was purified via column chromatography on silica gel, eluting with 20% EtOAc−hexane. Pure **5a** was obtained as a colorless oil (930 m g, 93%): ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 6 H), 3.05−3.14 (m, 8 H), 3.62−3.75 (m, 8 H), 7.42 (d, J = 9.2 Hz, 4 H), 7.67 (d, J = 9.2 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.4 (q), 42.4 (t), 51.4 (t), 52.6 (t), 56.3

(t), 59.0 (t), 127.1 (d), 129.7 (d), 135.2 (s), 143.8 (s). Anal. Calcd for $C_{22}H_{30}N_3S_2O_4Cl$: C, 52.84; H, 6.05. Found: C, 52.78; H, 6.35.

1-(3-Chloropropyl)-bis(*p*-toluene-4-sulfonyl)[1,4,7]-triazacyclononane (5b). The residue was purified via column chromatography on silica gel, eluting with 15% EtOAc−hexane. Pure **5b** was obtained as a colorless oil (966 mg, 94%): ^{1}H NMR ($CDCl_3$) δ 2.00 (t, J = 6.1 Hz, 2 H), 2.51 (s, 6 H), 2.82 (t, J = 6.0 Hz, 2 H), 2.94−2.94 (m, 4 H), 3.33 (s, 4 H), 3.58 (s, 2 H), 3.80 (t, J = 6.0 Hz, 4 H), 7.41 (d, J = 8.7 Hz, 4 H), 7.75 (d, J = 8.7 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.3 (q), 30.1 (t), 43.1 (t), 51.4 (t), 52.7 (t), 53.5 (t), 55.7 (t), 127.0 (d), 129.7 (d), 135.0 (s), 143.4 (s). HRMS (positive-ion FAB) Calcd for $C_{23}H_{32}N_3S_2O_4Cl$: [M + H]$^+$ m/z 514.1601. Found: [M + H]$^+$ m/z 514.1600. Anal. Calcd for $C_{23}H_{32}N_3S_2O_4Cl(H_2O)_{0.5}$: C, 52.81; H, 6.36. Found: C, 52.72; H, 6.49.

General Procedures for the Reaction of 5a and 5b with Azide. A mixture of compound **5a** or **5b** (3.3 mmol) and NaN_3 (6.93 mmol) in DMSO (20 mL) was heated to 90 °C for 4 h. The resulting mixture was poured into ice−water and extracted with ethyl ether (2 × 50 mL). The combined organic layers was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo.

1-(2-Azidoethyl)-4,7-bis(*p*-toluene-4-sulfonyl)[1,4,7]-triazacyclononane (6a). The crude **6a** could be used directly for the next step or purified via column chromatography on neutral alumina, eluting with 15% CH_2Cl_2−hexane. Pure **6a** (1.56 g, 96%) was thereby obtained as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 6 H), 2.82 (t, J = 6.2 Hz, 4 H), 2.99 (s, 2 H), 3.18−3.35 (m, 4 H), 3.38 (t, J = 6.2 Hz, 4 H), 3.54 (s, 2 H), 7.34 (d, J = 8.7 Hz, 4 H), 7.71 (d, J = 8.7 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.4 (q), 49.5 (t), 51.0 (t), 52.1 (t), 55.4 (t), 55.5 (t), 126.7 (d), 129.4 (d), 134.9 (s), 143.1 (s). Anal. Calcd for $C_{22}H_{30}N_6S_2O_4$: C, 52.16; H, 5.97. Found: C, 52.33; H, 6.10.

1-(3-Azidopropyl)-4,7-bis(*p*-toluene-4-sulfonyl)[1,4,7]-triazacyclononane (6b). The crude **6b** could be used directly for the next step or purified via column chromatography on neutral alumina, eluting with 15% CH_2Cl_2−hexane. Pure **6b** (1.54 g, 92%) was thereby obtained as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.84 (t, J = 5.1 Hz, 2 H), 2.52 (s, 6 H), 2.75 (t, J = 6.8 Hz, 2 H), 2.90−3.04 (m, 4 H), 3.30 (s, 4 H), 3.49−3.64 (s, 6 H), 7.41 (d, J = 8.6 Hz, 4 H), 7.53 (d, J = 8.6 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.4 (q), 27.0 (t), 49.1 (t), 51.5 (t), 52.7 (t), 53.8 (t), 55.5 (t), 127.1 (d), 129.7 (d), 135.0 (s), 143.5 (s). HRMS (positive-ion FAB) Calcd for $C_{23}H_{32}N_6S_2O_4$: [M + H]$^+$ m/z 521.2005. Found: [M + H]$^+$ m/z 521.1996. Anal. Calcd for $C_{23}H_{32}N_6S_2O_4(H_2O)_{0.5}$: C, 52.16; H, 6.28. Found: C, 52.02; H, 6.26.

General Procedure for the Reduction of Azides 6a and 6b. To a solution of the azides (4 mmol) in CH_3OH (20 mL) was added 10% Pd/C catalyst (100 mg). The resulting mixture was subjected to hydrogenation by agitation with excess H_2 (gas) at 25 psi in a Parr hydrogenator apparatus at ambient temperature for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo.

2-[4,7-Bis(*p*-toluene-4-sulfonyl)[1,4,7]triazacyclononan-1-yl]ethylamine (7a). The residue was purified via column chromatography on neutral alumina, eluting with 10% CH_3OH−EtOAC. Pure **7a** (1.79 g, 93%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.05−2.43 (m, 6 H), 2.69−2.95 (m, 10 H), 3.25 (s, 4 H), 3.49 (s, 4 H), 7.34 (d, J = 8.4 Hz, 4 H), 7.65 (d, J = 8.4 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 21.0 (q), 39.4 (t), 51.6 (t), 52.5 (t), 55.2 (t), 59.8 (t), 126.7 (d), 129.4 (d), 134.8 (s), 143.1 (s). Anal. Calcd for $C_{22}H_{32}N_4S_2O_4$: C, 54.98; H, 6.71. Found: C, 55.00; H, 7.13.

3-[4,7-Bis(*p*-toluene-4-sulfonyl)[1,4,7]triazacyclononan-1-yl]propylamine (7b). The residue was purified via column chromatography on neutral alumina, eluting with 10% CH_3OH−EtOAc. Pure **7b** (1.76 g, 91%) was thereby obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.67 (t, J = 6.2 Hz, 2 H), 1.95 (s, 2 H), 2.48 (s, 6 H), 2.67 (t, J = 5.3 Hz, 2 H), 2.82 (t, J = 5.6 Hz, 2 H), 2.95 (s, 4 H), 3.26 (s, 4 H), 3.53 (s, 4 H), 7.37 (d, J = 8.2 Hz, 4 H), 7.72 (d, J = 8.2 Hz, 4 H): ^{13}C NMR ($CDCl_3$) δ 21.3 (q), 31.2 (t), 40.1 (t), 51.3 (t), 52.4 (t), 54.9 (t), 55.4 (t), 126.9 (d), 129.6 (d), 135.0 (s), 143.3 (s). HRMS (positive-ion FAB) Calcd for $C_{23}H_{34}N_4S_2O_4$: [M + H]$^+$ m/z 495.2100. Found: [M + H]$^+$ m/z 495.2094. Anal. Calcd for $C_{23}H_{34}N_4S_2O_4(H_2O)_{0.5}$: C, 54.85; H, 7.00. Found: C, 54.47; H, 6.91.

General Procedure for Detosylation of 7a and 7b. Compound **7a** or **7b** (1 mmol) was dissolved in concentrated H_2SO_4 (5 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), and the combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo.

2-[1,4,7]Triazacyclononan-1-ylethylamine (8a). The crude **8a** was thereby obtained as a pale-yellow oil (159 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 2.27−2.41 (m, 16 H), 3.72 (4 H); ^{13}C NMR ($CDCl_3$) δ 39.4 (t), 45.8 (t), 46.0 (t), 52.4 (t), 59.7 (t). This material was observed to be unstable; accordingly, it was used immediately as obtained in the next step.

3-[1,4,7]Triazacyclononan-1-ylpropylamine (8b). The crude **8b** was thereby obtained as a colorless, viscous oil (164 mg, 89%): ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 3.2 Hz, 2 H), 1.48 (s, 4 H), 1.89−2.09 (m, 16 H); ^{13}C NMR ($CDCl_3$) δ 30.1 (t), 39.0 (t), 45.56 (t), 45.58 (t), 51.9 (t), 54.0 (t). HRMS (positive ion FAB) Calcd for $C_9H_{22}N_4$: [M + H]$^+$ m/z 187.1923. Found: [M + H]$^+$ m/z 187.1929.

General Procedure for Alkylation of Tetraamines 8a and 8b. To a suspension of **8a** or **8b** and K_2CO_3 in CH_3CN under argon was added dropwise *tert*-butyl bromoacetate, and the resulting mixture was heated at 65 °C for 24 h. The reaction mixture was allowed to cool gradually to ambient temperature and was filtered, and the filtrate was concentrated in vacuo.

[3-(4,7-Bis-*tert*-butoxycarbomethyl[1,4,7]triazacyclononan-1-ylethyl)-*tert*-butoxycarbonylmethylamino]-acetic Acid *tert*-Butyl Ester (9a). Compound **8a** (500 mg, 2.94 mmol), K_2CO_3 (913 mg, 11.76 mmol), and *tert*-butyl bromoacetate (2.86 g, 14.7 mmol) in CH_3CN (35 mL) afforded **9a** (888 mg, 48%) as a colorless oil after purification by chromatography on silica gel, eluting with 5% MeOH−CH_2Cl_2: ^{1}H NMR ($CDCl_3$) δ 1.38−1.72 (m, 36 H), 2.8 (s, 4 H), 3.05−3.73 (m, 20 H); ^{13}C NMR ($CDCl_3$) δ 27.4 (q), 47.9 (t), 49.3 (t), 50.7 (t), 52.3 (t), 53.6 (t), 55.2 (t), 56.2 (t), 81.2 (s), 81.3 (s), 169.5 (s), 169.8 (s). HRMS (positive ion FAB) Calcd for $C_{32}H_{60}N_4O_8$: [M + H]$^+$ m/z 629.4489. Found: [M + H]$^+$ m/z 629.4479.

[3-(4,7-Bis-*tert*-butoxycarbomethyl[1,4,7]triazacyclononan-1-ylpropyl)-*tert*-butoxycarbonylmethylamino]acetic Acid *tert*-Butyl Ester (9b). Compound **8b** (360 mg, 1.95 mmol), K_2CO_3 (1.08 g, 7.80 mmol), and *tert*-butyl bromoacetate (1.52 g, 7.82 mmol) in CH_3CN (23 mL) afforded **9b** (652 mg, 52%) as a colorless oil after purification by chromatography on silica gel, eluting with 4% MeOH−CH_2Cl_2: ^{1}H NMR ($CDCl_3$) δ 1.21−1.62 (m, 36 H), 2.00 (s, 2 H), 2.60−2.95 (m, 6 H), 3.03−3.84 (m, 18 H); ^{13}C NMR ($CDCl_3$) δ 22.7 (t), 27.9 (q), 49.2 (t), 51.4 (t), 52.0 (t), 52.7 (t), 54.1 (t), 55.9 (t), 57.7 (t), 81.1 (s), 81.3 (s), 170.2 (s), 170.4 (s). HRMS (positive-ion FAB) Calcd for $C_{33}H_{62}N_4O_8$: [M + H]$^+$ m/z 643.4646. Found: [M + H]$^+$ m/z 642.4641.

Deprotection of Tetra-*tert*-butyl Esters 9a and 9b. A solution of either **9a** or **9b** in dry 1,4-dioxane cooled in a ice−water bath was saturated with $HCl_{(g)}$ for 4 h, after which the mixture was warmed to ambient temperature and then stirred for 12 h. The precipitate was collected, washed with ethyl ether, and dissolved in water. The solution was then lyophilized to give **1** or **2** as a pale-yellow solid.

[2-(4,7-Biscarboxymethyl[1,4,7]triazacyclononan-1-ylethyl)carbonylmethyl-amino]acetic Acid Tetrahydrochloride (1). Compound **9a** (130 mg, 0.21 mmol) in 1,4-dioxane (20 mL) afforded **1** (78 mg, 92%) as a salt: ^{1}H NMR

($CDCl_3$) δ 3.21−3.45 (m, 10 H), 3.75−3.93 (m, 8 H), 4.63−4.78 (m, 8 H); ^{13}C NMR ($CDCl_3$) δ 49.6 (t), 50.7 (t), 51.2 (t), 51.6 (t), 55.6 (t), 56.9 (t), 169.7 (s), 170.2 (s). MS (positive-ion FAB) $[M + H]^+$ *m/e* 405. Anal. Calcd for $C_{16}H_{28}N_4O_8(HCl)_4(H_2O)_4$: C, 30.88; H, 6.48. Found: C, 30.71; H, 6.38.

[3-(4,7-Biscarboxymethyl[1,4,7]triazacyclononan-1-yl-propyl)carbonylmethylamino]acetic Acid Tetrahydrochloride (2). Compound **9b** (280 mg, 0.44 mmol) in 1,4-dioxane (30 mL) afforded **2** (162 mg, 88%) as a salt: ^1H NMR (D_2O, pD = 1) δ 3.23−3.62 (m, 8 H), 3.90 (s, 4 H), 4.21 (s, 4 H), 4.81 (s, 10 H); ^{13}C NMR (D_2O, pD = 1) δ 49.3 (t), 50.3 (t), 52.7 (t), 54.0 (t), 54.3 (t), 56.0 (t), 167.4 (s), 171.1 (s). MS (positive-ion FAB) *m/e* 419 $[M + H]^+$. Anal. Calcd for $C_{17}H_{30}N_4O_8(HCl)_4(H_2O)$: C, 35.07; H, 6.23. Found: C, 35.45; H, 6.15.

Kinetics. Formation kinetics of Y(III) complexes of **1**, **2**, and DOTA (Macrocyclics) was measured by monitoring the absorption at 652 nm. To a solution of YCl_3 (2 mL, 1.6 μM, atomic absorption standard solution, Aldrich) was added arsenazo(III) (5 μM, Sigma) in NH_4OAc (0.15 M, pH 4.0, metal-free). The resulting Y(III)−arsenazo(III) mixture was stirred for at least 2 h for complete equilibration and added to a solution of ligand (20 μL, 10 mM) in a cell. After mixing (<5 s), absorption of the solution at 652 nm (absorption wavelength of Y(III)-arsenazo(III))[6b] was immediately followed over time at room temperature using a HP 8452A Diode Array Spectrophotometer which was calibrated with a 5 μM arsenazo(III) solution. Without any ligand added, the absorption was 0.089 for the YCl_3−arsenazo(III) complex at 652 nm. The competition between arsenazo(III) (5 μM) and ligand (100 μM) for Y(III) (1.6 μM) was followed by the decrease in the absorbance at 652 nm.

Serum Stability. The ^{88}Y complexes of **1** and **2** were prepared by the addition of 350 μCi of ^{88}Y (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 were forced to completion by heating the reaction mixture at 80 °C for 18 h, after which they were 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 the free ^{88}Y. The pH of the ^{88}Y complex solutions was adjusted to 7.0 with PBS buffer, and 250 μCi of either complex was added to 1 mL of human serum incubated at 37 °C. An aliquot of the serum (5−10 μL) was taken at selected times (Figure 1) and analyzed by SE-HPLC. The serum stability of the ^{88}Y complexes was assessed by measuring the release of ^{88}Y radionuclide from the complexes to serum proteins using SE-HPLC with a TSK-3000 column. The column was eluted with PBS at a 1 mL/min flow rate.

In Vivo Biodistribution Studies. Female Balb/c mice were obtained from Charles River Laboratories (Wilmington, MA) at 4−6 weeks of age. The pH of the ^{86}Y-labeled ligands were adjusted to pH ~7.0 with 0.5 M sodium bicarbonate (pH 10.5) and diluted in phosphate-buffered saline. The radiolabeled ligands (5.9 μCi of ^{86}Y−DOTA, 5.0 μCi of ^{86}Y−**1**) were administered to the mice in 200 μL via tail vein injection. The mice (five per data point) were sacrificed by exsanguination at 0.5, 1, 4, 8, and 24 h. Blood and the major organs were harvested and wet-weighed, and the radioactivity was measured in a γ-scintillation counter (Minaxi-γ; Packard, Downers Grove, IL). The % ID/g was determined for each tissue. The values presented are the mean and standard error of the mean for each tissue. All animal experiments were performed in compliance with current regulations and guidelines of the U.S. Department of Agriculture and the NIH Animal Research Advisory Committee.

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