Novel Bifunctional Macrocyclic Chelating Agents Appended with a Pendant-Type Carboxymethylamino Ligand and Nitrobenzyl Group and Stability of the $^{88}Y^{III}$ Complexes

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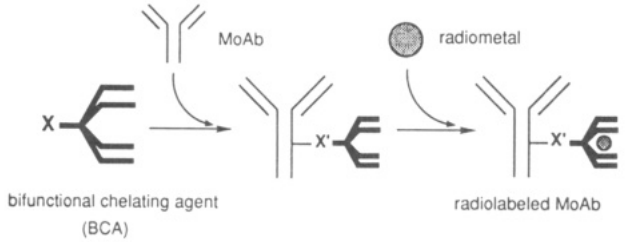
There have been a number of approaches to radiometal-labeled monoclonal antibodies (MoAb) as novel agents targeted for tumor imaging and cancer treatment during the past decade.[1] For development of an effective radiolabeled MoAb, good chelating agents, which couple the radionuclide to the MoAb, are most critical.

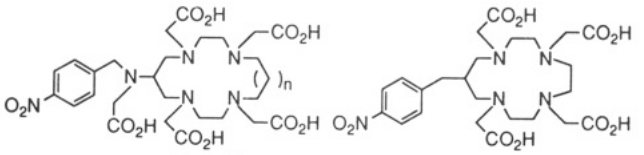


The chelating agent must form a stable radiometal complex because any liberated radiometal may be taken up by normal tissue and may cause radiation damage to nontarget cells. Further, such a compound must have a reactive site (X) by which it can be chemically linked to the antibody protein. Thus, the chelating agent must be bifunctional. Bifunctionalized EDTA or diethylenetriaminepentaacetic acid (DTPA) have been widely used for labeling MoAbs with radionuclides such as ^{111}In, ^{67}Ga, ^{90}Y, ^{67}Cu, and ^{212}Bi.[2] These acyclic bifunctional chelating agents (BCAs), however, did not form radionuclide complexes which were stable in vivo owing to acid- or cation-promoted dissociation.[3] Recently, several modified BCAs have been developed for greater complex stability in vivo. Among them are backbone-substituted DTPAs[3,4] and macrocyclic BCAs. In particular, some macrocyclic BCAs (e.g. 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)) have displayed high selectivity for particular radiometals and these complexes showed remarkable kinetic and thermodynamic inertness in vivo.[5]

However, we found that these macrocyclic BCAs often suffer from a disadvantage of extremely slow metal complexation compared with acyclic BCAs such as DTPA.[6] In our preliminary experiment with Y^{III}, it took 1–2 days for complete chelation of Y^{III} with 2-(4-nitrobenzyl)-DOTA[5b] at room temperature, while the half-life of $^{90}Y^{III}$, one of the most useful candidates for radioimmunotherapy (E_{max} = 2.3 MeV), is 2.6 days.[7]

Earlier, we reported that when appended with a pyridyl pendant donor, a cyclam ring, the [14]aneN$_4$ (pyridylcyclam, 5-(2-pyridyl)-1,4,8,11-tetraazacyclotetradecane) exhibits an elevated rate of complex formation with Ni^{II} (where the apparent second-order rate constant (M^{-1} s^{-1}) of Ni^{II} complexation at pH 6.5 and 25 °C is 0.12 for pyridylcyclam, against 4.2×10^{-3} for cyclam).[8] It was postulated that the pyridyl pendant donor plays an important kinetic role in Ni^{II} cyclam complexation. Pendant donors might be helpful in enhancing the radiometal complexation as well.

In the present study, we have developed a new synthetic route for pendant-type macrocyclic BCAs such as **1** and **2**, which contain a carboxymethylamino group appended to the macrocyclic system. The nitrobenzyl group is reduced to the corresponding aniline which in turn can be converted into a protein-reactive functional group such as isothiocyanate, whenever desired.[9] We compared **1** with a reference, nonpendant-type macrocyclic BCA **3** (6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotridecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (Bz-TRTA)) in the rate of complex formation with yttrium(III) and stability of the resulting complexes in human serum. We used $^{88}Y^{III}$ for laboratory studies instead of $^{90}Y^{III}$ to take advantage of its long half-life (106.6 days) and γ-emitting properties.



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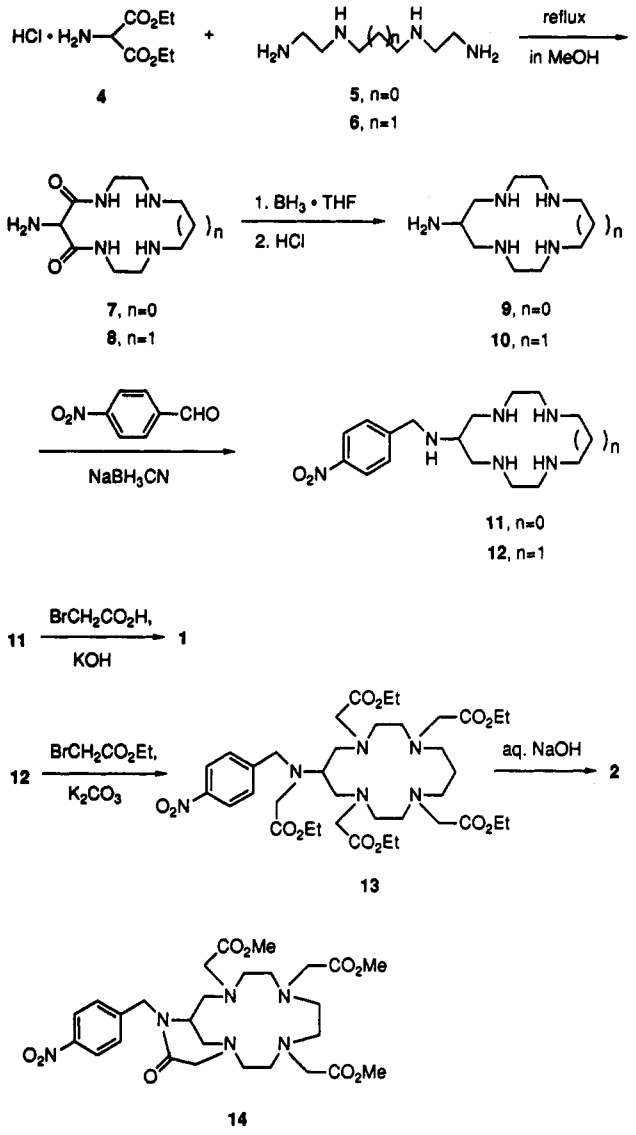
Scheme I



Results and Discussion

Syntheses of Pendant-Type BCAs. A new synthetic procedure for the pendant BCA 1 via the amino-substituted azamacrocycle intermediate 9 was developed (Scheme I). A dilute solution of diethyl aminomalonate hydrochloride (4) and 1,8-diamino-3,6-diazaoctane (5) in methanol was refluxed for 3 days to give dioxoaza cyclic amine 7 in 34% yield. Reduction of the dioxoaza cyclic amine with BH_3–THF afforded the corresponding amine 9 in 87% yield. The amino-substituted azamacrocycle 9 was reacted with 4-nitrobenzaldehyde in the presence of $NaBH_3CN$ to give 4-nitrobenzylamino-substituted azamacrocycle 11 in 79% yield.[10] Subsequent N-alkylation of the pendant-substituted azamacrocycle to form the pendant BCA 1 was accomplished in 44% yield by using bromoacetic acid and KOH. N-Alkylation of 11 with methyl, ethyl, *tert*-butyl, or benzyl bromoacetates (K_2CO_3/DMF) did not give the corresponding pentasubstituted macrocyclic polyamine. Either a wrong product 14 was indicated with methyl bromoacetate (data not shown) or the reaction was very slow with other esters. Ring-expanded congener 2 was synthesized by the same process via the intermediate 10, thus proving the versatility of this procedure as a process for such macrocycles as amino-substituted azamacrocycles and pendant BCAs. N-Alkylation of 12 to obtain 2 was better accomplished by a two-step process, a reaction with ethyl bromoacetate (K_2CO_3/DMF) followed by base hydrolysis (NaOH in aqueous MeOH).

Table I. The Time-Dependent Chelating Reaction of Macrocyclic BCA with $^{88}Y^{III}$ in pH 5 Buffer at 25 °C

macrocyclic BCA	(radioactivity chelated/radioactivity added) × 100 (%)				
	0 h	0.25 h	0.5 h	1.0 h	2.0 h
1	0	96.2	96.4	97.8	98.1
3	0	60.7	81.8	93.4	97.5

Synthesis of Nonpendant-Type BCA. Bz-TRTA (3) was prepared by following the reported procedure[11] with minor modification (see Experimental Section).

Rate of ^{88}Y Complex Formation. In order to evaluate the effect of the pendant donor with our pendant-type BCA on the rate of complex formation, we compared the rate of radionuclide $^{88}Y^{III}$ chelation of pendant-type BCA 1 with nonpendant-type BCA 3 which has the same macrocycle backbone. We selected 1 rather than 2 for the comparison because 2 which has the TETA backbone is thought to form a less stable complex with Y^{III} than 1 which has the TRTA backbone (stability constants K for the formation of 1:1 Y^{III} complexes: TRTA, $\log K = 19.6$; TETA, $\log K = 16.3$[5e]). The congener 2 may be suitable for chelation of copper(II).[5d] The proportion of the chelated [$^{88}Y^{III}$]BCA among the total $^{88}Y^{III}$ added to the reaction system was calculated from the results of paper chromatographic separation[12] of the radioactivity into free ($^{88}Y^{III}$) and chelated ([$^{88}Y^{III}$]BCA).

Table I shows the proportion of the chelated radioactivity at various times after initiation of complex formation between the pendant-type BCA 1 or nonpendant-type BCA 3 and $^{88}Y^{III}$. The time necessary to reach the complete chelation of nonpendant-type BCA 3 was about 2 h, while that of pendant-type BCA 1 was less than 15 min. This result evidently indicates that the carboxymethylamino pendant group enhances the rate of $^{88}Y^{III}$ complexation with BCAs. The rate enhancement observed in the pendant-type BCA 1 may be discussed as follows: $^{88}Y^{III}$ ion may be captured more quickly, though loosely, by the pendant donor carboxymethylamino group which is conformationally more flexible because of its position off the rigid macrocycle backbone than solely by the backbone carboxymethyl groups which constitute a well-fixed rigid structure. The local metal concentration near the BCA backbone may consequently be raised and the rate of ultimate complexation involving the four backbone carboxymethyl groups will be enhanced.

Stability of Complex in Serum. The BCA complexes of radionuclides must be stable in the body in order to avoid undesirable radiation damage to nontarget cells when they are utilized in radiolabeled MoAbs as pharmaceuticals. In order to mimic the in vivo condition, stability of the complex was tested in human serum which contains biological proteins (albumin, transferrin, etc.) radionuclides may be bound. Figure 1 shows the radioactivity retained in BCAs during incubation of complexes in human serum at 37 °C. The stability of $^{88}Y^{III}$ complex of pendant-type BCA 1 was found to be similar to that of nonpendant-

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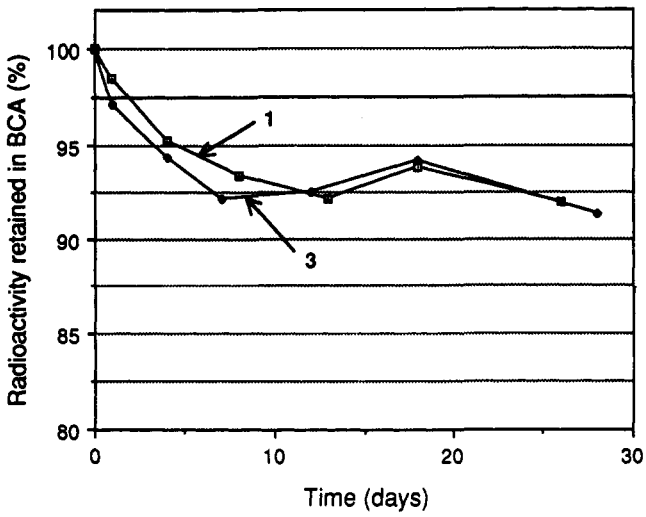

Figure 1. Stability of ^{88}Y–BCA complexes in human serum at 37 °C.

type BCA 3; both retained more than 90% of radioactivity for over 3 weeks. The carboxymethylamino pendant donor of 1 apparently has little effect on the overall stability of the BCA 1 complex in the present test.

In conclusion, the carboxymethylamino-pendant group accelerates the rate of Y^{III} complexation with BCA. A pendant-type macrocyclic BCA may open a way to new BCA's useful for radiolabeling of proteins such as MoAbs.

Experimental Section

General Methods. MeOH was dried over molecular sieves 3A. THF was distilled from sodium benzophenone ketyl. Starting materials, 1,8-diamino-3,6-diazaoctane (5) and 1,9-diamino-3,7-diazanonane (6) (Kanto Chemical Co., Inc., Tokyo), were purified by recrystallization as corresponding hydrochloride salts and then by distillation as free forms prior to use. The other reagents obtained commercially were used without further purification. Human serum was prepared from blood collected from healthy men in the usual way. Melting points were determined on a micro melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained at 270 and 68 MHz, respectively. High-resolution molecular secondary ion mass spectra (HRSIMS) were performed using either glycerol (G) or 3-nitrobenzyl alcohol/2-hydroxyethyl disulfide (NBA/HED) as matrix. Column chromatography was carried out on silica gel (Daisogel IR-60). Syntheses of 6-aminocyclam-5,7-dione (8) and 6-aminocyclam (10) are described separately.[13] The purity of the compounds were certified by either combustion analysis or by ^1H and/or ^{13}C NMR spectral determination (see the supplementary materials).

6-Amino-1,4,8,11-tetraazacyclotridecane-5,7-dione (7). A solution of diethyl aminomalonate hydrochloride (4) (10.0 g, 47.2 mmol) and 1,8-diamino-3,6-diazaoctane (5) (6.9 g, 47.2 mmol) in 1 L of absolute methanol was refluxed for 3 days. After evaporation of the solvent, the residue was purified by silica gel chromatography (eluent: $CHCl_3$–MeOH–25% aqueous NH_3, ratio from 100:10:1 to 100:20:2). Precipitation from EtOH–HCl gave 7·3HCl as a white solid (6.35 g). A water solution of the solid of 7·3HCl was loaded on a column of Dowex 1X-8 anion-exchange resin (free form). The column was eluted with water. Alkaline fractions were collected and water was removed under reduced pressure to give a residue. It was recrystallized from a mixture of CH_3CN and MeOH to give 7 (3.73 g, 34%) as colorless needles: mp 186–187 °C; IR (KBr) 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5–2.3 (br, 4 H, CNHC and NH_2), 2.55–2.68 (m, 6 H, NCH_2), 2.75–2.82 (m, 2 H, NCH_2), 3.38–3.47 (m, 4 H, $CONCH_2$), 4.13 (s, 1 H, COCHCO), 7.5–7.8 (br, 2 H, CONH); ^{13}C NMR ($CDCl_3$) δ 39.77, 47.76, 49.17, 58.37, 170.82; HRSIMS (G) calcd for $C_9H_{20}N_5O_2$ ($M + H^+$) 230.1617, found 230.1621. Anal. Calcd for $C_9H_{19}N_5O_2$: C, 47.15; H, 8.35; N, 30.54. Found: C, 47.54; H, 8.67; N, 30.44.

6-Amino-1,4,8,11-tetraazacyclotridecane (9). A solution of BH_3 in THF (Aldrich Chemical Co., Inc., 1 M, 360 mL) was added dropwise to a stirred suspension of 7 (2.72 g, 11.9 mmol) in THF (150 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was refluxed for 14 h. Water (50 mL) and concd HCl (50 mL) was added slowly to this mixture at 0 °C, and then this mixture was refluxed for 4 h. After concentration of the solution to one-third of the volume in vacuo, the resulting precipitate was filtered. The filtrate was washed with ether. After evaporation of the aqueous solution, the residue was dissolved in water and was loaded on a column of Dowex 1X-8 anion-exchange resin (free form). The column was eluted with water. Alkaline fractions were collected and water was removed under reduced pressure to give an oil. Treatment of the oil with 47% aqueous HBr in EtOH gave 9·5HBr as a white solid (5.71 g). A water solution of the solid of 9·5HBr was loaded on a column of Dowex 1X-8 anion-exchange resin (free form) and the column was eluted with water. Alkaline fractions were collected and water was removed under reduced pressure to give a colorless oil of 9 (2.08 g, 87%): ^1H NMR ($CDCl_3$) δ 1.7–2.4 (br, 6 H, CNHC and NH_2), 2.60–2.84 (m, 16 H, NCH_2), 3.02–3.10 (m, 1 H, CCHC); ^{13}C NMR ($CDCl_3$) δ 47.35, 47.64, 48.89, 50.39, 56.26; HRSIMS (G) calcd for $C_9H_{24}N_5$ ($M + H^+$) 202.2032, found 202.2016.

6-[*N*-(4-Nitrobenzyl)amino]-1,4,8,11-tetraazacyclotridecane (11). To a solution of 9 (1.01 g, 5.0 mmol) in dry MeOH (60 mL) were added under stirring 1 M HCl in MeOH (10 mL, 10 mmol), 4-nitrobenzaldehyde (1.5 g, 10 mmol), and $NaBH_3CN$ (0.31 g, 5.0 mmol) in this order at rt under a nitrogen atmosphere. The solution was stirred at rt for 3 h. Then the solution was poured into 30 mL of 1 M HCl and the mixture was washed with three portions of CH_2Cl_2. The aqueous solution was brought to alkaline (pH >9) with 1 M NaOH and was extracted with five portions of CH_2Cl_2. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The resulting residue was purified by silica gel chromatography (eluent: $CHCl_3$–MeOH–25% aqueous NH_3, ratio from 100:20:2 to 100:50:15) to give 11 (1.33 g, 79%) as a pale brown oil: ^1H NMR ($CDCl_3$) δ 1.9–2.4 (br, 5 H, CNHC), 2.65–2.84 (m, 17 H, NCH_2 and CCHC), 3.95 (s, 2 H, $ArCH_2N$), 7.53 (d, J = 8.90 Hz, 2 H, ArH), 8.17 (d, J = 8.58 Hz, 2 H, ArH); ^{13}C NMR ($CDCl_3$) δ 47.31, 47.73, 48.98, 51.09, 52.92, 56.50, 123.59, 128.55, 147.01, 148.69; HRSIMS (G) calcd for $C_{16}H_{29}N_6O_2$ ($M + H^+$) 337.2352, found 337.2362.

6-[*N*-(4-Nitrobenzyl)amino]-1,4,8,11-tetraazacyclotetradecane (12). Following the procedure described for the synthesis of 11, 43 mg of 10 was converted to 53 mg (75%) of 12 as pale yellow needles: mp 101.0–102.0 °C; ^1H NMR ($CDCl_3$) δ 1.68–1.76 (m, 2 H, CCH_2C), 1.9–2.3 (br, 5 H, CNHC), 2.61–2.82 (m, 17 H, NCH_2 and CCHC), 3.94 (s, 2 H, $ArCH_2N$), 7.53 (d, J = 8.57 Hz, 2 H, ArH), 8.17 (d, J = 8.58 Hz, 2 H, ArH); ^{13}C NMR ($CDCl_3$) δ 29.45, 49.07, 49.18, 50.39, 51.21, 53.64, 56.39, 123.59, 128.57, 147.02, 148.75; HRSIMS (G) calcd for $C_{17}H_{31}N_6O_2$ ($M + H^+$) 351.2508, found 351.2532.

6-[*N*-(Carboxymethyl)-*N*-(4-nitrobenzyl)amino]-1,4,8,11-tetraazacyclotridecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid (1). A solution of bromoacetic acid (167 mg, 1.2 mmol) in water (2 mL) was brought to pH 10 with 7 M KOH below 5 °C. To this solution was added a solution of 11 (67 mg, 0.2 mmol) in EtOH (3 mL) and the mixture was heated at 70 °C for 4 h. The pH of the mixture was maintained ca. 10 with 7 M KOH. After cooling, the solution was acidified to pH 1.5 with 47% aqueous HBr and then was extracted with two portions of ether. After the aqueous solution was evaporated, the resulting residue was dissolved in water (1 mL). The solution was loaded on a column of Amberlite 200C cation-exchange resin (free form) and the column was eluted with water (400 mL) followed by 2% aqueous NH_3 (300 mL). The fraction-containing eluent of 2% aqueous NH_3 was evaporated. The resulting residue was purified by silica gel chromatography (eluent: $CHCl_3$–MeOH–25% aqueous NH_3 = 6:3:1) to give 1 (55 mg, 44%) as a glassy solid: IR (KBr) 1676 cm^{-1}; ^1H NMR (D_2O, pD 1) δ 2.75–4.20 (m, 27 H, NH_2 and CCHC), 4.36 (br, 2 H, $ArCH_2N$), 7.71 (d, J = 8.58 Hz, 2 H, ArH), 8.31 (d, J

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= 8.57 Hz, 2 H, ArH); HRSIMS (NBA/HED) calcd for $C_{26}H_{39}N_6O_{12}$ (M + H$^+$) 627.2626, found 627.2698.

6-[*N*-[(Ethoxycarbonyl)methyl]-*N*-(4-nitrobenzyl)amino]-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic Acid Tetraethyl Ester (13). To a solution of 12 (0.50 g, 1.4 mmol) in DMF (20 mL) with stirring was added K_2CO_3 (1.18 g, 8.6 mmol) and the resulting mixture was stirred for 1 h at rt under a nitrogen atmosphere. Ethyl bromoacetate (2.39 g, 14.3 mmol) was added to the reaction mixture which then was stirred for a further 3 h at rt. To the resulting solution was added water (50 mL) and the mixture was extracted with two portions of ethyl acetate. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel chromatography (eluent: $CHCl_3$–MeOH, ratio from 100:1 to 10:1) followed by centrifugal partition chromatography (CPC Model LLN, Sanki Engineering Ltd.) with a solvent system of hexane–AcOEt–MeOH–H_2O (20:12:18:7) to give 13 (337 mg, 30%) as a pale yellow oil: IR (film) 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22–1.30 (m, 15 H, CCH_3), 1.42–1.49 (m, 2 H, CCH_2C), 2.54–2.86 (m, 17 H, NCH_2C and CCHC), 3.16–3.68 (m, 10 H, NCH_2-CO), 3.93 (s, 2 H, $ArCH_2N$), 4.08–4.19 (m, 10 H, OCH_2C), 7.67 (d, J = 8.57 Hz, 2 H, ArH), 8.17 (d, J = 8.58 Hz, 2 H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.13, 14.23, 14.27, 25.43, 50.64, 51.25, 51.56, 51.70, 54.09, 55.01, 55.85, 58.80, 60.11, 60.15, 60.58, 123.36, 129.58, 147.10, 147.83, 171.54, 171.79; HRSIMS (NBA/HED) calcd for $C_{37}H_{61}N_6O_{12}$ (M + H$^+$) 781.4347, found 781.4424.

6-[*N*-(Carboxymethyl)-*N*-(4-nitrobenzyl)amino]-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic Acid (2). To a solution of 13 (390 mg, 0.5 mmol) in MeOH (20 mL) was added 2 M NaOH (5 mL, 10 mmol) and the solution was stirred for 6 h at rt. The reaction mixture was acidified to pH 1.5 with 5 M HCl and then was extracted with two portions of ether. The aqueous solution was concentrated in vacuo and the resulting residue was dissolved in water (1 mL). The solution was loaded on a column of Amberlite 200C cation-exchange resin (free form) and the column was eluted with water (400 mL) and then with 2% aqueous NH_3 (300 mL). The fraction that contained the eluent of 2% aqueous NH_3 was evaporated in vacuo to result a residue. The residue that contained 2 was purified by silica gel chromatography (eluent: $CHCl_3$–MeOH–25% aqueous NH_3 = 6:3:1) to give a sample of 2. It was precipitated from water to give a white solid of 2 (161 mg, 50%): IR (KBr) 1597 cm^{-1}; ^{1}H NMR (D_2O, pD 5) δ 1.75–2.08 (m, 2 H, CCH_2C), 2.75–3.68 (m, 27 H, NCH_2 and CCHC), 4.05 (s, 2 H, $ArCH_2N$), 7.65 (d, J = 8.25 Hz, 2 H, ArH), 8.22 (d, J = 7.92 Hz, 2 H, ArH); HRSIMS (NBA/HED) calcd for $C_{27}H_{41}N_6O_{12}$ (M + H$^+$) 641.2782, found 641.2732.

6-(4-Nitrobenzyl)-1,4,8,11-tetraazacyclotridecane-*N,N',N'',N'''*-tetraacetic Acid (3).[11] Briefly, refluxing a solution of diethyl (4-nitrobenzyl)malonate[5a] (29.5 g, 0.10 mol) and 5 (14.6 g, 0.10 mol) in 2.0 L of dry MeOH for 13 d gave 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotridecane-5,7-dione (18.83 g, 54%) [a pale yellow solid; IR (KBr) 1670 cm^{-1}; ^{1}H NMR (D_2O, pD 4) δ 2.98–3.38 (m, 12 H, NCH_2 and $CONCH_2$), 3.69–3.78 (m, 3 H, $ArCH_2$ and COCHCO), 7.47 (d, J = 8.25 Hz, 2 H, ArH), 8.15 (d, J = 8.90 Hz, 2 H, ArH); ^{13}C NMR (D_2O, pD 4) δ 33.83, 38.56, 44.92, 47.88, 56.31, 124.77, 130.74, 147.13, 147.43, 171.84; HRSIMS (G) calcd for $C_{16}H_{24}N_5O_4$ (M + H$^+$) 350.1828, found 350.1910].

The dioxo compound (3.49 g, 10 mmol) was reduced with BH_3–THF (1 M, 100 mL) to give 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotridecane (2.19 g, 68%) [colorless crystals; mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (br, 4 H, NH), 2.48–2.81 (m, 19 H, $ArCH_2$, ArCCH, and NCH_2), 7.34 (d, J = 8.58 Hz, 2 H, ArH), 8.15 (d, J = 8.57 Hz, 2 H, ArH); ^{13}C NMR (D_2O/HCl) δ 37.48, 37.77, 44.97, 45.40, 47.09, 54.21, 124.81, 131.06, 147.36, 148.04; HRSIMS (G) calcd for $C_{16}H_{28}N_5O_2$ (M + H$^+$) 322.2243, found 322.2290].

Subsequently, N-alkylation of the azamacrocycle (321 mg, 1.0 mmol) with methyl bromoacetate (765 mg, 5.0 mmol) in the presence of K_2CO_3 (2.76 g, 20.0 mmol) in DMF gave 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotridecane-*N,N',N'',N'''*-tetraacetic acid tetramethyl ester (329 mg, 54%) [a pale yellow oil; IR (film) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.14 (br, 1 H, ArCCH), 2.35–2.46 (m, 2 H, $ArCH_2$), 2.55–2.97 (m, 16 H, NCH_2), 3.36–3.62 (m, 8 H, NCH_2CO), 3.66–3.76 (m, 12 H, CO_2CH_3), 7.37 (d, J = 8.24 Hz, 2 H, ArH), 8.14 (d, J = 8.55 Hz, 2 H, ArH); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 38.18, 39.41, 50.79, 51.38, 51.81, 52.45, 54.90, 56.97, 57.53, 123.48, 129.99, 146.37, 149.48, 172.00; HRSIMS (NBA/HED) calcd for $C_{28}H_{44}N_5O_{10}$ (M + H$^+$) 610.3088, found 610.2997].

Finally, the tetramethyl ester (0.33 g, 0.54 mmol) was hydrolyzed with NaOH in aqueous MeOH to give 3 (0.25 g, 83%) [a white solid; IR (KBr) 1605 cm^{-1}; ^{1}H NMR (D_2O, pD 5) δ 2.62 (br, 1 H, ArCCH), 2.77 (d, J = 7.26 Hz, 2 H, $ArCH_2$), 3.1–3.8 (m, 24 H, NCH_2), 7.49 (d, J = 8.58 Hz, 2 H, ArH), 8.14 (d, J = 8.58 Hz, 2 H, ArH); ^{13}C NMR (D_2O, pD 5) δ 33.24, 37.55, 51.71, 51.80, 54.16, 56.28, 57.26, 59.28, 124.94, 131.08, 147.51, 147.81, 174.48, 174.62; HRSIMS (G) calcd for $C_{24}H_{36}N_5O_{10}$ (M + H$^+$) 554.2462, found 554. 2448].

Stock Solution of $^{88}Y^{III}$ in Ammonium Acetate Buffer (pH 5). To a solution (100 μL) of $^{88}Y^{III}$ (yttrium-88, 0.368 mCi/mL in 1 mL of 0.23 M HCl, specific activity of 1.43×10^4 mCi/mg; contaminants Al ~1.0 ppm, Mg ~0.1 ppm, Zn ~0.5 ppm, Ca ~0.2 ppm, Cu ~0.05 ppm, Si ~0.5 ppm; Amersham Japan Ltd.) was added 200 μL of distilled water and 600 μL of 0.1 M ammonium acetate buffer (pH 5). The solution was adjusted to pH 5 with 32 μL of 0.5 M aqueous NH_3.

Rate of Complex Formation. A stock solution of $^{88}Y^{III}$ in ammonium acetate buffer (45 μL, 1.5×10^{-12} mol, 1.78 μCi) was added to a solution of a chelator (15 μL, 3×10^{-4} M, 4.5×10^{-9} mol) in distilled water. The mixture was incubated at 25 °C. At appropriate time intervals, a 4-μL aliquot of incubation mixture was analyzed for complex formation, as described below, by using paper chromatography.

Stability of $^{88}Y^{III}$ Complex in Human Serum. A stock solution of ^{88}Y in ammonium acetate buffer (90 μL, 3×10^{-12} mol, 3.55 μCi) was added to a solution of a chelator (30 μL, 3×10^{-4} M, 9×10^{-9} mol) in distilled water. The mixture was incubated at rt for 24 h and the pH of the mixture was raised to 7 with 0.5 M aqueous NH_3 (2.5 μL). The resulting mixture was analyzed for binding, as described below, by using paper chromatography. Then a 60-μL aliquot of the mixture was mixed with 540 μL of human serum in plastic tube. The tube was placed in an incubator kept at 37 ± 1 °C. At appropriate time intervals, a 10-μL aliquot of incubation mixture was analyzed using paper chromatography.

Paper Chromatography Analysis. The method described by Subramanian and Wolf was followed.[11] The paper chromatography system consisted of filter paper (51B, Toyo Roshi Kaisha Ltd.) and a mixture of 2% (w/v) ammonium acetate in ion-exchanged water (pH 7.4) and CH_3CN (with a ratio of 1:1, v/v) as the developing solvent. An aliquot of a sample solution was spotted on the end of paper strip. The paper was air-dried and developed in the solvent. After air-drying, the paper strip was cut into 1-cm pieces and the radioactivity of each piece was counted in a γ-counter (ARC-361, Aloka). $^{88}Y^{III}$–chelator complex migrated with R_f 0.3–1.0, while unchelated free $^{88}Y^{III}$ remained near the origin.

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Supplementary Material Available: Copies of ^{1}H NMR spectra for compounds 7, 9, 11, 12, 1, 13, and 2 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.