Novel Pendant-Type Macrocyclic Bifunctional Chelating Agents: (Carboxymethyl)amino Derivatives of 2-(4-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic Acid and Their Complex Formation with Yttrium(III)

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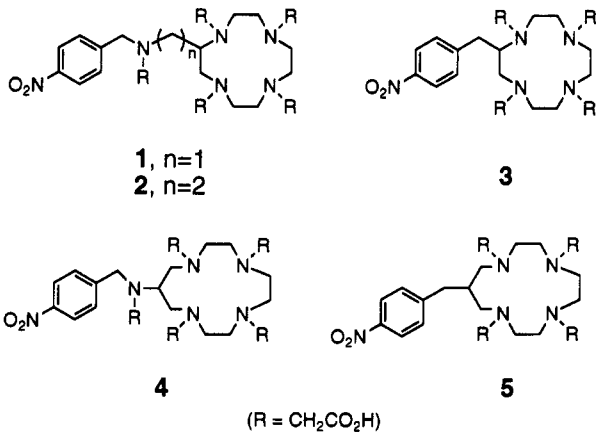
Introduction

For effective radiolabeled monoclonal antibodies (MoAb) usable for tumor imaging and cancer therapy,[1] development of good chelating agents, which couple the radionuclide to the MoAb, is most critical. In radioimmunotherapy, yttrium-90 (^{90}Y), which has a physical half-life of 2.7 days and is a pure β-emitter of high energy (E_{max} = 2.28 MeV), is considered a suitable radionuclide.[2] Earlier, we found that DOTA (1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid), a most favorable binder of yttrium, suffers from the disadvantage of extremely slow metal complexation compared with acyclic chelating agent such as DTPA.[3] We have reported that chelator **4**, a macrocyclic bifunctional chelating agent (BCA) with a structure of *p*-NO_2-Bz-TRTA (6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotridecane-*N,N',N'',N'''*-tetraacetic acid) appended with a (carboxymethyl)amino "pendant", formed an $^{88}Y^{III}$ complex 10 times faster than *p*-NO_2-Bz-TRTA (**5**), a nonpendant-type chelator.[4] It was postulated, therefore, that the (carboxymethyl)amino pendant donor plays an important kinetic role on Y^{III}-macrocyclic BCA complexation. Furthermore, these $^{88}Y^{III}$ complexes of **4** and **5** remained similarly stable in human serum at 37 °C. Therefore, a pendant donor might be helpful in accelerating the Y^{III} complexation, but has little effect on the stability of complexes *in vivo* conditions.

In the present study, we have explored a new synthetic method for pendant-type *p*-NO_2-Bz-DOTA, **1** and **2**, which contain a (carboxymethyl)amino group appended to the DOTA structure via a methylene (**1**) and an ethylene (**2**) spacer, respectively. We then compared **1** and **2** with **3** (*p*-NO_2-Bz-DOTA), a nonpendant-type reference, in the complexation kinetics with Y^{III} using ligand displacement reaction and the stability of the $^{88}Y^{III}$ complexes in human serum.

Results and Discussion

Syntheses of Pendant-Type BCAs. Construction of a polyazamacrocyclic ring is most crucial for successful synthesis of a macrocyclic BCA. Earlier reported cyclization methodologies for preparing 12-membered polyazamacrocycles include reactions of deprotonated tosylamides with tosylates (Richman–Atkins cyclization)[5-7] and of diamine with BOC-protected amino disuccinimido esters.[8] Use of these methodologies for BCA syntheses adds steps for protection and activation making the total procedure longer. The bimolecular cyclization between an imino diester and a polyamine without particular protection or activation features the present synthetic approach, as shown in Schemes I and II. This cyclization methodology which we originally developed[9] is simpler and more convenient than previously reported methods[10] and is applicable to preparation of other pendant functions or ring-expanded congeners with appropriate polyamines or polyimino diesters. In fact, we have prepared imidazole- and hydroxymethylene-pendant cyclenes from histidine and serine, respectively.[11]



Acyl chloride **6**[12] was allowed to react with *p*-nitrobenzylamine to obtain amide **7** in 80% yield. Bromination[13] of **7** gave bromide **8** in 83% yield. The bromide **8** was treated with glycine ethyl ester to give *N*-(4-nitrobenzyl)-amido-substituted imino diester **9** in 84% yield. A dilute solution of the imino diester **9** and equimolar diethylenetriamine in MeOH was heated at reflux for 5 days to give *N*-(4-nitrobenzyl)amido-substituted dioxoazamacrocycle **10** in 10% yield. Reduction of the dioxoazamacrocycle **10** with BH_3 afforded the corresponding amine **11** in 54% yield. Subsequent *N*-alkylation of the pendant-substituted azamacrocycle to the pendant BCA **1** was accomplished with bromoacetic acid and KOH in 14% yield.

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(1) (a) Parker, D. *Chem. Soc. Rev.* **1990**, *19*, 271. (b) Yuanfang, L.; Chuanchu, W. *Pure Appl. Chem.* **1991**, *63*, 427.

(2) Humm, J. L. *J. Nucl. Med.* **1986**, *27*, 1490.

(3) Kodama, M.; Koike, T.; Mahatma, A. B.; Kimura, E. *Inorg. Chem.* **1991**, *30*, 1270.

(4) Takenouchi, K.; Watanabe, K.; Kato, Y.; Koike, T.; Kimura, E. *J. Org. Chem.* **1993**, *58*, 1955.

(5) Moi, M. K.; Meares, C. F.; DeNardo, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 6266.

(6) Renn, O.; Meares, C. F. *Bioconjugate Chem.* **1992**, *3*, 563.

(7) Cox, J. P. L.; Craig, A. S.; Helps, I. M.; Jankowski, K. J.; Parker, D.; Eaton, M. A. W.; Millican, A. T.; Millar, K.; Beeley, N. R. A.; Boyce, B. A. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2567.

(8) McMurry, T. J.; Brechbiel, M.; Kumar, K.; Gansow, O. A. *Bioconjugate Chem.* **1992**, *3*, 108.

(9) (a) Takenouchi, K.; Watanabe, K.; Hara, T.; Kato, Y.; Kimura, E. Jpn. Kokai Tokkyo Koho JP 03, 112, 969 [91, 112, 969], 1991; *Chem. Abstr.* **1991**, *115*, 948. (b) Takenouchi, K.; Watanabe, K.; Hara, T.; Kato, Y.; Kimura, E. Jpn. Kokai Tokkyo Koho JP 03, 197, 468 [91, 197, 468], 1991; *Chem. Abstr.* **1992**, *116*, 898.

(10) A comparison of the present method with reported ones with regard to the number of steps and the total yield (%) from commercially available materials in the syntheses of compound **22** (common intermediate for preparation of *p*-NO_2-Bz-DOTA **3**) is as follows: the present method (see Scheme III), three steps with 9.8%; the method in ref 6, eight steps with 7.7%; the method in ref 8, six steps with 11.2%.

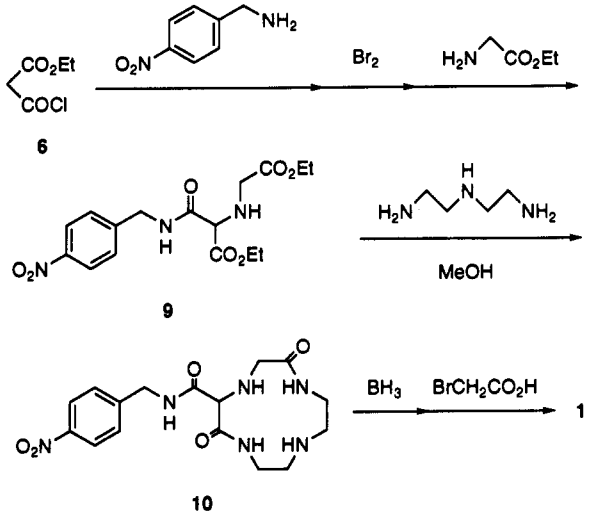
(11) Kimura, E.; Nakamura, I. Unpublished results.

(12) Svendsen, A.; Boll, P. M. *J. Org. Chem.* **1975**, *40*, 1927.

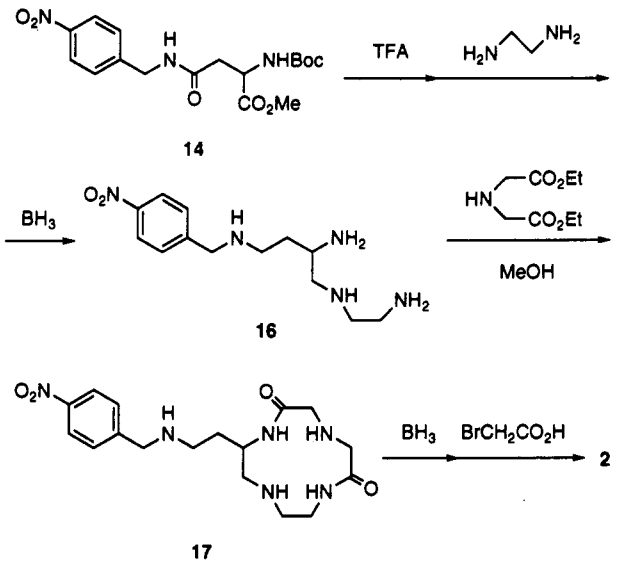
(13) Suzuki, J.; Suzuki, K.; Sekiya, M. *Chem. Pharm. Bull.* **1974**, *22*, 965.

0022-3263/93/1958-6895$04.00/0

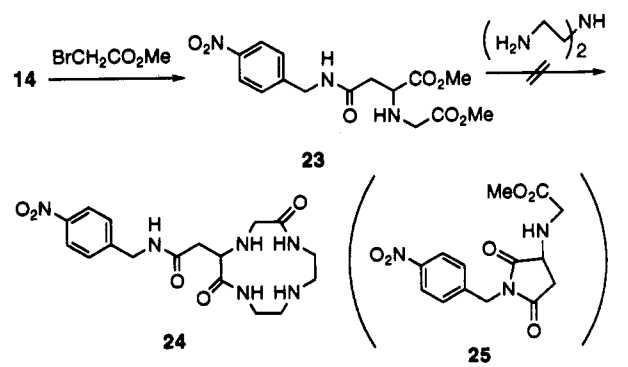
Scheme I



Scheme II



The congener **2**, in which a DOTA structure is linked with a (carboxymethyl)amino ligand via an ethylene spacer, was independently prepared with the same cyclization strategy (Scheme II). Our initial attempt to obtain dioxoazamacrocycle **24** via a cyclization reaction of *N*-(4-nitrobenzyl)amido-substituted imino diester **23** and diethylenetriamine was unsuccessful owing to preferential intracyclization of **23** to give succinimide derivative **25**.



Then, we turned to a cyclization reaction of tetraamine **16** which was prepared from **14** via two steps in 34% yield.

Scheme III

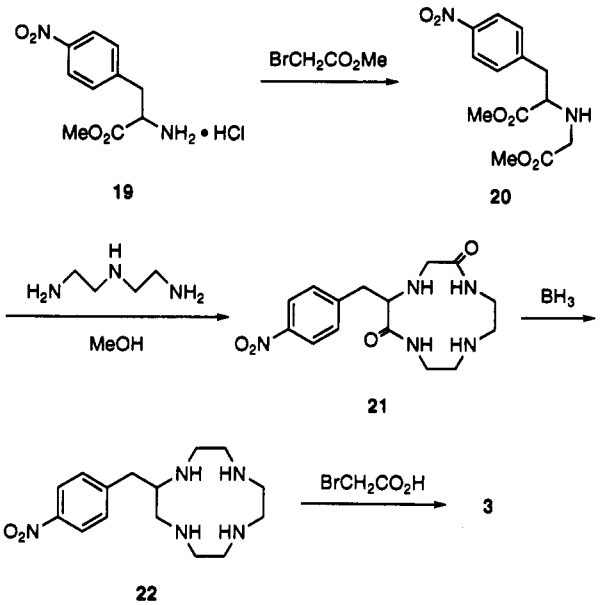


Table I. Pseudo First-Order Rate Constants k_{obs}' for Y^{III}-Macrocyclic BCA Complexes Formation (Reaction 1 in the Text) in pH 6.5 Buffer at 37 °C[a]

macrocyclic BCA	$\times 10^{-3}\ k_{obs}'$ (min^{-1})
1	15.2
2	5.8
3	7.0

[a] In all the confidence limits are within 20%. $-d[Y^{III}\text{-arsenazo III}]/dt = k_{obs}'[Y^{III}\text{-arsenazo III}]$. Initial concentrations of arsenazo III, Y^{III}, and ligand are 2.5×10^{-5}, 2.5×10^{-6}, and 1.0×10^{-4} M, respectively.

The reaction of **16** with iminodiacetic acid ethyl ester gave pendant-substituted dioxoazamacrocycle **17** in 9% yield. Reduction of the dioxoazamacrocycle **17** with BH_3 gave the corresponding amine **18** (84%), and subsequent *N*-alkylation with bromoacetic acid yielded **2** in 14% yield.

Synthesis of Nonpendant-Type BCA. *p*-NO_2-Bz-DOTA (**3**) was prepared by the same methodology as for BCA **1** synthesis (Scheme III). *p*-Nitrophenylalanine methyl ester (**19**)[14] was treated with methyl bromoacetate to give 4-nitrobenzyl-substituted imino diester **20** in a quantitative yield. Cyclization of the imino diester **20** with diethylenetriamine gave dioxoazamacrocycle **21** in 16% yield. Reduction of the resulting dioxo compound **21** with BH_3 gave the corresponding azamacrocycle **22** in 61% yield. Exhaustive *N*-alkylation of **22** with bromoacetic acid afforded *p*-NO_2-Bz-DOTA (**3**) in 46% yield.

Rate of Yttrium Complex Formation. In order to evaluate the effect of the pendant donor on the rate of complex formation, we compared the Y^{III} complexation kinetics of pendant-type BCA **1** and **2** with nonpendant-type BCA **3** which has the common macrocycle skeleton. We have determined relative rates using the ligand displacement reaction (1) (see Experimental Section), as earlier reported.[3]

Pseudo-first-order kinetics were observed in all the cases. Table I shows the conditional rate constants k_{obs}' at pH 6.5 and 37 °C. The pendant-type BCA **1** formed an Y^{III} complex about twice faster than the nonpendant-type BCA **3**. The rate enhancement with **1** was apparently due to

(14) Shchukina, M. N.; Sycheva, T. P. *Zh. Obshch. Khim.* **1952**, *22*, 1879; *Chem. Abstr.* **1953**, *47*, 5375d.

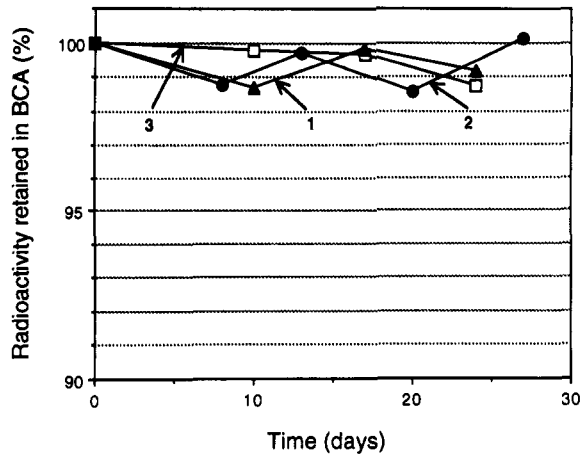
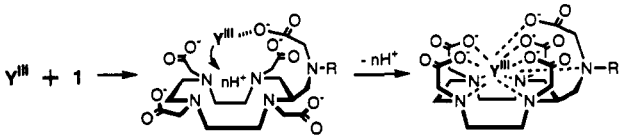


Figure 1. Stability of $^{88}Y^{III}$–BCA complexes in human serum at 37 °C. In all cases the confidence limits are within 2%.

the effect of the (carboxymethyl)amino pendant donor, which, however, was not as dramatic as we observed in 13-membered macrocycle pendant BCA 4.[4] On the other hand, 2 reacted with Y^{III} at almost the same rate as 3. The distance from the pendant donor to the center of macrocycle may be a critical factor in achieving the rate enhancement.



Stability of Complex in Serum. The stability of the complexes in human serum was determined by following our previously reported procedure.[4] Figure 1 shows the radioactivity retained in BCAs during incubation of complexes in human serum at 37 °C. The stabilities of $^{88}Y^{III}$ complexes of pendant-type BCA 1 and 2 were found to be similar to that of nonpendant-type BCA 3; there was no measurable loss of $^{88}Y^{III}$ from any of those three complexes for over 3 weeks. The (carboxymethyl)amino pendant donors in 1 and 2 apparently have no negative effect on the overall stability of the complexes.

In conclusion, the rate acceleration by a (carboxymethyl)amino-pendant group observed in the Bz-TRTA system[4] prevailed in the present DOTA system, though the extent of acceleration in the DOTA system was smaller. The extent also depended on the length of the spacer between the pendant group and the macrocyclic ring.

Experimental Section

General Method. CH_2Cl_2 was distilled from calcium hydride. MeOH and DMF were dried over molecular sieves 3A and 4A, respectively. THF was distilled from sodium benzophenone ketyl. Deionized water (>18 MΩ/cm) was used throughout. The other reagents commercially obtained were used without further purification. Melting points were determined on a micro melting apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained at 270 and 68 MHz, respectively, and their peak assignments were included in the supplementary material. High-resolution molecular secondary ion mass spectra (HRSIMS) were obtained using the following matrixes: glycerol (G), glycerol/dithiothreitol/dithioerythritol (G/DTT/DTE), 2-hydroxyethyl disulfide/*m*-nitrobenzyl alcohol (HED/NBA), glycerol/0.1% trifluoroacetic acid (G/TFA). Column chromatography was carried out on silica gel (Daisogel IR-60).

***N*-(4-Nitrobenzyl)malonamic Acid Ethyl Ester (7).** Ethyl (chlorocarbonyl)acetate (6)[12] (31.72 g, 0.211 mol) in dry CH_2Cl_2 (120 mL) was added dropwise over 45 min to a stirred, ice-cooled suspension of *p*-nitrobenzylamine hydrochloride (38.54 g, 0.204 mol) and triethylamine (43.4 g, 0.429 mol) in dry CH_2Cl_2 (250 mL). The suspension was stirred for 3 h at rt, triethylamine hydrochloride was filtered off, and the filtrate was washed with saturated Na_2CO_3, diluted HCl, and brine. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was recrystallized from benzene to give 7 (44.60 g, 80%) as colorless needles: mp 88.0–89.0 °C; IR (KBr) 1740, 1645 cm^{-1}; HRSIMS (G) calcd for $C_{12}H_{15}N_2O_5$ ($M + H^+$) 267.0981, found 267.0943.

2-Bromo-*N*-(4-nitrobenzyl)malonamic Acid Ethyl Ester (8). The following procedure was after the method of Suzuki et al.[13] Bromine (26.77 g, 0.168 mol) in AcOH (300 mL) was added dropwise to a stirred solution of 7 (44.60 g, 0.168 mol) in a mixture of AcOH (1.05 L) and Ac_2O (150 mL) at 8–10 °C, and the solution was stirred for an additional 2 h at the same temperature. The solution was evaporated *in vacuo*, the residue was purified by silica gel chromatography (the eluent: *n*-hexane/AcOEt, the ratio from 3:1 to 1:1) to give 8 (48.20 g, 83%) as colorless crystals: mp 130.5–131.0 °C; IR (KBr) 1750, 1640 cm^{-1}; HRSIMS (G/DTT/DTE) calcd for $C_{12}H_{14}N_2O_5Br$ ($M + H^+$) 345.0086, found 345.0032.

2-(((Ethoxycarbonyl)methyl)amino)-*N*-(4-nitrobenzyl)-malonamic Acid Ethyl Ester (9). A solution of 8 (23.5 g, 68.1 mmol) in dry DMF (300 mL) was added to a stirred suspension of glycine ethyl ester hydrochloride (19.01 g, 0.136 mol) and triethylamine (24.1 g, 0.238 mol) in dry DMF (200 mL) at rt under a nitrogen atmosphere. The reaction mixture was heated at 50 °C for 19 h. Water (200 mL) was added, and the resulting solution was extracted with four portions of AcOEt and two portions of CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated *in vacuo*. The residue was purified by silica gel chromatography (the eluent: *n*-hexane/AcOEt, the ratio from 1:1 to 1:2) to give 9 (21.0 g, 84%) as a pale yellow oil: IR (neat) 1740, 1670 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{16}H_{22}N_3O_7$ ($M + H^+$) 368.1458, found 368.1436.

3-(3-(4-Nitrophenyl)-2-aza-1-oxopropyl)-2,6-dioxo-1,4,7,-10-tetraazacyclododecane (10). A solution of 9 (51.4 g, 0.14 mol) and diethylenetriamine (14.4 g, 0.14 mol) in dry MeOH (2.8 L) was heated at reflux for 5 d under a nitrogen atmosphere. After concentration of the solution to one-third of the volume *in vacuo*, the resulting precipitate was collected by filtration. This precipitate was washed with MeCN and MeOH to give 10 (5.18 g, 10%) as a pale yellow solid: IR (KBr) 1670 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{16}H_{23}N_6O_5$ ($M + H^+$) 379.1730, found 379.1740.

2-(3-(4-Nitrophenyl)-2-azapropyl)-1,4,7,10-tetraazacyclododecane (11). A solution of BH_3 in THF (Aldrich Chemical Co., Inc., 1 M, 90 mL) was added dropwise to a stirred suspension of 10 (1.14 g, 3 mmol) in THF (100 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was heated at reflux for 1 d. Water (30 mL) and concd HCl (30 mL) was added slowly to the mixture at 0 °C, and then the mixture was heated at reflux for 14 h. After concentration of the solution to one-third of the volume *in vacuo*, the resulting precipitate was filtered off. The filtrate was washed with ether. After the aqueous solution was evaporated *in vacuo*, the resulting 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 result an oil. This oil was purified by centrifugal partition chromatography (CPC Model LLN, Sanki Engineering Ltd.) with a solvent system of $CHCl_3$–MeOH–H_2O (5:3:5) and then by silica gel chromatography (the eluent: $CHCl_3$–MeOH–25% aqueous NH_3, the ratio from 100:20:2 to 100:30:6) to give 11 (0.54 g, 54%) as a pale yellow oil: HRSIMS (G) calcd for $C_{16}H_{29}N_6O_2$ ($M + H^+$) 337.2352, found 337.2351.

2-(*N*-(Carboxymethyl)-3-(4-nitrophenyl)-2-azapropyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid (1). A solution of bromoacetic acid (278 mg, 2.0 mmol) in water (3 mL) was brought to pH 10 with 7 M KOH at below 5 °C. To this solution was added a solution of 11 (67.3 mg, 0.2 mmol) in

EtOH (3 mL), and the mixture was heated at 70 °C for 7 h. During the reaction, the pH of the mixture was kept about 10 by the addition of 7 M KOH. After cooling, the pH of the solution was adjusted to 5 with 47% HBr. The resulting precipitate was separated from the solution by centrifugation. This precipitate was purified by HPLC using a C_{18} column (YMC Co., Ltd., A-343) with a solvent system of 0.5% (v/v) TFA in distilled water–MeOH (7:3) at a flow rate of 7.5 mL/min. The product had a retention time of 40.5 min. The combined fractions from the HPLC were evaporated *in vacuo*, and the resulting water solution of the product was lyophilized to give **1** (18 mg, 14%) as a white powder: IR (KBr) 1680 cm^{-1}; HRSIMS (G/TFA) calcd for $C_{26}H_{39}N_6O_{12}$ (M + H^+) 627.2626, found 627.2661.

***N*-(*tert*-Butoxycarbonyl)aspartic Acid α-Methyl Ester (13).** To a solution of *N*-(*tert*-butoxycarbonyl)aspartic acid β-benzyl ester (**12**) (BACHEM Feinchemikalien AG, 32.3 g, 0.1 mol) in CH_2Cl_2 (200 mL) was added a solution of diazomethane in ether (*ca.* 0.6 M, 170 mL, generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[15]). After addition of a small amount of acetic acid to the reaction mixture for decomposition of excess diazomethane, this solution was washed with saturated $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give methyl ester of **12** (33.7 g) as a white solid. To a solution of this solid in dry MeOH (800 mL) was added 10% Pd/C (3.37 g), and the solution was stirred for 3.5 h at rt under hydrogen atmosphere. After removal of Pd/C by filtration, the solution was evaporated *in vacuo* to give **13** (24.7 g, 100%) as a white solid, which was used in the next step without purification: mp 138–140 °C; IR (KBr) 1742, 1705 cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)aspartic Acid α-Methyl Ester β-(4-Nitrobenzyl)amide (14).** Under a nitrogen atmosphere, a solution of **13** (24.73 g, 0.10 mol) in dry DMF (400 mL) was cooled on ice. To this solution was added *N*,*N*′-carbonyldiimidazole (24.32 g, 0.15 mol), and the mixture was stirred for 1 h in an ice bath. A mixture of *p*-nitrobenzylamine hydrochloride (18.86 g, 0.1 mol) and triethylamine (10.1 g, 0.1 mol) in dry DMF (300 mL) was added to the reaction mixture which then was stirred further for 3 h in an ice bath. To the resulting solution was added water (1.5 L), and the mixture was extracted with three portions of AcOEt. The combined extracts were washed successively with saturated $NaHCO_3$, 10% citric acid, and brine and then dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by silica gel chromatography (the eluent: *n*-hexane/AcOEt, the ratio from 1:1 to 1:2) to give a sample of **14**. It was recrystallized from benzene to give colorless needles of **14** (27.82 g, 73%): mp 134.5–135.5 °C; IR (KBr) 1748, 1690, 1655 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{17}H_{24}N_3O_7$ (M + H^+) 382.1614, found 382.1668.

5,9-Diamino-1-(4-nitrophenyl)-3,6-dioxo-2,7-diazanonane (15). **14** (30.0 g, 78.7 mmol) was dissolved in trifluoroacetic acid (200 mL), and this solution was stirred for 2 h at rt. After removal of the solvent *in vacuo*, the resulting residue was dissolved in dry MeOH (300 mL). Ethylenediamine (42.3 g, 0.79 mol) was added to the solution, and the mixture was stirred for 2 h at rt. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel chromatography (the eluent: $CHCl_3$–MeOH–25% aqueous NH_3, the ratio from 100:10:1 to 100:20:2) to give **15** (20.17 g, 83%) as a pale yellow oil: IR (neat) 1655 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{13}H_{20}N_5O_4$ (M + H^+) 310.1515, found 310.1517.

5,9-Diamino-1-(4-nitrophenyl)-2,7-diazanonane (16). Following the procedure described for the synthesis of **11**, 7.80 g of **15** was converted to 2.90 g (41%) of **16** as a pale yellow oil: HRSIMS (G/TFA) calcd for $C_{13}H_{24}N_5O_2$ (M + H^+) 282.1930, found 282.1926.

8-(4-(4-Nitrophenyl)-3-azabutyl)-2,6-dioxo-1,4,7,10-tetraazacyclododecane (17). A solution of **16** (2.90 g, 10.3 mmol) and iminodiacetic acid diethyl ester (14.4 g, 0.14 mol) in dry MeOH (500 mL) was heated at reflux for 10 d under a nitrogen atmosphere. After evaporation of the solvent, the residue was purified by silica gel chromatography (the eluent: $CHCl_3$–MeOH–25% aqueous NH_3 = 100:20:2) to give **17** (0.33 g, 9%) as a pale yellow solid: IR (KBr) 1649 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{17}H_{27}N_6O_4$ (M + H^+) 379.2094, found 379.2141.

2-(4-(4-Nitrophenyl)-3-azabutyl)-1,4,7,10-tetraazacyclododecane (18). Following the procedure described for the synthesis of **11**, 334 mg of **17** was converted to 259 mg (84%) of **18** as a pale yellow oil: HRSIMS (G) calcd for $C_{17}H_{31}N_6O_2$ (M + H^+) 351.2508, found 351.2495.

2-(*N*-(Carboxymethyl)-4-(4-nitrophenyl)-3-azabutyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid (2). Following the procedure described for the synthesis of **1** (HPLC purification of **2** was carried out using same column with a solvent system of 0.5% (v/v) TFA in distilled water–MeOH (9:1) at a flow rate of 8.0 mL/min, the product had a retention time of 24.9 min), 309 mg of **18** was converted to 32.4 mg (14%) of **2** as a white powder: IR (neat) 1678 cm^{-1}; HRSIMS (HED/NBA) calcd for $C_{27}H_{41}N_6O_{12}$ (M + H^+) 641.2782, found 641.2820.

***N*-((Methoxycarbonyl)methyl)-4-nitrophenylalanine Methyl Ester (20).** To a suspension of 4-nitrophenylalanine methyl ester hydrochloride (**19**)[14] (26.1 g, 0.10 mol) in DMF (200 mL) was added triethylamine (10.1 g, 0.10 mol), and this mixture was stirred at rt for 0.5 h. The resulting precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in dry DMF (300 mL), and to this solution was added methyl bromoacetate (45.9 g, 0.30 mol) and triethylamine (30.3 g, 0.30 mol) at rt under nitrogen atmosphere. The solution was stirred at rt for 2 h. The resulting precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt (300 mL), and this solution was washed with water, dried (Na_2SO_4), and evaporated to give **20** (29.6 g, 100%) as a pale yellow oil, which was used in the next step without purification: IR (neat) 1740 cm^{-1}; FD-MS calcd for $C_{13}H_{16}N_2O_6$ (M^+) 296, found 296.

3-(4-Nitrobenzyl)-2,6-dioxo-1,4,7,10-tetraazacyclododecane (21). Following the procedure described for the synthesis of **10** (reflux for 11 d), 26.75 g of **20** was converted to 4.70 g (16%) of **21** as a pale yellow solid: IR (KBr) 1660, 1640 cm^{-1}; HRSIMS (G/TFA) calcd for $C_{15}H_{22}N_5O_4$ (M + H^+) 336.1672, found 336.1636.

2-(4-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane (22). Following the procedure described for the synthesis of **11**, 6.70 g of **21** was converted to 3.75 g (61%) of **22** as orange crystals (recrystallized from MeCN): mp 110.0–111.0 °C; HRSIMS (G) calcd for $C_{15}H_{26}N_5O_2$ (M + H^+) 308.2086, found 308.2082.

2-(4-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid (3). A solution of bromoacetic acid (1.39 g, 10.0 mmol) in water (15 mL) was brought to pH 10 with 7 M KOH at below 5 °C. To this solution was added a solution of **23** (0.614 g, 2.0 mmol) in EtOH (20 mL), and the mixture was heated at 70 °C for 4.5 h. During the reaction, the pH of the mixture was kept about 10 by the addition of 7 M KOH. After cooling, the pH of the solution was adjusted to about 3.4 with 47% HBr. The resulting precipitate was separated from the solution by filtration and dried *in vacuo* to give **3** (0.50 g, 46%) as a white solid: IR (neat) 1680 cm^{-1}; HRSIMS (G/DTT/DTE) calcd for $C_{23}H_{34}N_5O_{10}$ (M + H^+) 540.2306, found 540.2329.

Kinetic Measurements. The time scan measurements were carried out in the same manner as described in the previous paper.[3] The complexation kinetics for **1**–**3** were compared on the basis of the reaction (1) under the same conditions (*i.e.*, the

$$\underset{(\lambda_{max}\ 653\ nm)}{\text{arsenazo III}\cdot Y^{III}} + \underset{\text{(a large excess)}}{\text{chelator}} \xrightarrow{k_{obs}} \text{arsenazo III} + \text{chelator}\cdot Y^{III} \quad (1)$$

same reactants, concentration, and buffer pH 6.5, 37 °C). A solution of chelator (300 μL, 2 × 10^{-4} M) in deionized water was treated with chelate resin (DIAION, CR 10, H^+/Na^+ = 1/1 form) in a batchwise operation. The resulting solution was added to the mixture of arsenazo III (Tokyo Kasei Kogyo Co., Ltd., Tokyo, 5 × 10^{-5} M) and Y^{III} ($YCl_3 \cdot 6H_2O$, Aldrich, 5 × 10^{-6} M) in HEPES buffer (300 μL, 5 × 10^{-2} M, pH 6.5). The ligand displacement rates of Y^{III} (2.5 × 10^{-6} M)/arsenazo III (2.5 × 10^{-5} M) with **1**–**3** (all in 1.0 × 10^{-4} M) were followed by the decrease in absorbance at 653 nm (A_{653}). This process followed pseudo-

(15) *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 250.

first-order kinetics with rate constants k_{obs}' as indicated all by the good exponential decays with time.

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Supplementary Material Available: ^{1}H and/or ^{13}C NMR spectra and their peak assignments for all compounds described in the Experimental Section (35 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.