Convenient Syntheses of Bifunctional Metal Chelates

Matthew M. Hayward, James C. Adrian, Jr.,† and Alanna Schepartz*

Department of Chemistry, Yale University, New Haven, Connecticut 06511

Received February 22, 1995

The ability of ethylenediaminetetraacetic acid (EDTA) and its derivatives to form stable complexes with a variety of metal ions allows for their use as probes in various areas in chemistry, biology, and medicine. Derivatives of EDTA-Fe, for example, are effective cleavage reagents used to investigate biomolecular structure and function. Protein and nucleic acid cleavage experiments have been used to probe biomolecule tertiary structure and drug binding sites, as well as to identify folding intermediates and to investigate interactions with other macromolecues.[1] Complexation of radioactive metals, alternatively, with these EDTA reagents has found extensive utility in medicine.[2] Accordingly, there have been several syntheses of reagents capable of delivering and attaching these chelates to various macromolecules.[3] Here we report the facile synthesis of three novel reagents useful for linking an EDTA chelate covalently to a protein or nucleic acid-borne thiol. Pyridine disulfide

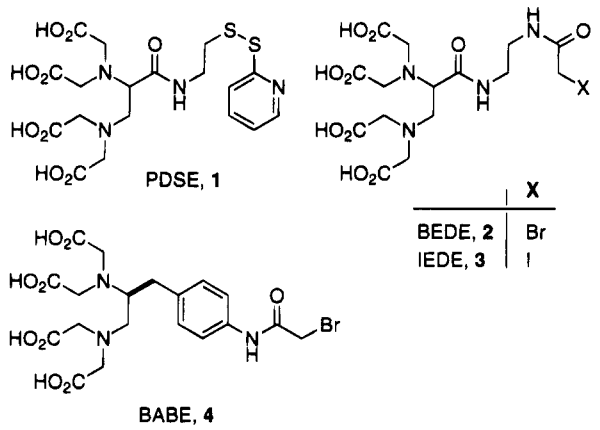


Figure 1.

1 may be joined reversibly to a biomolecule thiol upon disulfide exchange; α-haloacetamide compounds **2** and **3** are irreversible alkylating agents. Also, an improved synthesis of (*S*)-1-[4-(bromoacetamido)benzyl]-EDTA, compound **4**, is presented. An important aspect of these four reagents is substitution at the 2-position of the EDTA. This allows for the involvement of all four carboxylates in metal complexation, increasing the kinetic and thermodynamic stability of the resultant complex.[4]

Compound **1** was synthesized in four steps as illustrated in Scheme 1. Commercially available 2,3-diaminopropanoic acid **5** was converted in two steps into carboxylic acid **6** in 52% yield according to the method of Arya *et al.*[5] Compound **6** was then treated with S-(2-pyridylthio)cysteamine hydrochloride[1j] in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and *N*-hydroxysuccinimide (NHS) to provide tetraester **7** in 29% yield. We found that **7** could be deprotected in almost quantitative yield by the action of anhydrous trifluoroacetic acid to provide tetraacid **1** as a pure solid.

The synthetic strategy used to prepare α-halo acetamides **2** and **3** is shown in Scheme 2. 2,3-Diaminopropanoic acid **5** was converted in three steps into the dihydrochloride salt of diamine **8** in 78% yield under standard protocols. Exhaustive alkylation of **8** with *tert*-butyl bromoacetate afforded **9** in 80% yield. Removal of the benzyl carbamate upon catalytic hydrogenation followed by treatment with an excess of bromoacetyl bromide or the NHS ester of iodoacetic acid[6] afforded tetra *tert*-butyl esters **12** and **13**, respectively.[7] The *tert*-butyl esters were deprotected in near quantitative yield by the action of trifluoroacetic acid to generate reagents **2** and **3**.

The synthetic strategy used to prepare (*S*)-1-[4-(bromoacetamido)benzyl]-EDTA, **4**, is shown in Scheme 3. Commercially available 4-nitrophenylalanine was converted into amide **16** following the precedent of DeRiemer.[3b] Borane reduction of **16** gave diamine **17** in 83% yield which was then tetraalkylated as above with

† Present Address: Chemistry Department, Union College, Schenectady, NY 12306.

(1) **Protein Cleavage.** (a) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 2457–2458. (b) Schepartz, A.; Cuenoud, B. *J. Am. Chem. Soc.* **1990**, *112*, 3247–3249. (c) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249–3250. (d) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1991**, *113*, 1859–1861. (e) Cuenoud, B.; Tarasow, M.; Schepartz, A. *Tetrahedron Lett.* **1992**, *33*, 895–898. (f) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578–10582. (g) Ermacora, M. R.; Delfino, J. M.; Cuenoud, B.; Schepartz, A.; Fox, R. O. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6383–6387. (h) Hayward, M. M.; Schepartz, A. In *Perspectives in Medicinal Chemistry*; Testa, B.; Kyburz, E., Fuhrer, W., Giger, R., Eds.; Verlag Helvetica Chimica Acta: Basel, 1993; Chapter 32, pp 501–512. **Protein–Nucleic Acid Cleavage.** (i) Sluka, J. P.; Horvath, S. J.; Glasgow, A. C.; Simon, M. I.; Dervan, P. B. *Biochemistry* **1990**, *29*, 6551–6561. (j) Ebright, Y. W.; Chen, Y.; Pendergrast, P. S.; Ebright, R. H. *Biochemistry* **1992**, *31*, 10664–10670. (k) Ebright, Y. W.; Chen, Y.; Ludescher, R. D.; Ebright, R. H. *Bioconjug. Chem.* **1993**, *4*, 219–225. (l) Saluz, H. P.; Jost, J. P. *Crit. Rev. Euk. Gene Expression* **1993**, *3*, 1–29. **Nucleic Acid Cleavage.** (m) Dervan, P. B. *Science* **1986**, *232*, 464–471. (n) Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73–75. (o) Sigman, D. S.; Chen, C. B. *Annu. Rev. Biochem.* **1990**, *59*, 207–236. (p) Han, H.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4955–4959. (q) Han, H.; Schepartz, A.; Pellegrini, M.; Dervan, P. B. *Biochem.* **1994**, *33*, 9831–9844.

(2) (a) Sundberg, M. W.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Med. Chem.* **1974**, *17*, 1304–1307. (b) Meares, C. F.; Wensel, T. G. *Acc. Chem. Res.* **1984**, *17*, 202–209. (c) Brechbiel, M. W.; Ganow, O. A.; Atcher, R. W.; Schlom, J; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772–2781. (d) Mukkala, V.-M.; Mikola, H.; Hemmilä, I. *Anal. Biochem.* **1989**, *176*, 319–325. (e) Studer, M.; Kroger, L. A.; DeNardo, S. J.; Kukis, D. L.; Meares, C. F. *Bioconjug. Chem.* **1992**, *3*, 424–429.

(3) (a) Yeh, S. M.; Sherman, D. G.; Meares, C. F. *Anal. Biochem.* **1979**, *100*, 152–159. (b) DeRiemer, L. H.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Lab. Compd. Radiopharm.* **1981**, *18*, 1517–1534. (c) Altman, J.; Shoef, N.; Wilchek, M.; Warshawsky, A. *J. Chem. Soc., Perkin Trans. 1* **1983**, 365–368. (d) Altman, J.; Shoef, N.; Wilchek, M.; Warshawsky, A. *J. Chem. Soc., Perkin Trans. 1* **1984**, 59–62. (e) Warshawsky, A.; Altman, J.; Kahana, N.; Arad-Yellin, R.; Desch, A.; Hasson, H.; Shoef, N.; Gottlieb, H. *Synthesis* **1989**, 825–829. (f) Warshawsky, A.; Altman, J.; Arad-Yellin, R.; Gottlieb, H.; Desch, A.; Kahana, N.; Shoef, N.; Wilchek, M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1781–1786. (g) Sluka, J. P.; Griffin, J. H.; Mack, D. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 6369–6374. (h) Studer, M.; Meares, C. F. *Bioconjug. Chem.* **1992**, *3*, 420–423. (i) Ebright, Y. W.; Chen, Y.; Ludescher, R. D. and Ebright, R. H. *Bioconjug. Chem.* **1993**, *4*, 219–225. (j) Richardson, P. L.; Gross, M. L.; Light-Wahl, K. J.; Smith, R. D.; Schepartz, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2133–2138.

(4) (a) Margerum, D. W.; Bydalek, T. J. *Inorg. Chem.* **1963**, *2*, 683–688. (b) Carr, J. D.; Libby, R. A.; Margerum, D. W. *Inorg. Chem.* **1967**, *6*, 1083–1088. (c) Martel, A. E.; Smith, R. M. *Critical Stability Constants;* Plenum Press: New York, 1974; Vol. 1.

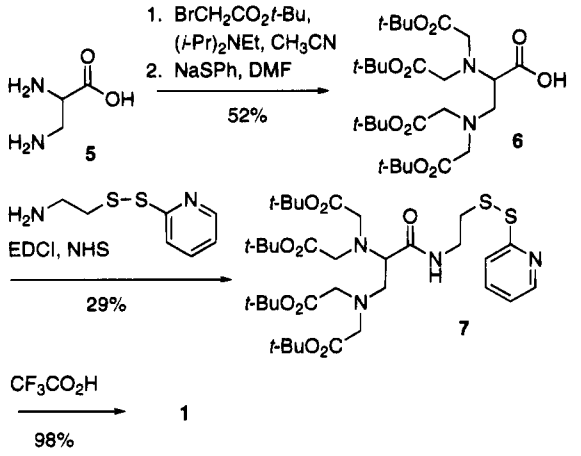
(5) Arya, R.; Gariépy, J. *Bioconjug. Chem.* **1991**, *2*, 323–326.

(6) Santi, D. V.; Cunnion, S. O. *Biochem.* **1974**, *13*, 481–485.

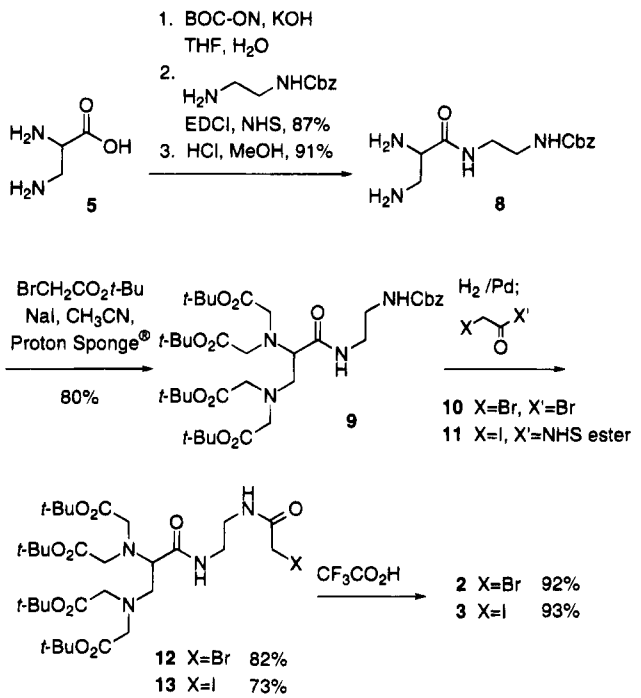
(7) α-Halo compounds **12**, **13**, **2**, and **3** reacted readily with 4-(4-nitrobenzyl)pyridine, a convenient TLC test for alkylating reagents and reactive halogens. See Thomas, J. J.; Kim, J. H.; Mauro, D. M. *Arch. Environ. Contam. Toxicol.* **1992**, *22*, 219–227.

0022-3263/95/1960-3924$09.00/0

Scheme 1



Scheme 2



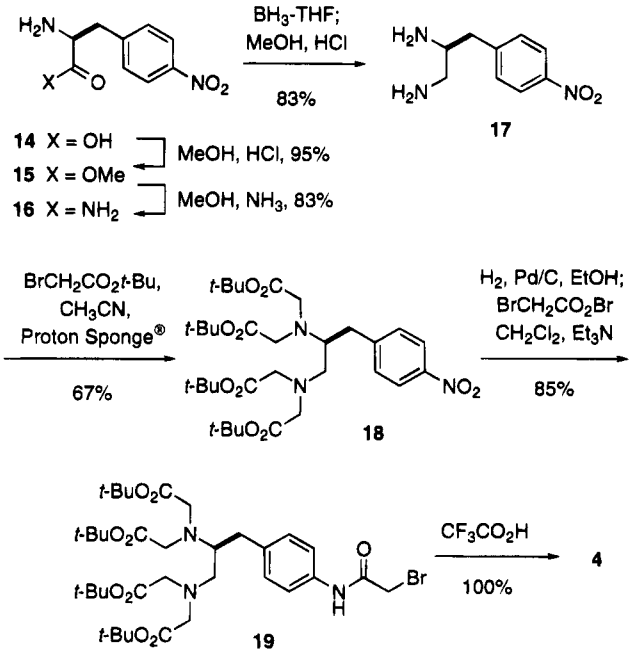
tert-butyl bromoacetate to achieve tetraester **18** in 67% yield. Reduction of the nitro group via catalytic hydrogention followed by reaction with bromoacetyl bromide afforded **19** in 85% yield. Finally, removal of the *tert*-butyl esters with trifluoroacetic acid yielded **4** in quantitative yield. This method eliminates several difficult and time consuming steps from the published procedure by waiting until the final step to unmask the troublesome tetraacid moiety.

We have described the convenient synthesis of four reagents useful for the conjugation of EDTA to proteins and nucleic acids. Disulfide-EDTA **1** is easily prepared, stable, thiol specific, and removable upon treatment with dithiothreitol or other reducing agents. The α-haloacetamide-EDTA compounds **2**–**4** are easily prepared alkylation reagents. The syntheses described here have been performed on gram scale and may be adapted easily to afford a homologous series of macromolecule cleavage agents.

Experimental Section

General. All reactions were carried out under a positive atmosphere of dry N_2 or Ar unless indicated otherwise. Proton and carbon magnetic resonance spectra were recorded at 250, 300, or 490 MHz and chemical shifts are expressed in ppm. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60F-254 (0.25 mm) analytical glass plates. E. Merck silica gel 60 (230–400 mesh) was used for flash chromatography. High pressure liquid chromatography (HPLC) was conducted on a Waters two head pump with a Waters 490E UV detector. The specific column used is noted where applicable. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Tetrahydrofuran, acetonitrile, and methylene chloride were purified and dried according to standard procedures.[8] All other commercially available reagents and solvents were reagent grade and used without further purification unless otherwise noted.

Scheme 3



[[2-[Bis-[(*tert*-butoxycarbonyl)methyl]amino]-1-[[2-(pyridin-2-yldithio)ethyl]carbamoyl]-ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (7). To a stirred solution of acid **6** (1.1 g, 1.9 mmol)[5] in acetonitrile (30 mL) at rt was added *N*-hydroxysuccinimide (241 mg, 2.1 mmol). The resulting mixture was cooled in an ice bath, and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (476 mg, 2.5 mmol) was added. The ice bath was removed after 2 h and the stirring continued for an additional 2 h. To this mixture was added a mixture of *S*-(2-pyridylthio)cysteamine hydrochloride[10] (516 mg, 2.3 mmol) and Et_3N (1.4 mL 9.5 mmol) in $CHCl_3$ (35 mL). After 20 h the reaction mixture was diluted with $CHCl_3$ (100 mL) and filtered and the filtrate washed sequentially with 0.1 M H_3PO_4, $NaHCO_3$ (sat.), and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to a brown oil. The oil was purified by flash chromatography (SiO_2: 60% hexane/ethyl acetate) to afford a total of 400.0 mg (29%) of **7** as a pale yellow oil. The oil slowly decomposes in the air and should be stored in the dark at −20 °C under inert atmosphere if not used immediately: IR (neat) 3316, 3041, 2969, 2931, 1729, 1666, 1572, 1517, 1444, 1415, 1391, 1365, 1284, 1221, 1148, 1041, 983, 912, 847, 758, 732 cm^{-1}; 250 MHz ^{1}H-NMR (acetone-d_6) δ 8.56 (s, 1 H), 8.45 (d, J = 6.0 Hz, 1 H), 7.85–7.79 (m, 2 H), 7.18 (m, 1 H), 3.56 (m, 12 H), 3.25 (dd, J = 5.0 Hz, J = 14.0 Hz, 1 H), 2.97 (t, J = 7.0 Hz, 2 H), 1.44 (s, 36 H); 63 MHz ^{13}C-NMR ($CDCl_3$) δ 172.7, 171.1, 170.3, 160.0, 149.4, 136.7, 120.4, 119.5, 80.8, 80.7, 63.8, 56.2, 54.2, 54.1, 38.1, 27.9; HRMS (FAB, matrix: *p*-NOBA–methanol), *m/z* calcd for $C_{34}H_{56}N_4O_9S_2$ (M + 1) 729.3570, measured 729.3644.

[[2-[Bis(carboxymethyl)amino]-1-[[2-(pyridin-2-yldithio)ethyl]carbamoyl]ethyl](carboxymethyl)amino]-acetic Acid (1). A solution of the tetraester **7** (396.0 mg, 0.5 mmol) in trifluoroacetic acid (3.0 mL) was stirred at rt. After

(8) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.

22 h the trifluoroacetic acid was removed to afford a beige solid which was taken up in distilled water (5 mL) and lyophilized to give 266.5 mg (98%) of the tetraacid **1** as an off-white solid. Further purification of a 26.0 mg aliquot by reverse phase HPLC afforded 21.0 mg of the tetraacid **1** as a white solid: mp, yellow at 120 °C, dec >140 °C; retention time = 20.9 min (linear gradient 4–25% acetonitrile from 0–30 min, 0.06% trifluoroacteic acid; Vydac Protein & Peptide C_{18} column, 1 mL per min, 290 nm). IR (KBr) 3415, 3248, 3018, 2548, 1726, 1663, 1402, 1198, 1140, 984, 963, 896, 873, 765, 719, 669 cm^{-1}; 250 MHz ^{1}H-NMR (D_2O) δ 8.51 (dd, J = 1.0 Hz, J = 6.0 Hz, 1 H), 8.24 (dt, J = 1.0 Hz, J = 7.0 Hz, J = 8.0 Hz, 1 H), 8.20 (d, J = 8.0 Hz, 1 H), 7.63 (dt, J = 1.0, J = 6.0 Hz, J = 7.0 Hz, 1 H), 4.02 (s, 4 H), 3.89 (t, J = 8.0 Hz, 1 H), 3.64 (s, 4 H), 3.55 (d, J = 8.0 Hz, 2 H), 3.45 (m, 2 H), 2.94 (t, J = 6.0 Hz, 2 H); 123 MHz ^{13}C-NMR (D_2O) δ 175.2, 170.4, 169.5, 156.1, 144.2, 143.8, 124.8, 123.6, 59.4, 56.6, 54.0, 52.1, 37.7, 37.4; HRMS (FAB, matrix: *p*-NOBA–methanol), m/z calcd for $C_{18}H_{24}N_4O_9S_2$ (M + 1) 505.0985, measured 505.1036.

[[1-[[2-[(Benzyloxycarbonyl)amino]ethyl]carbamoyl]-2-[bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (9). A stirred heterogeneous mixture of 2,3-diamino-*N*-[[(benzyloxycarbonyl)amino]ethyl]propionamide dihydrochloride (**8**) (2.0 g, 5.7 mmol),[3f] Proton-Sponge (8.6 g, 40.1 mmol), and NaI (900 mg, 6.3 mmol) in acetonitrile (40 mL) was heated at reflux for 2 h. To this hot mixture was added *tert*-butyl bromoacetate (4.8 mL, 29.5 mmol). After 22 h, heating was discontinued and the reaction mixture was cooled to rt and diluted with $CHCl_3$ (100 mL). A white precipitate was removed by filtration. The filtrate was washed sequentially with iced 2 N HCl, $NaHCO_3$ (sat.), and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a dark brown oil. Flash chromatography of the oil (SiO_2: 5% methanol: CH_2Cl_2) afforded a total of 3.6 g (80%) of the tetraester **9** as an amber glass: IR (neat) 3332, 2976, 2931, 1730, 1675, 1522, 1454, 1391, 1366, 1248, 1226, 1150, 990, 844, 744, 697 cm^{-1}; 250 MHz ^{1}H-NMR ($CDCl_3$) δ 8.62 (bs, 1 H), 7.32, (bs, 5 H), 5.98 (bs, 1 H), 5.08 (s, 2 H), 3.6–3.2 (m, 14 H), 2.88 (dd, J = 8.0 Hz, J = 14.0 Hz, 1 H), 1.44 (s, 18 H), 1.41 (s, 18 H); 63 MHz ^{13}C-NMR ($CDCl_3$) δ 173.0, 171.5, 170.6, 156.6, 136.8, 128.3, 128.0, 127.8, 81.2, 81.0, 66.4, 64.2, 56.4, 54.6, 54.5, 41.2, 39.4, 28.1, 28.0; HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{37}H_{60}N_4O_{11}$ (M + 1) 737.4339, measured 737.4394.

[[2-[Bis[(*tert*-butoxycarbonyl)methyl]amino]-1-[[2-[(bromoacetyl)amino]ethyl]carbamoyl]ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (12). A heterogeneous mixture of tetraester **9** (500 mg, 0.68 mmol) and palladium black (200 mg) was stirred in 2.0 mL of THF under a hydrogen atmosphere (1 atm). After 2 h the reaction mixture was filtered through Celite into a stirred solution of bromoacetyl bromide (72 μL, 0.82 mmol), triethylamine (95 μL, 0.68 mmol), and THF (1 mL); the Celite was washed with THF (5 mL). After 30 min the product was purified by flash chromatography (SiO_2: 30% hexane:EtOAc) to give 403 mg (82%) of tetraester **12** as a pale yellow oil: IR (neat) 3312, 3080, 2978, 2931, 1731, 1668, 1533, 1429, 1391, 1365, 1220, 1147, 989, 847, 738 cm^{-1}; 250 MHz ^{1}H-NMR (acetone-d_6) δ 8.42 (bs, 1 H), 7.53, (bs, 1 H), 3,86 (s, 2 H), 3.60–3.44 (m, 9 H), 3.35–3.29, (m, 4 H), 3.24 (dd, J = 6.0 Hz, J = 14.0 Hz, 1 H), 2.95 (dd, J = 8.0 Hz, J = 14.0 Hz, 1 H), 1.46 (s, 18 H), 1.45 (s, 18 H); 63 MHz ^{13}C-NMR ($CDCl_3$) δ 173.4, 171.5, 170.5, 166.2, 81.2, 81.1, 64.0, 56.5, 54.6, 54.4, 40.7, 38.5, 28.7, 28.1, 28.0; HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{31}H_{55}N_4O_{10}Br$ (^{79}Br, M + 1) 723.3182, measured 723.3229.

[[2-[Bis(carboxymethyl)amino]-1-[[2-[(bromoacetyl)amino]ethyl]carbamoyl]ethyl](carboxymethyl)amino]acetic Acid (2). A solution of tetraester **12** (100 mg, 0.14 mmol) in anhydrous trifluoroacetic acid (2.0 mL) was stirred at rt. After 14 h, the trifluoroacetic acid was removed to afford a white foam which was then dissolved in H_2O (2 mL) and lyophilized to afford 63 mg (92%) of tetraacid **2** as a flocculent white solid: mp red at 130 °C, decomposition >165 °C; IR (KBr) 3395, 3081, 2958, 2532, 1719, 1657, 1559, 1398, 1220, 950, 890, 670 cm^{-1}; 250 MHz ^{1}H-NMR (D_2O) δ 4.04 (s, 4 H), 3.86 (t, J = 8.0 Hz, 1 H), 3.79 (s, 2 H), 3.63 (s, 4 H), 3.56 (d, J = 8.0 Hz, 2 H), 3.26 (s, 4 H); 123 MHz ^{13}C-NMR (D_2O) δ 175.2, 170.2, 170.1, 169.1, 59.8, 56.3, 54.3, 52.0, 38.9, 28.1; HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{15}H_{23}N_4O_{10}Br$ (^{79}Br, M + 1) 499.0676, measured 499.0688.

[[2-[Bis[(*tert*-butoxycarbonyl)methyl]amino]-1-[[2-[(iodoacetyl)amino]ethyl]carbamoyl]ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (13). A heterogeneous mixture of tetraester **9** (200 mg, 0.26 mmol) and palladium black (100 mg) was stirred in 2.0 mL of THF under a hydrogen atmosphere (1 atm). After 1 h the reaction mixture was filtered through Celite into a stirred mixture of succinimide iodoacetate (68 mg, 0.3 mmol) and $NaHCO_3$ (anhyd) (53 mg, 0.64 mmol) and dioxane (1 mL); the Celite was washed with dioxane (5 mL). After 15 min the reaction mixture was concentrated *in vacuo* to afford a viscous yellow-brown oil. Flash chromatography of the oil (SiO_2: 30% hexane:EtOAc) afforded a total of 146 mg (73%) of tetraester **13** as a pale yellow oil; IR (neat) 3311, 3074, 2970, 2929, 1732, 1669, 1533, 1453, 1392, 1366, 1289, 1247, 1223, 1153, 989, 846, 743 cm^{-1}; 250 MHz ^{1}H-NMR (acetone-d_6) δ 8.39 (bs, 1 H), 7.46, (bs, 1 H), 3.71 (s, 2 H), 3.60–3.40 (m, 9 H), 3.35–3.15, (m, 5 H), 2.95 (dd, J = 8.0 Hz, J = 14.0 Hz, 1 H), 1.46 (s, 18 H), 1.45 (s, 18 H); 63 MHz ^{13}C-NMR ($CDCl_3$) δ 173.4, 171.7, 170.6, 167.7, 81.4, 81.2, 64.1, 56.6, 54.7, 54.3, 40.7, 38.6, 28.7, 28.1, –0.9; HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{31}H_{55}N_4O_{10}I$ (M + 1H$^+$) 771.2964, measured 771.3064.

[[2-[(Bis(carboxymethyl)amino]-1-[[2-[(iodoacetyl)amino]ethyl]carbamoyl]ethyl](carboxymethyl)amino]acetic acid (3): mp tan at 110 °C, decomposition > 140 °C; IR (KBr) 3293, 3000, 2546, 1723, 1646, 1544, 1397, 1212, 957, 900, 676 cm^{-1}; 250 MHz ^{1}H-NMR (D_2O) δ 3.95 (s, 4H), 3.86 (t, J = 8.0 Hz, 1 H), 3.63 (s, 4 H), 3.62 (s, 2 H), 3.54 (d, J = 8.0 Hz, 2 H), 3.27–3.21 (m, 4 H); 123 MHz ^{13}C-NMR (D_2O) δ 175.2, 172.2, 170.2, 169.2, 59.8, 56.4, 54.3, 52.1, 38.8, –2.2; HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{15}H_{23}N_4O_{10}I$ (M + 1H$^+$) 547.0461, measured 547.0511.

3-(4-Nitrophenyl)propane-1,2-diamine Dihydrochloride (17).[2c] Into THF (120 mL) at 4 °C was added amide **16** (1.0 g, 4.8 mmol).[3b] To this solution was added 1.0 M BH_3–THF (24 mL, 24 mmol) over a 15 min period. The ice bath was removed and the mixture was brought to reflux. After 20 h the reaction was cooled to rt and placed in an ice bath, and MeOH (15 mL) was slowly added. The reaction was allowed to warm to rt after which the solvents were removed. MeOH was again added to the residue and removed by evaporation. EtOH (30 ml) was added to the residue, saturated with HCl(g), and brought to reflux. After 2 h the reaction was stoppered and left at 4 °C for 15 h. The diamine dihydrochloride precipitated and was isolated by filtration as an off-white solid (1.068 g, 83%): 300 MHz ^{1}H-NMR (MeOD) δ 8.10 (d, J = 8.7Hz, 2H) 7.40 (d, J = 8.7Hz, 2H), 3.96 (m, 1H), 3.83 (m, 1H), 3.64 (m, 2H), 3.49 (m, 1H); 75 MHz ^{13}C-NMR (MeOD) δ 147.5, 141.8, 130.3, 123.6, 50.3, 40.5, 35.6.

(*S*)-[[1-[[Bis[(*tert*-butoxycarbonyl)methyl]amino]methyl]-2-(4-nitrophenyl)ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (18). To a mixture of diamine dihydrochloride **17** (100 mg, 0.37 mmol), NaI (62 mg, 0.41 mmol), and Proton-Sponge (640 mg, 2.98 mmol) in CH_3CN (8 mL) was added *tert*-butyl bromoacetate (0.325 mL, 3.73 mmol). The reaction was brought to reflux in the dark. After 50 h the CH_3CN was removed and the resulting brown oil was dissolved in EtOAc (80 mL), washed with H_2O and brine, and dried over Na_2SO_4. The solvent was removed and the crude product purified by flash chromatography (silica: 15% EtOAc:hexanes) to give 0.163 g (67%) tetraester as a bright yellow oil: IR (neat) 2965, 2917, 1731, 1599, 1518, 1471, 1448, 1388, 1366, 1342, 1145, 1223, 1153, 987, 848, 743 cm^{-1}; 300 MHz ^{1}H-NMR ($CDCl_3$): δ 8.07 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 8.7 Hz, 2H), 3.47 (m, 8H), 2.97 (m, 4H), 2.44 (m, 1H), 1.39 (m, 36H). 76 MHz ^{13}C-NMR ($CDCl_3$) δ 170.8, 170.4, 148.7, 146.0, 130.0, 123.0, 80.8, 80.7, 62.9, 56.2, 55.6, 55.5, 53.2, 36.9, 27.8. HRMS (FAB, matrix: *p*-NOBA–methanol) m/z calcd for $C_{33}H_{56}N_3O_{10}$ (M + 1H$^+$) 652.3733, measured 652.3811.

(*S*)-[[1-[[Bis[(*tert*-butoxycarbonyl)methyl]amino]methyl]-2-[4-[(2-bromoacetyl)amino]phenyl]ethyl][(*tert*-butoxycarbonyl)methyl]amino]acetic Acid *tert*-Butyl Ester (19). A heterogeneous mixture of tetraester **18** (53 mg, 8.04 × 10^{-5} mol) and 10% Pd/C (10 mg) was stirred in EtOH (8 mL) under a hydrogen atmosphere (1 atm). After 1 h the mixture was filtered through Celite and the solvent removed to afford 50 mg of the

aryl amine. The brown oil was dissolved in CH_2Cl_2 (1 mL), and Et_3N (11 μL, 8.04×10^{-5} mol) and bromoacetyl bromide (8 μL, 8.80×10^{-5} mol) were added. After 5 min the product was purified by flash chromatography (silica: 30% EtOAc:hexanes) to give 51 mg (85%) product: IR (neat) 3302, 3185, 3118, 2984, 2920, 1742, 1607, 1538, 1514, 1365, 1243, 1150, 982, 912, 841, 725cm^{-1}; 300 MHz ^{1}H-NMR ($CDCl_3$): δ 8.07 (s, 1 H), 7.39 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 4.02 (s, 2 H), 3.44 (m, 8H), 3.38 (m, 1H), 2.63–2.93 (m, 3H), 2.53 (m, 1H), 1.41 (m, 36H); 75 MHz ^{13}C-NMR ($CDCl_3$) δ 171.5, 171.1, 135.4, 130.8, 129.7, 120.0, 81.1, 80.9, 63.0, 56.4, 55.2, 53.3, 36.0, 29.9, 28.2; HRMS (FAB, matrix: p-NOBA–methanol) m/z calcd for $C_{35}H_{56}N_3O_9Br$ (^{79}Br, M + 1) 742.3280, measured 742.3291.

(*S*)-[[1-[[Bis(carboxymethyl)amino]methyl]-2-[4-[(2-bromoacetyl)amino]phenyl]ethyl](carboxymethyl)amino]-acetic Acid (4). To bromoacetamide **19** (50 mg, 6.73×10^{-5} mol) was added trifluoroacetic acid (5 mL). The reaction was stirred in the dark for 15 h at which time the trifluoroacetic acid was removed by evaporation to give a quantitative yield of **4** as its trifluoroacetic acid salt: IR (KBr) 3370(br), 2984, 2545, 1736, 1712, 1608, 1542, 1512, 1410, 1330, 1195, 1140, 979, 902, 796, 718cm^{-1}; 490 MHz ^{1}H-NMR (D_2O) δ 7.37 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 3.97 (m, 6H), 3.60 (m, 5H), 3.27 (m, 2H), 3.07 (m, 1H), 2.64 (m, 1H); 75 MHz ^{13}C-NMR (D_2O) δ 174.0, 169.0, 135.6, 133.8, 129.8, 121.9, 60.4, 55.8, 54.5, 52.2, 32.4, 29.6, 28.9; HRMS (FAB, matrix: thioglycerol) m/z calcd for $C_{19}H_{24}N_3O_9Br$ (^{79}Br, M + 1) 518.0696, measured 518.0775.

Acknowledgment. This work was supported by the NIH, with additional support provided by Merck, Rohm and Haas, Sterling Winthrop, Pfizer, Zeneca, and Hoffmann-LaRoche. J.A. is a National Institutes of Health Postdoctoral Fellow. M.H. was supported by a Department of Education grant. A.S. is an Eli Lilly Fellow, a David and Lucile Packard Foundation Fellow, a Camile and Henry Dreyfus Teacher–Scholar, an Alfred P. Sloan Research Fellow, a 1995 Arthur C. Cope Scholar, and a National Science Foundation Presidential Young Investigator.

Supplementary Material Available: ^{1}H-NMR and ^{13}C-NMR spectra of compounds **1**–**4**, **7**, **9**, **12**, **13**, **18**, and **19** (10 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.

JO950338P