A Conceptual Approach to the Synthesis of Bifunctional EDTA Analogs: EDTA-Extended Polyamides

N. Kahana, R. Arad-Yellin, and A. Warshawsky*

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

Received January 3, 1994 (Revised Manuscript Received June 1, 1994®)

A conceptual approach to the synthesis of ϵ-carboxyl and ϵ-amino polyamide-linked EDTAs is proposed (Scheme 1), starting from ϵ-carboxy vicinal diamine blocked by Cbz or Boc groups (A, Scheme 1), followed by extension along the chain to form polyamides with ϵ-carboxy groups (A_2, A_3, ..., A_n) or amine groups (B_1, B_2, ..., B_n). The A_n series can be converted to acidic EDTAs (AE_n series) or basic EDTAs (BE_n series). This depends on the selection of the protecting groups to offer exclusive protection of the amine and acid functions. The choice of the protecting groups (three categories a–c in Scheme 1) provides an exclusive synthetic methodology. This is further exploited for conversion of acidic polyamide-linked EDTA (AE_n) to either acidic or basic EDTA homologs (see Scheme 2). Scheme 4 proposes methodology for synthesis of terminally blocked bis-EDTA polyamides.

1. Introduction

Bifunctional EDTA analogs are characterized by their ability to bind a wide range of ions, including radioactive tracers and fluorescent ions. Hence they have been introduced by Meares and co-workers[1] as an approach to covalent attachment of metal chelates to macromolecules[2,3] for the purpose of radiolabeling of antibodies.[4] The importance of this topic has urged us and others to introduce new methodologies for the synthesis of bifunctional chelating agents.

In previous contributions from this laboratory[5–7] we have described a general methodology for the synthesis of EDTA analogs, starting from substituted imidazoles through Bamberger ring cleavage to vicinal diamines and followed by alkylation with bromoacetic acid benzyl esters, to provide hydrophobic EDTA analogs with carbobenzoxy (Cbz) protection on the amine terminal.

Meares and co-workers have introduced new macrocyclic analogs of EDTA and have shown that they possess much higher binding constants.[8] Dervan and his group have shown that EDTA–Fe cleaves at highly localized sites on DNA restriction fragments and plasmids[9–10] and have recently introduced EDTA derivatives for the synthesis of protein–EDTA via Merrifield-type solid-state synthesis[11] and proposed their use in affinity cleaving.

We now wish to propose a wide conceptual approach to the synthesis of bifunctional EDTA analogs, carrying acidic or basic functionalities. In Scheme 1, we present a framework of reactions leading to "matrix" of bifunctional EDTA analogs. The actual synthesis of selected compounds from this "matrix" is demonstrated in Scheme 3. In building this new conceptual approach, we have borrowed from the methodology and methods practiced in the field of peptide synthesis.[12]

The extension of our previous work to a general method for the synthesis of EDTA analogs with polyamide (polypeptide) side chains and ϵ-functionality (amine or carboxylic acid) may also be viewed as a general method for the synthesis of polypeptides with terminal metal chelating EDTA groups (EDTA-extended polypeptides).

2. Methodology (Scheme 1)

Scheme 1 shows 4,5-diaminovaleric acid (A_1) as the starting compound in a special case; where a $-CH_2CH_2-$ unit bridges the diamino and CO_2H functionalities. Of course, Scheme 1 also represents larger aliphatic or other bridging units.

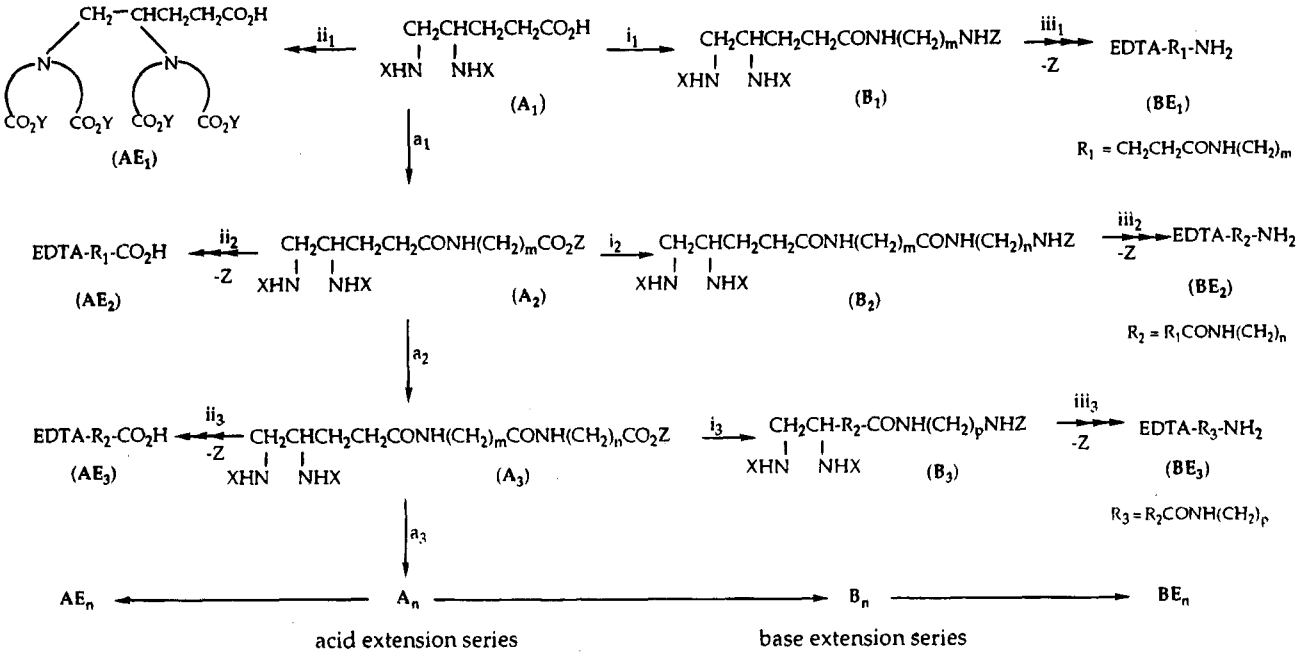
Starting with a diprotected compound (A_1), it is possible to produce the amide-linking bond by reaction with an amine. By choosing amines with protected ϵ-carboxylic acids, it is possible to progress via steps a_1, a_2, a_3, ..., a_n in the acid-extension series (deblocking Z and acid activation), producing compounds A_2, A_3, ..., A_n. By choosing monoprotected diamine and following steps i_n (deprotecting Z and acid activation), the triprotected triamines (B_1, B_2, ..., B_n) can be synthesized.

® Abstract published in *Advance ACS Abstracts*, July 1, 1994.

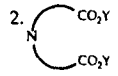
(1) Sundberg, M. W.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Med. Chem.* **1974**, *17*, 1304–1307.
(2) Eckelman, W. C.; Paik, C. H.; Reba, C. *Cancer Res.* **1980**, *40*, 3036–3042.
(3) Hwang, K. J.; Wase, A. W. *Biochim. Biophys. Acta* **1978**, *512*, 54–71.
(4) Meares, C. F.; Goodwin, D. S.; Leung, C. S-H.; Girgis, A. Y.; Silvester, D. J.; Nunn, A. D.; Lavender, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, 3803–3806.
(5) (a) Altman, J.; Shoef, N.; Wilchek, M.; Warshawsky, A. *J. Chem. Soc. Perkin Trans.* **1983**, 365–368. (b) Altman, J.; Shoef, N.; Wilchek, M.; Warshawsky, A. *J. Chem. Soc., Perkin Trans.* **1984**, 59–62. (c) Altman, J.; Shoef, N.; Wilchek, M.; Warshawsky, A. *Isr. J. Chem.* **1986**, *27*, 29–32.
(6) Warshawsky, A.; Altman, J.; Kahana, N.; Arad-Yellin, R.; Deshe, A.; Hasson, H.; Shoef, N.; Gottlieb, H. *Synthesis* **1989**, *11*, 825–829.
(7) Warshawsky, A.; Altman, J.; Arad-Yellin, R.; Gottlieb, H.; Deshe, A.; Kahana, N.; Shoef, N.; Wilchek, M. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1781–1786.
(8) (a) Moi, M. K.; Meares, C. F.; DeNardo, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 6266–6267. (b) Meares, C. F.; Moi, M. K.; Diril, H.; Kukis, D. L.; McCall, M. J.; Deshpande, S. V.; DeNardo, S. J.; Snook, D.; Epenetos, A. A. *Br. J. Cancer Suppl.* **1990**, *62*, 21–26. (c) Moi, M. K.; DeNardo, S. J.; Meares, C. F. *Can. Res. Suppl.* **1990**, *50*, 7895–7935. (d) McCall, M. J.; Diril, H.; Meares, C. F. *Bioconjugate Chem.* **1990**, *1*, 222–226. (e) Mathias, C.; Welch, M.; Green, M. A.; Diril, H.; Meares, C. F.; Gropler, R. J.; Bergman, S. *J. Nucl. Med.* **1991**, *32*, 475–480.
(9) Schultz, P. G.; Taylor, J. S.; Dervan, P. B. J. *J. Am. Chem. Soc.* **1982**, *104*, 6861–6863.
(10) Dervan, P. B. *Science* **1986**, *232*, 464–471.
(11) Sluka, J. P.; Griffin, J. H.; Mack, D. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 6369–6374.
(12) Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; p 48.
(13) Fieser, L. F.; Fieser, M. *Reagents For Organic Synthesis*; John Wiley and Sons Inc.: New York, 1967; p 82.

0022-3263/94/1959-4832$04.50/0

Scheme 1. A General Scheme for Synthesis of ϵ-Carboxy and ϵ-Amino Polyamide-Linked EDTAs



1. (a) non selective extensions if X=Cbz, Y=Bz, Z=Bz
(b) selective extensions if X=Cbz, Y=Bz, Z=t-Bu
(c) selective extensions if X=Boc, Y=t-Bu, Z=Bz

2.  represents $N(CH_2CO_2Y)_2$

The selection of protecting groups is straight from classical peptide synthesis chemistry. We chose benzyl (Bz), carbobenzoxy (Cbz), and *tert*-butyloxycarbonyl (Boc) as reagent triades, which can be removed very easily and selectively. Both Bz and Cbz can be removed under hydrogenation over Pd, and the Boc under mild acidic conditions.

The removal of the X protecting group in the acid extension series A_n or the base extension series B_n is followed by exhaustive alkylation with bromoacetic acid esters ($BrCH_2CO_2Y$), (step ii_n from A_n and iii_n from B_n), where Y is selected to be a Bz or *tert*-butyl (t-Bu) group. Here, two situations exist. In the first, X, Z, and Y are chosen so as to allow total deblocking of the EDTA extended acids AE_n or bases BE_n. Thus, under a (see Scheme 1), X = Cbz, Z = Bz, and Y = Bz, total deprotection is possible. This strategy is applicable to the synthesis of **both** acid EDTA (AE_n) or base EDTA (BE_n) series. Under b and c, selective deprotection is available. Thus, the Y and Z functionalities could be removed independently, leaving Y or Z intact.

The result is that interesting synthetic possibilities ensue. In the acid EDTA series AE_n, deprotection of Z allows further activation and reaction with any amine, including those in the B_n series (after deprotection) or with any of the amines in the BE_n series. This is demonstrated in Scheme 2 for one of the compounds in the AE_n series. Selective deprotection of the ϵ-CO_2Z group in AE_2 to the ϵ-CO_2H group, followed by activation and coupling with a monoprotected diamine, allows synthesis of the blocked EDTA compound in the BE_n series, namely BE_2. Alternatively, coupling with an ϵ-aminoalkanoic acid ester allows synthesis of the next homolog in the AE_n series, namely AE_3.

3. Results and Discussion

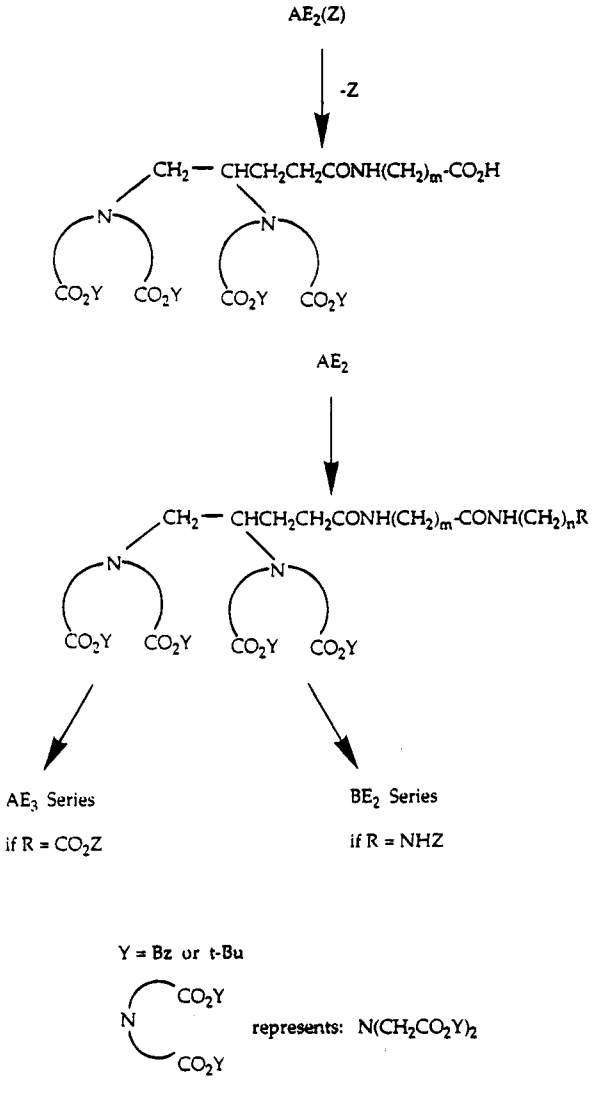
The key elements illustrated in Scheme 1 and 2 have been implemented successfully, and actual synthetic examples are shown in Scheme 3. The numbering of the compounds in Scheme 3 also provides a cross-reference to the general terminology presented earlier. We have chosen to demonstrate the principles without attempting to optimize reaction conditions or product yields.

Scheme 3 shows the synthesis of A_1, X = Cbz (**2**). The *N*-hydroxysuccinimide ester of **2** is coupled with 6-aminohexanoic acid *tert*-butyl ester, to A_2, (X = Cbz, Z = t-Bu), (**3**). Removal of the Cbz group by H_2/Pd to compound **4**, followed by alkylation with bromoacetic acid benzyl ester, produces the acidic-blocked polyamide-linked EDTA, $AE_2(Z)$ (Y = Bz, Z = t-Bu), (compound **5**). ($AE_n(Z)$ is the Z-carboxy derivative of AE_n.) Selective deprotection of the ϵ-CO_2-t-Bu group to ϵ-CO_2H group leads to AE_2 (compound **6**), which can be converted to its NHS ester **7** or coupled directly as the carboxyisobutyryl mixed anhydride to compound **8** [$AE_3(Z)$ in Schemes 1 and 2].

It is important to emphasize that the conclusions reached regarding polyamide-linked EDTA should be applicable to polypeptide-linked EDTA compounds. We believe these will be a class of metal complexing ligands of great biological significance which deserves further synthetic attempts.

Attempts were made to synthesize the parent compound in the acidic EDTA series, namely, AE_1: 4,5-bis-

Scheme 2. Conversion of Acidic Polyamide-Linked EDTA'S to Acidic or Basic EDTA Homologs in the BEn or AEn Series (See Scheme 3 for Real Examples)



[bis[(benzyloxycarbonyl)methyl]amino]valeric acid and its *tert*-butyl ester, from 4,5-diaminovaleric acid and its *tert*-butyl ester (which was synthesized by adoption of a transesterification procedure, using *tert*-butyl acetate and perchloric acid). Neither attempt, in different solvents using inorganic or organic bases, resulted in the desired product in any substantial yields. The experiments were repeated at least twice and separation by column chromatography was attempted. The reason for this failure may be a result of internal hydrogen bonding between the CO_2H and the NH_2 groups and the formation of 5- or 6-membered rings due to intramolecular amidation reaction. A steric difficulty should also be considered.

Another option for the methodology is demonstrated in Scheme 4. This is the proposed synthesis of terminally bis-EDTA compounds (DE_n)—blocked polyamides from coupling of acidic EDTA (AE_1) with basic EDTA (BE_1). This, of course, corresponds with the whole series of compounds presented in Scheme 1.

4. Experimental Section

Materials and Methods. All organic solvents were A.R. grade or distilled solvents. Reagents used were as follows: carbobenzoxy chloride (Aldrich, 95%); *N*-hydroxysuccinimide (Kodak); *N,N'*-dicylclohexylcarbodiimide (Merck, >98%); 10% Pd/C (Merck); benzyl bromoacetate (Aldrich, 96%); *N,N,N',N'*-tetramethyl-1,8-naphthalenediamine (Aldrich); sodium iodide (Merck, 99.5%); trifluoroacetic acid (Merck, 99%); isobutyl chloroformate (Aldrich, 98%); methylmorpholine (Merck, 98%); *tert*-butyl acetate (Fluka, 99%).

TLC was carried out on Merck Kieselgel 60F plates with EtOAc–hexane or $CHCl_3$–MeOH–NH_3 as the eluants. The dyeing reagents were basic aqueous 1% $KMnO_4$ and ethanolic 0.2% ninhydrin solution. Flash chromatography was carried out on 40–63-μm silica gel 60 (Merck No. 9385) with EtOAc–hexane as the eluent.

4,5-Bis[(benzyloxycarbonyl)amino]valeric Acid (Scheme 3, Compound 2; Scheme 1, A_1). Carbobenzoxy chloride (3.13 mL, 22 mmol) and 5 N NaOH (4.4 mL, 22 mmol) were added simultaneously from two dropping funnels to a magnetically stirred solution of 4,5-diaminovaleric acid (2.05 g, 10 mmol) in distilled H_2O (6 mL) and 5 N NaOH (6 mL, 30 mmol) that was cooled to 0 °C.

The temperature was allowed to rise to ambient. After 2 days THF (100 mL) and 5% $NaHCO_3$ (100 mL) were added and stirring was continued for a further 2 days. THF was removed under vacuum and the aqueous phase was washed twice with ether (2 × 100 mL) and acidified with 10% HCl to produce a white precipitate, which was extracted with EtOAc (3 × 150 mL). The EtOAc solution was dried over Na_2SO_4 and evaporated to give the product **2** as a white powder (3.62 g, 90% yield), which was crystallized from EtOAc to produce white crystals of **2** (2.88 g), mp 135–7 °C. ^{1}H NMR: δ (MeOD) 7.31 (10H, m); 5.06, 5.05 (4H, 2s); 3.8–3.65 (1H, m); 3.3–3.15 (2H, m); 2.38 (2H, t, J = 7.4 Hz); 1.9–1.6 (2H, m). ^{13}C NMR: δ ($CDCl_3$ + MeOD) 176.37, 158.02, 157.62, 136.93, 129.01, 128.61, 128.42, 128.34, 67.32, 67.26, 51.87, 45.42, 45.30, 31.02, 27.85. IR: ν_{max} (KBr) 3337 (N–H stretching); 1689 (C=O stretching, NH*C*O_2Bz) cm^{-1} (CO_2H peak is not distinguished). Elemental analysis: $C_{21}H_{24}N_2O_6$ requires: C, 62.99, H, 6.04, N, 7.00; found C, 62.96, H, 6.25, N, 7.06.

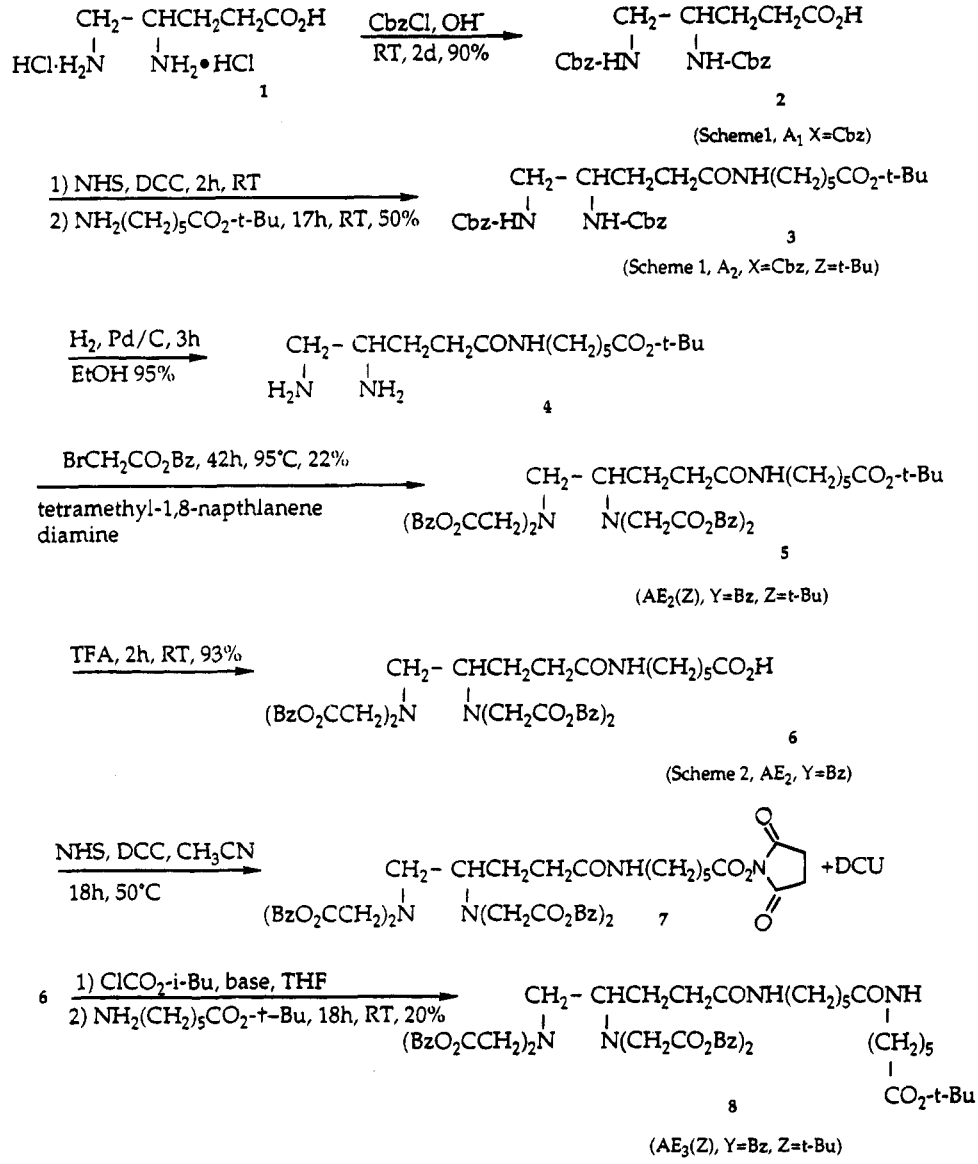
***N*-[5-(*tert*-Butyloxycarbonyl)pentyl]-4,5-bis[(benzyloxycarbonyl)amino]valeramide (Scheme 3, Compound 3; Scheme 1, A_2).** Compound **2** (2 g, 5 mmol) was dissolved in freshly distilled (over P_2O_5) CH_3CN (100 mL), and *N*-hydroxysuccinimide (0.63 g, 5.5 mmol) was added. The reaction mixture was cooled to 0 °C and dicyclohexylcarbodiimide (1.24 g, 6 mmol) was added. After 1 h of stirring at 0 °C the ice bath was removed and the reaction mixture stirred for an additional 2 h at rt.

A solution of *tert*-butyl 6-aminohexanoate (0.94 g, 5 mmol) in $CHCl_3$ (100 mL, dried over alumina) and Et_3N (2.1 mL, 15 mmol) was added to the reaction mixture, which was then stirred for 17 h at room temperature.

Dicyclohexylurea was filtered off and the filtrate evaporated under reduced pressure. EtOAc (100 mL) and H_2O (100 mL) were added to the residual white solid. After shaking, the layers were separated and the aqueous layer extracted with EtOAc (100 mL). The combined EtOAc layers were washed with 10% $NaHCO_3$ (100 mL) and H_2O (100 mL), dried over Na_2SO_4, and evaporated to give a white solid (2.67 g), which was flash chromatographed on silica gel using 30% hexane–EtOAc as starting eluent. The product **3** (A2), a white solid (1.4 g, 50% yield) was eluted with 15% hexane–EtOAc, mp 119–119.5 °C. ^{1}H NMR: δ ($CDCl_3$) 7.30 (10H, m); 6.19 (1H, br s); 5.60 (1H, br d); 5.48 (1H, br s); 5.06 (4H, s); 3.75–3.60 (1H, m); 3.35–3.10 (4H, m); 2.19 (4H, t, J = 7.4 Hz); 1.9–1.65 (2H, m); 1.56 (2H, m); 1.5–1.4 (2H, m); 1.43 (9H, s); 1.35–1.25 (2H, m). ^{13}C NMR: δ ($CDCl_3$) 173.78, 173.22, 157.95, 157.69, 137.14, 137.08, 129.17, 128.79, 129.69, 128.61, 80.81, 67.53, 67.40, 52.67, 45.52, 40.05, 36.02, 33.57, 29.79, 29.04, 28.78, 27.00, 25.26. IR: ν_{max} (KBr) 3318 (N–H stretching); 1730 [C=O stretching, *C*O_2C(CH_3)$_3$]; 1687 (C=O stretching, NH*C*O_2Bz); 1642 (C=O stretching, NH*CO*) cm^{-1}. Elemental analysis: $C_{31}H_{43}N_3O_7$ requires: C, 65.36, H, 7.61, N, 7.38; found: C, 65.0, H, 7.6, N, 7.3.

***N*-[5-(*tert*-Butyloxycarbonyl)pentyl]-4,5-diaminovaleramide (Scheme 3, Compound 4).** Compound **3** (A_2) (1.19 g, 2.1 mmol) in ethanol (100 mL) was hydrogenated over 10% Pd/C (0.1 g) at atmospheric pressure for 3 h. The reaction

Scheme 3. Synthesis of Selected Compounds of Schemes 1 and 2



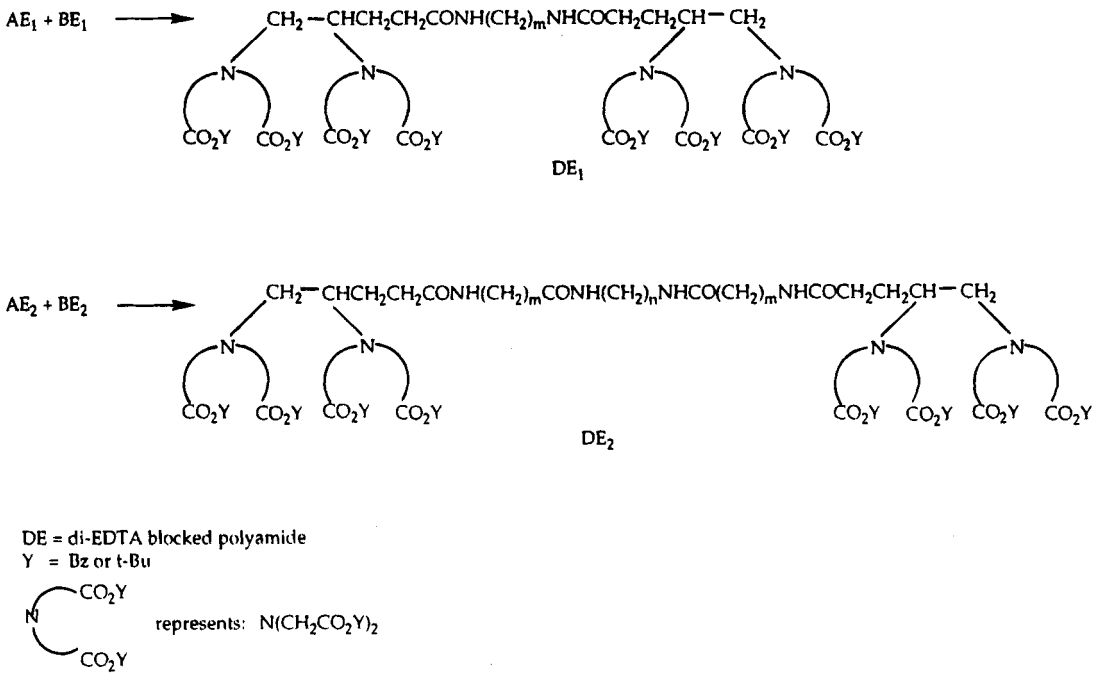
mixture was filtered and evaporated to give the product **4** as a light brown oil (0.59 g, 94% yield). ^{1}H NMR: δ ($CDCl_3$) 6.75–6.40 (1H, broad t); 3.5–2.95 (2H, m); 2.95–2.4 (7H, broad s); 2.21 (4H, t, J = 7 Hz); 1.9–1.1 (8H, m); 1.43 (9H, s). IR: ν_{max} (KBr) 3276 (N–H stretching, *NH*$_2$, *NH*); 1733 [C=O stretching, *CO*$_2$C(CH_3)$_3$]; 1646 (C=O stretching, *CO*NH; N–H bending, NH_2) cm^{-1}. MS: *m/e* 302.2 (MH^+). The compound was used immediately in the next step.

***N*-[5-(*tert*-Butyloxycarbonyl)pentyl]-4,5-bis[bis[(benzyloxycarbonyl)methyl]amino]valeramide [Scheme 3, Compound 5; AE$_2$(Z)].** *N,N,N′,N′*-Tetramethylnaphthalene-1,8-diamine (2.18 g, 10.2 mmol) and NaI (0.15 g, 1 mmol) were added to a solution of **4** (0.58 g, 1.9 mmol) in CH_3CN (freshly distilled on P_2O_5, 3 mL) and the reaction mixture was placed in a silicon oil bath at 95 °C.

Benzyl 2-bromoacetate (1.6 ml, 10.2 mmol) was added, and the mixture was refluxed under N_2 for 42 h and then cooled to room temperature. The solid was filtered off and washed with $CHCl_3$. The filtrate and washing were evaporated, and the residual oil was washed (×3) with ethyl acetate–hexane (1:9) to remove excess benzyl bromoacetate. The solvent was decanted and the residue (2.14 g, brown oil) was flash chromatographed on silica gel using 0.25% MeOH–$CHCl_3$ as eluant to give the product as a yellow-brown oil **5** (0.38 g, 22% yield). ^{1}H NMR: δ ($CDCl_3$) 7.32 (20H, m); 6.37 (1H, br s); 5.09, 5.07 (8H, 2s); 3.53 (8H, s); 3.10 (2H, m); 2.9–2.5 (3H, m); 2.4–2.25 (2H, m); 2.19 (2H, t, J = 7.6 Hz); 1.8–1.2 (8H, m); 1.43 (9H, s). IR: ν_{max} (neat): 3500–3200 (N–H stretching); 1745 (C=O stretching, *CO*$_2$C$_7$H$_7$; 1731 [C=O stretching, *CO*$_2$C-(CH_3)$_3$]; 1666 (C=O stretching, *CO*NH) cm^{-1}. Elemental analysis: $C_{51}H_{63}N_3O_{11}$ requires: N, 4.70; found: N, 4.66. MS: *m/e* 894.2 (MH^+).

***N*-(5-Carboxypentyl)-4,5-bis[bis[(benzyloxycarbonyl)methyl]amino]valeramide (Scheme 3, Compound 6; Scheme 1, AE$_2$).** Compound **5** (0.246 g) was dissolved in TFA (4 mL), and the solution was stirred for 2 h. During the first few minutes evolution of CO_2 was observed. TFA was removed under high vacuum, $CHCl_3$ (5 mL) was added and the TFA salt of **6** was neutralized by adding 0.1 N NaOH (aq) (~10 mL) to reach pH = 6.5. The two layers were separated (after addition of NaCl), and the organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum to give the product as a yellow-brown oil (0.214 g, 93% yield). ^{1}H NMR: δ ($CDCl_3$) 7.32 (20H, m); 6.47 (1H, br t); 5.08, 5.07 (8H, 2s); 3.56, 3.54, 3.52 (8H, 3s); 3.2–3.05 (2H, m); 2.9–2.5 (3H, m); 2.35–2.15 (4H, m); 1.85–1.2 (8H, m). ^{13}C NMR: δ ($CDCl_3$) 178.26, 174.29, 172.76, 171.65, 136.24, 136.17, 129.23, 129.21, 129.00, 128.98, 128.96, 67.16, 67.03, 61.23, 56.79, 55.92, 53.53, 39.75, 34.70, 33.94, 29.73, 27.52, 26.94, 25.07. IR: ν_{max} (neat) 3600–3150 (N–H stretching); 1740 (C=O stretching, *CO*$_2$C$_7$H$_7$); 1647 (C=O stretching, *CO*NH) cm^{-1}. Elemental analysis: $C_{47}H_{55}N_3O_{11}$ requires: N, 5.01; found: N, 5.01. MS: *m/e* 838.3 (MH^+).

Scheme 4. Proposed Scheme for Synthesis of Terminally Bis-EDTA-Blocked Polyamides



***N*-[5-[(Succinimidooxy)carbonyl]pentyl]-4,5-bis[bis-[(benzyloxycarbonyl)methyl]amino]valeramide (Scheme 3, Compound 7).** The acid **6** (35 mg, 0.042 mmol) was dissolved in CH_3CN (1 mL, distilled over P_2O_5); *N*-hydroxysuccinimide (4.8 mg, 0.042 mmol) in CH_3CN (0.25 mL) was added and the reaction mixture was cooled to 0 °C. DCC (8.6 mg, 0.042 mmol) was added and after 3 h of stirring at 0 °C the system was transferred to the refrigerator for an additional 18 h. Since, by TLC, the starting material was still present, NHS (1 mg) and DCC (5 mg) were added, followed by a further amount of DCC (4.5 mg) being added after 3 h of stirring at room temperature. The solid (DCU) was filtered off and washed with $CHCl_3$. The filtrate and washing were combined, washed with H_2O (×2) to remove excess NHS, dried over Na_2SO_4, and evaporated to give 45 mg of a yellow-brown oil that is the product + DCU. ^1H NMR: δ ($CDCl_3$) 7.31 (20H, s); 6.00–5.65 (1H, m); 5.07 (8H, s); 3.53 (8H, s); 3.35–2.95 (3H, m); 2.75 (4H, s); 2.40–2.05 (4H, m); 2.05–1.00 [DCU + (8H, m)].

***N*-[5-[[5-(*tert*-butyloxycarbonyl)pentyl]carbamoyl]-pentyl]-4,5-bis[bis[(benzyloxycarbonyl)methyl]amino]-valeramide (Scheme 3, Compound 8; AE3(Z), Y = Bz, Z = t-Bu).** A solution of the acid **6** (0.03 g, 0.036 mmol) in THF (0.3 mL, distilled over $LiAlH_4$) was placed in an ice–NaCl bath at −14 °C. *N*-Methylmorpholine (0.004 g, 0.04 mmol) in THF (0.1 mL) was added, followed by isobutyl chloroformate (0.005 g, 0.036 mmol) in THF (0.2 mL). The reaction mixture was stirred for 1 min and then *tert*-butyl 6-aminohexanoate (0.0067 g, 0.036 mmol) in THF (0.2 mL) was added. The ice–NaCl bath was removed and the reaction mixture stirred for 18 h at room temperature. The solvent was removed in vacuum, EtOAc was added, and the organic solution washed with distilled H_2O, 0.5 M $NaHCO_3$, and saturated NaCl (aq), and then dried over Na_2SO_4, filtered, and evaporated to give 0.028 g of a pale yellow oil, which was then chromatographed on 2 TLC plates (20 × 20 cm, 0.2-mm thickness) using 5% MeOH–$CHCl_3$ as eluant. The product **8** (7 mg, 20% yield) was obtained as a yellow-brown oil. ^1H NMR: δ ($CDCl_3$) 7.32 (20H, m); 6.47 (1H, br t); 5.68 (1H, br t); 5.09, 5.07 (8H, 2s); 3.57, 3.53 (8H, 2s); 3.25–3.05 (4H, m); 2.85–2.50 (3H, m); 2.20 (4H, t, J = 7.4 Hz); 2.13 (2H, t, J = 7.4 Hz); 1.8–1.2 (14H, m); 1.43 (9H, s). IR: ν_{max} (neat) 3295 (N–H stretching); 1740 (C=O stretching, $CO_2C_7H_7$); 1734 [C=O stretching, $CO_2C(CH_3)_3$]; 1652, 1646 (C=O stretching, CONH ×2) cm^{-1}. MS: *m/e* 1007.3 (MH^+).

6-[(Benzyloxycarbonyl)amino]hexanoic Acid [Cbz-NH-$(CH_2)_5CO_2H$]. A solution of the 6-aminohexanoic acid (13.12 g, 0.1 mol) in distilled H_2O (30 mL) and 5 N NaOH (20 mL, 0.1 mol) was cooled to 0 °C.

Added simultaneously from two dropping funnels during 90 minutes were carbobenzoxy chloride (16 mL, 0.11 mol) and 5 N NaOH (22 mL, 0.11 mol) plus distilled H_2O (30 mL), while the solution pH was kept around 10. The ice bath was removed and the reaction mixture stirred for an additional 90 min. The solution was washed with ether (4 × 50 mL) to remove excess of carbobenzoxy chloride, acidified with 5 N HCl (20 mL), and extracted with ether (3 × 40 mL). The ether solution was dried over $MgSO_4$ and evaporated to obtain the product (24.4 g, 92% yield) as a white solid. ^1H NMR: δ ($CDCl_3$) 7.35, 7.34 (5H, 2s); 5.09 (2H, s); 5.00 (1H, br s); 3.2–3.05 (2H, m); 2.33 (2H, t, J = 7.4 Hz); 1.7–1.55 (2H, m); 1.55–1.45 (2H, m); 1.45–1.3 (2H, m). ^{13}C NMR: δ ($CDCl_3$) 178.97, 156.56, 136.55, 128.50, 128.09, 66.65, 40.80, 33.87, 29.54, 26.11, 24.27.

***tert*-Butyl 6-[(Benzyloxycarbonyl)amino]hexanoate [Cbz-NH-$(CH_2)_5CO_2$-t-Bu].** 6-[(Benzyloxycarbonyl)amino]-hexanoic acid (14 g, 0.053 mol) in CH_2Cl_2 (150 mL) was placed in two pressure bottles. H_2SO_4 (0.36 mL, 98%) was added to each bottle, followed by cooling in dry ice–acetone bath. Isobutylene (30 mL), which was condensed in a dry ice–acetone bath, was added to each bottle. The bottles were closed and their temperature allowed to rise to room temperature. After 4 days, the bottles were cooled in a dry ice–acetone bath and opened. Na_2CO_3 (12 mL, 1 M) was added to each bottle without stirring. After 1 h, the two solutions were combined, the layers were separated, and the organic layer was evaporated. The residue was dissolved in EtOAc (200 mL), washed with 1 M Na_2CO_3, followed by H_2O, dried over $MgSO_4$, and evaporated to obtain the products as a liquid (12.35 g, 70% yield). ^1H NMR: δ ($CDCl_3$) 7.33 (5H, s); 5.09 (2H, s); 4.46 (1H, broad m); 3.18 (2H, m); 2.20 (2H, t, J = 6.7 Hz); 1.77–1.25 (6H, m); 1.43 (9H, s).

***tert*-Butyl 6-Aminohexanoate [$H_2N(CH_2)_5CO_2C(CH_3)_3$].** *tert*-Butyl 6-[(benzyloxycarbonyl)amino]hexanate (12.32 g, 0.038 mol) in EtOH (100 mL) was transferred to two 250-mL round-bottomed flasks; 10% Pd/C (0.21 g) was added to each flask and the reaction mixture was hydrogenated under atmospheric pressure (~840 mL of H_2 was adsorbed). After filtration and evaporation of the solvent, the crude material was distilled (83–84 °C, 1.5 mmHg) to obtain a transparent liquid (5.27 g, 73% yield). ^1H NMR: δ ($CDCl_3$) 2.69 (2H, t, J = 7.0 Hz); 2.22 (2H, t, J = 7.4 Hz); 1.90 (2H, s); 1.60 (2H, m); 1.5–1.4 (2H, m); 1.44 (9H, s); 1.4–1.3 (2H, m). ^{13}C NMR: δ ($CDCl_3$) 173.44,

80.28, 42.23, 35.81, 33.60, 28.44, 26.67, 25.22. Elemental analysis: $C_{10}H_{21}NO_2$ requires: N, 7.48; found: N, 7.67.

4,5-Diaminovaleric Acid *tert*-Butyl ester. (Based on procedures of Bodansky[12] and Fieser[13] by transfer esterification with $HClO_4$ as catalyst.) Compound **1** (0.205 g, 1 mmol) was stirred for 22 h at room temperature in 16 mL of *tert*-butyl acetate and 0.22 mL of $HClO_4$. $NaHCO_3$ (0.84 g, 10 mmol) was added and the solvent removed under high vacuum while stirring (warming of the system should be avoided). The oily solid obtained was triturated with $CHCl_3$ and ether, followed by drying under high vacuum to obtain the product (0.23 g, >100% yield)—a very hygroscopic off-white solid. TLC: R_f 0.37 ($CHCl_3$–MeOH–NH_3 8:2:0.5).

Attempted Synthesis of 4,5-Bis[bis(benzyloxycarbonyl)methyl]amino]valeric Acid (Scheme 1, AE_1). The synthesis was attemped from 4,5-diaminovaleric acid and inorganic bases in different solvents: CH_3CN, $CHCl_3$, 1,2-DCE.

Na_2CO_3 (0.42 g, 4 mmol) and NaI (0.45 g, 0.3 mmol) were added to a solution of **1** (0.103 g, 0.5 mmol) in 0.75 mL of distilled H_2O or in 5 N NaOH (0.3 mL, 1.5 mmol). CH_3CN (0.5 mL) or 1,2-DCE (1.5 mL) were added, followed by benzyl bromoacetate (0.48 mL, 3 mmol), and the mixture was stirred for 21 h at rt, 17 h at 50 °C, or 17 h at 91 °C. After cooling, the mixture was neutralized with 10% HCl; $CHCl_3$ was added and the two phases were checked by TLC and NMR.

The organic phase contained benzyl bromoacetate and its hydrolyzed product, and the aqueous phase contained the starting material or its cyclization product.

Another reaction was conducted with CH_3CN as solvent (1.5 mL) and Na_2HPO_4 (0.568 g, 4 mmol) as base, 17 h at 92 °C. Only starting material and/or its cyclization product were recovered.

The stability of benzyl bromoacetate against 5% Na_2CO_3 at 25 °C/50 °C was checked and appears to be good.

Attempted Synthesis of *tert*-Butyl 4,5-Bis[bis[(benzyloxycarbonyl)methyl]amino]valerate. The synthesis was attempted from 4,5-diaminovaleric acid *tert*-butyl ester in the presence of *N,N,N',N'*-tetramethyl-1,8-naphthalenediamine and ethyl diisopropylamine.

N,N,N',N'-Tetramethylnaphthalendiamine (0.74 g, 3.4 mmol) or ethyldiisopropylamine (0.54 ml, 3.2 mmol) and NaI (0.051 g, 0.3 mmol) were added to a solution of diaminovaleric acid *tert*-butyl ester (0.122 g, 0.65 mmol) in 1.5 mL CH_3CN (freshly distilled over P_2O_5). The reaction flask was placed in a 94 °C silicon bath under N_2; benzyl bromoacetate (0.55 mL, 3.4 mmol) was added and the mixture was stirred for 21 h and 48 h. The white solid was filtered off, and the solution was evaporated to obtain a brown oil, which upon washing with 10% EtOH–hexane was almost completely dissolved. This solution was flash chromatographed but no product was recovered. Also, the undissolved material did not appear to contain the product (NMR).

Acknowledgment. We thank Varda Weintraub for synthesizing a larger batch of *tert*-butyl 6-aminohexanoate. Prof. M. Wilcheks' continued interest and suggestions are kindly acknowledged. We thank Shelly Litvin for help in the preparation of this manuscript.