Bifunctional Chelating Agents, Part 3:† 4,5-Bis[di(carboxymethyl)amino]-*N*-(aminoalkyl)valeramide, an EDTA with an Amine Functionality and Amide-type Linker Arm

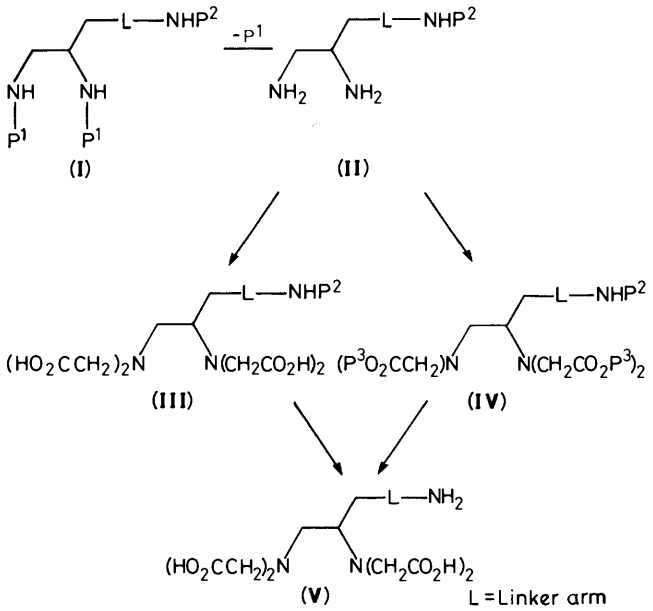
Abraham Warshawsky,[a,*] Janina Altman,[b] Rina Arad-Yellin,[a] Hugo E. Gottlieb,[c] Abraham Deshe,[a] Nava Kahana,[a] Nurit Shoef,[a] and Meir Wilchek[b]
Departments of Organic Chemistry[a] and Biophysics,[b] The Weizmann Institute of Science, Rehovot 76100, Israel
[c] *Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel*

Synthesis of bifunctional EDTA compounds with an ε-amino functionality linked through an amidoalkyl linker group derived from the previously described 4,5-diaminovaleric acid. Protection of the vicinal diamine with Boc groups and chain extension with monobenzyloxycarbonylalkylamines. Removal of the Boc groups and alkylation with bromoacetic acid (or esters), or further removal of the Z groups provides the amino EDTA compounds (**8**).

Chelating agents conjugated to antibodies are being used extensively for detection and localization of cancerous cells in organs.[1-6]

We have previously described compounds containing, in addition to the EDTA‡ moiety, a carboxylic acid group [*e.g.* 1-(2-carboxyethyl)-EDTA][7] which is used to couple the molecule to an antibody. However, since all five carboxy groups react, the metal binding strength of the chelating group is reduced. Since, for effective localization, a strong chelating agent is required (to ensure the metal is not lost in the body fluids before reaching the target cells), chelating agents containing functional groups other than a carboxy group are required. Attempts to synthesize 2-aminoethyl-EDTA from 1-tosylhistamine[8] or 1-isobutyryl-histamine,[9,10] revealed difficulties in obtaining a good match of protecting groups for the vicinal diamine (P^1 in Scheme 1) and P^2 of the ε-amino group. The main difficulty was removal of the *N*-tosyl[8] or *N*-isobutyryl group[10] in the last step [Scheme 1, (**III**) ⟶ (**IV**) or (**III**) ⟶ (**V**)].

Here, we describe the synthesis of ε-aminoalkyl-EDTA of type (**V**) where the linker arm includes an amide bond. This approach provides not only an excellent solution to the dilemma discussed above, but also allows the synthesis of many linker-type ε-amino-EDTA compounds.



Scheme 1. General synthesis of functional ε-amino EDTA compounds with a linker arm (L) between EDTA and amino functionalities

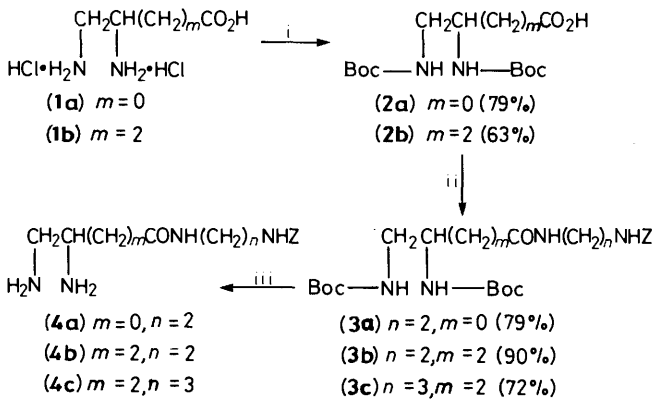
Results and Discussion (see Schemes 2 and 3)

Compound (**1b**)[7] was allowed to react with an excess of di-t-butyl oxydiformate to give di-4,5-bis(Bocamino)valeric acid (**2b**) under conditions described for various amino acids,[11-12] including histamine.[8-10] Next, (**2b**) was coupled with *N*-Z-propane-1,3-diamine hydrochloride,[14] using the DCC/NHS method,[15,16] the product *N*-Z-aminopropyl-4,5-bis(Bocamino)-valeramide (**3c**) was obtained after flash chromatography on silica. Similarly, (**2b**) reacted with *N*-Z-ethylenediamine hydrochloride[14] and yielded *N*-Z-amino-4,5-bis(Bocamino)-valeramide (**3b**).§ The same sequence of reactions was repeated on 2,3-diaminopropionic acid dihydrochloride (**1a**), yielding 2,3-bis(Bocamino)propionic acid (**2a**), which was not isolated but allowed to react further to give *N*-Z-aminoethyl-2,3-bis(Bocamino)propionamide (**3a**).

The diamine, 4,5-diamino-*N*-(Z-aminopropyl)valeramide (**4c**) was also tetra-alkylated with methyl 2-bromoacetate in MeCN, using *N,N,N',N'*-tetramethylnaphthalene-1,8-diamine[17] as a proton sponge, to provide 4,5-bis[di(methoxycarbonylmethyl)amino]-*N*-(Z-aminopropyl)valeramide (**6c**). Removal of the Z group gave, quantitatively, 4,5-bis[di(methoxycarbonylmethyl)amino]-*N*-(aminopropyl)valeramide (**7c**) which was characterized only by n.m.r. and immediately hydrolysed to (**8c**).

The tetra-alkylation of vicinal diamines of type **II** (Scheme 1) to ε-amino protected EDTA of type **III** is usually conducted in aqueous or partially aqueous solvents, in the presence of inorganic salts and is characterized by very low yields and great difficulties in the isolation of pure products, requiring chromatographic separation as in the case of 1-(*p*-nitrophenyl)-EDTA[18] and 1-(*p*-carboxymethoxybenzyl)-EDTA.[19] Otherwise various isolation techniques are used, including crystallization.[7,20,21] The low yields and the cumbersome isolation methods are

† Part 2, ref. 1.
‡ EDTA = ethylenediaminetetra-acetic acid.
§ The terms Boc (t-butoxycarbonyl) and Z (benzyloxycarbonyl) are used for ease of discussion.



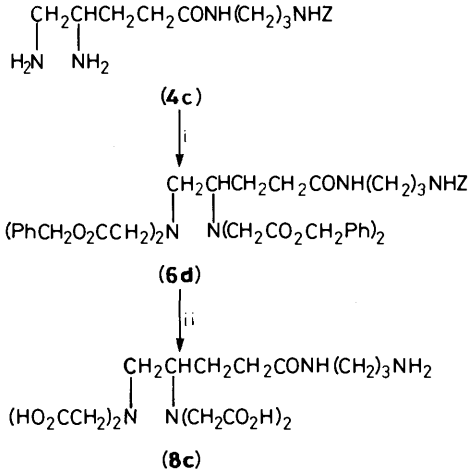
Scheme 2. *Reagents:* i, $(Bu^tCO_2)_2O$; ii, DCC, NHS; iii, HCl, TFA

a burden in the completion of the reaction steps (II) ⟶ (III) ⟶ (V) (Scheme 1) particularly if step (II) ⟶ (III) is conducted in aqueous media and step (III) ⟶ (V) in anhydrous media.

The alkylation of the vicinal diamine (II) with bromoacetic acid esters, leading to the EDTA esters of type (IV), provides a more satisfactory way to (V), especially if P^2 and P^3 are chosen so that both are removed in one step.

The well-known protection of amines as benzyl carbamates[22,23] and of carboxylic acid benzyl esters,[22,24,25] suggested the simultaneous use of Z for the protection of the amine (P1) and Bz for the protonation of the carboxy group (P3 in Scheme 1). Recently, diethylenetriaminepenta-acetic acid was protected as the tetrabenzyl ester and applied in preparing peptides carrying chelating iminodiacetate groups.[25]

Synthesis of Hydrophobic EDTA Benzyl Esters (*Scheme 4*).—The protection of the ε-amine group with Z and the carboxylic acid with Bz provides hydrophobic high molecular weight (see Scheme 1), extractable compounds of type (IV). They are u.v.-detectable, readily characterized, and easy to separate on t.l.c. plates. In addition, they are readily purified by flash chromatography. Removal of all five blocking groups in (IV), under mild hydrogenation conditions in organic polar media, leads to the desired ε-amino substituted EDTA (V). This scheme is demonstrated in the alkylation of diaminovaleramide



Scheme 4. *Reagents:* i, $BrCH_2CO_2CH_2Ph$, Proton Sponge; ii, H_2, POL

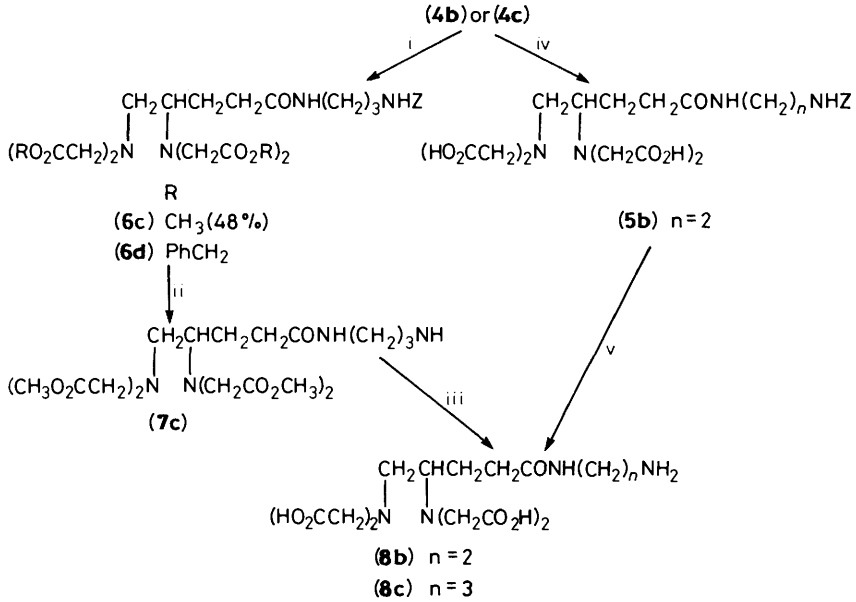
(4c) to 4,5-bis[di(benzyloxycarbonylmethyl)amino]-*N*-(benzylcarbonylaminopropyl)valeramide (6d), followed by hydrogenolysis to 4,5-bis[di(carboxymethyl)amino]-*N*-(aminopropyl)-valeramide (8c) (Scheme 4).

Thus, the simultaneous protection of the amine, in the substrate to be alkylated, by Z and the carboxylic group in the alkylating agent by Bz provides a facile and coherent solution to the synthesis of ε-amino-N^1,N^1,N^2,N^2-tetra-acetic acid-1,2-ε-diamines.

Structural assignment of the synthesized compounds by ^1H n.m.r. is somewhat limited and usually hard, because of the presence of highly labile protons of the amine and carboxy types, and also water of hydration molecules. ^{13}C N.m.r. was therefore an important tool in the structural assignment. In addition to the information provided in the Experimental section, complete ^{13}C n.m.r. assignments are provided in Table 1 (for numbering, see the Figure).

Experimental

General.—Di-t-butyl oxydiformate (Fluka), *N*-hydroxysuccinimide (Aldrich), dicyclohexylcarbodi-imide (DCC) (Riedel



Scheme 3. *Reagents:* i, $BrCH_2CO_2R$, CH_3CN, Proton sponge; ii, Pd/C, H^+, CH_3OH; iii, OH^-; iv, $BrCH_2CO_2H$, pH 10.5; v, Pd, cyclohexane

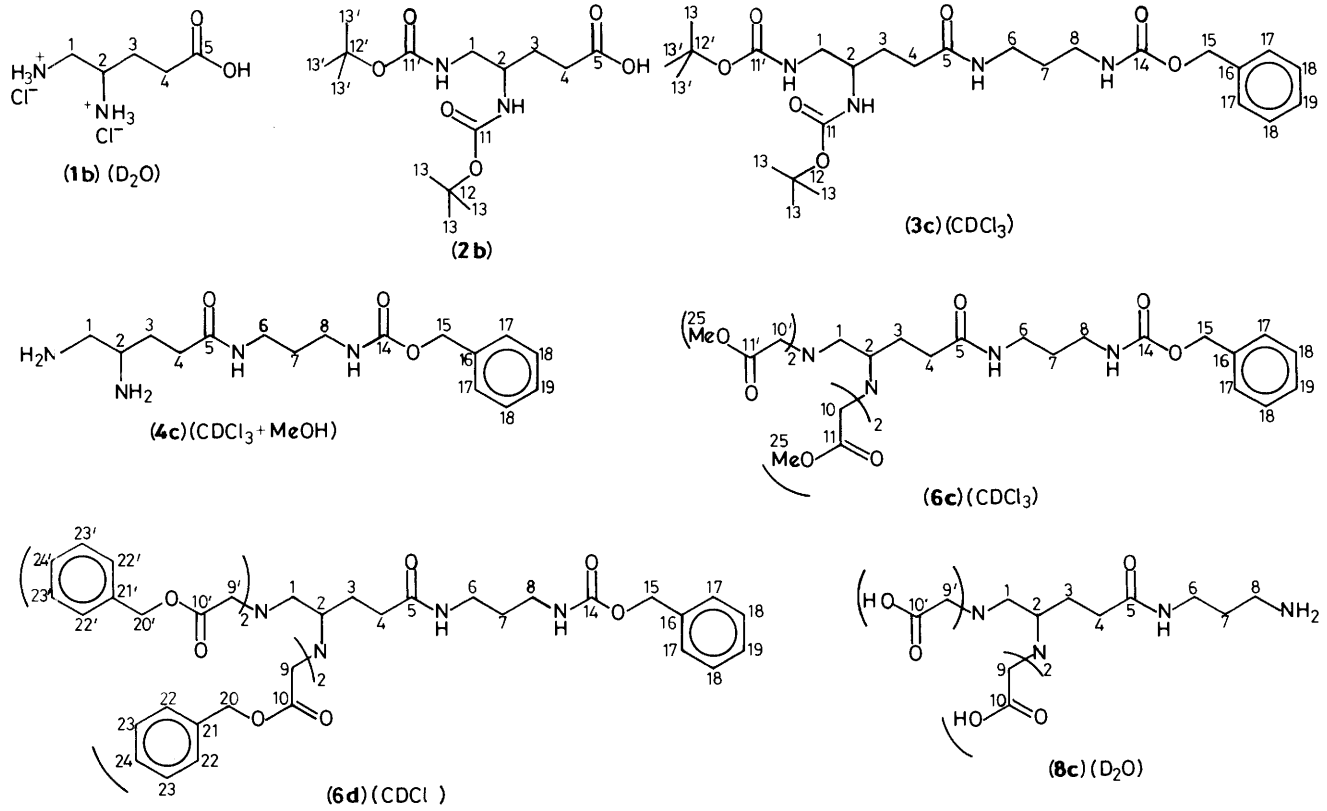


Figure. Carbon numbering for compounds shown in the Table

De Haen). *N*-Z-propane-1,3-diamine and *N*-Z-ethane-1,2-diamine hydrochloride were prepared according to W. B. Lawson *et al.*,[14] from ethane-1,2-diamine and propane-1,3-diamine (99%) (Aldrich) and benzyloxycarbonyl chloride (Biomakor), *N,N,N',N'*-tetramethylnaphthalene-1,8-diamine (Aldrich), methyl 2-bromoacetate (Merck) and palladium black (Fluka). T.l.c. was performed on Merck Kieselgel 60F 254 plates with the eluants: ethyl acetate, and chloroform–methanol (95:5). The dyeing reagents were: basic aqueous 1% $KMnO_4$ solution and ethanolic 0.2% ninhydrin solution. Flash chromatography was carried out by the method of W. C. Still,[26] using 40—63 μm silica gel (Merck No. 9385) and EtoAc or $CHCl_3$ and $CHCl_3$–MeOH (99:1) as eluants. M.p.s were determined on a Gallenkamp apparatus and are corrected. ^1H N.m.r. spectra were recorded on a Varian FT80A spectrometer. I.r. spectra were obtained on Matteson CYGNUS 25 FTIR spectrophotometer. ^1H N.m.r. spectra were run at 80 MHz in a Varian FT80A instrument. ^{13}C N.m.r. spectra were obtained at 75.5 MHz on a Bruker AM-300, with tetramethylsilane as an internal reference for $CDCl_3$ solutions and MeOH (δ_C = 49.5) for D_2O solutions. The assignments were aided by multiplicities and $^{13}C \times {}^1H$ correlations resulting from off-resonance decoupled spectra. Waters h.p.l.c. system includes: Model 6000A solvent delivery pump; model UGK injector; model 480 UV-VIS detector; RP-C18 μ-Bondpak 300 × 46 mm. H.p.l.c. analyses were performed on an RP column with THF–H_2O (3:1) and u.v. detection at 235 nm.

N,N-*Di-t-butoxycarbonyl*-4,5-*diaminovaleric Acid* (**2b**) *and* N,N-*Di-t-butoxycarbonyl*-2,3-*diaminopropionic Acid* (**2a**).—A solution of the diamino acid (**1b**) (6.10 g, 30 mmol) in 0.5M KOH (180 ml) was cooled to 0 °C and a solution of di-t-butyl oxydiformate (19.48 g, 90 mmol) in dioxane (120 ml) was added; the pH was maintained at 7. The stirred reaction mixture was kept at −4 °C overnight after which a further equivalent of di-t-butyl oxydiformate (6.55 g, 30 mmol) in dioxane (10 ml) and 0.5M KOH (80 ml) were added. The solution was stirred for 24 h at room temperature and then diluted with water (750 ml) and extracted with ether (× 6). The aqueous phase was acidified with 15% citric acid to pH ~4 and extracted with ether (× 3). The combined extracts were washed with saturated brine, dried ($MgSO_4$), and evaporated under reduced pressure to give an oil which was extracted overnight with hexane (120 ml). The filtered extract was then evaporated under reduced pressure to yield the product (**2b**), as a white powder (6.24 g), m.p. 130—131.5 °C;[27] $\delta_H(CDCl_3)$ 6.30—5.76 (1 H, br s, NH), 5.16—4.76 (1 H, br s, NH), 3.86—3.40 (1 H, m, CH), 3.17 (2 H, t, *J* 8 Hz, CH_2NH), 2.43 (2 H, t, *J* 9 Hz, CH_2CO_2), 2.00—1.60 (2 H, m, CH_2), 1.43 (18 H, s, CH_3); $\delta_C(CDCl_3)$ 44.60, 51.17, 27.96, 30.62, 177.16 (skeletal carbons), 156.57 (CO), 79.65 (O-C), and 128.36 (Bu^t); $\nu_{max.}$(KBr) 3 368 (NH), 1 720 (CO_2H), and 1 685 cm^{-1} (CO, $NHCO_2Bu^t$) (Found: C, 54.5; H, 8.25; N, 8.55. $C_{15}H_{28}N_2O_6$ requires C, 54.20; H, 8.49; N, 8.43%).

Product (**2a**) was prepared by the same procedure from 2,3-diaminopropionic acid and was used for the next step without purification.

N-*Benzyloxycarbonylaminopropyl*-2,3-*bis*(*t-butoxycarbonylamino*)*propionamide* (**3a**), N-*Benzyloxycarbonylaminoethyl*-4,5-*bis*(*t-butoxycarbonylamino*)*valeramide* (**3b**), *and* N-*Benzyloxycarbonylaminopropyl*-4,5-*bis*(*t-butoxycarbonylamino*)*valeramide* (**3c**).—N^4,N^5-Di-Boc-4,5-diaminovaleric acid (**2b**), (6 g, 18 mmol) was dissolved in MeCN (360 ml) and *N*-hydroxysuccinimide (NHS) (2.28 g, 20 mmol) was added. The reaction mixture was cooled to 0 °C and dicyclohexylcarbodi-imide (DCC) (4.53 g, 22 mmol) was added. After the mixture had been stirred for 2 h at 0 °C, the ice-bath was removed and the reaction mixture was allowed to warm to room temperature; it was then stirred for an additional 2 h. A solution of *N*-Z-propane-1,3-diamine hydrochloride (4.38 g, 18 mmol), or *N*-Z-ethane-1,3-

Table. ^{13}C N.m.r. assignments for compounds described in Schemes 2 and 3

No.	(1b)	(2b)	(3c)	(4c)	(6c)	(6d)	(8c)	
1	41.22	44.60	44.35	48.09	56.32	56.22	54.71	skeletal carbons
2	49.93	51.17	51.39	52.79	60.43	60.57	59.50	
3	25.72	27.96	29.15	30.87	26.72	26.91	27.29	
4	29.90	30.62	33.02	33.00	33.26	33.26	32.49	
5	176.91	177.16	173.16	173.87	174.20	174.13	175.32	
6			36.15	36.31	36.16	35.97	36.76	
7			29.92	29.71	29.80	29.78	22.79	
8			37.88	37.94	37.81	37.69	37.68	
9					55.16	55.01	53.39	
9′					55.16	55.01	56.56	
10					172.53	171.89	172.28	
10′					172.53	171.89	173.29	
11		156.57	156.79					vicinal diamine protecting group
11′		156.78	156.79					
12		79.65	79.47					
12′		79.65	79.47					
13		28.36	28.38					
13′		28.36	28.38					
14			156.79	157.08	156.65	156.65		ε-amino protecting group
15			66.58	66.64	66.48	66.51		
16			136.61	136.59	136.72	136.69		
17			128.00	128.05	127.94	127.87		
18			128.44	128.48	128.42	128.37		
19			128.00	127.90	127.94	127.92		
20						66.36		carboxyl protecting group
20′						66.51		
21						135.48		
21′						135.54		
22,22′						128.25		
23,23′						128.52		
24						128.25		
25								

diamine hydrochloride (18 mmol) in $CHCl_3$ (360 ml) and Et_3N 10 ml, 72 mmol) was added to the reaction mixture which was then stirred overnight at room temperature. Dicyclohexylurea was filtered off and the filtrate evaporated under reduced pressure. The residue was dissolved in EtOAc and the solution washed with distilled water. The layers were separated and the aqueous phase was washed with EtOAc, which was added to the organic phase. The EtOAc layer was washed with saturated $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated. The residue (10.3 g) was flash chromatographed on silica gel, using 30% hexane–EtOAc as starting eluant. The product was eluted with EtOAc and was obtained as a white solid.

N-*Benzyloxycarbonylaminopropyl*-2,3-*bis*(*t-butoxycarbonyl-amino*)*propionamide* (**3a**) (79%), m.p. 159—160 °C: $\delta_H(CDCl_3)$ 7.34 (5 H, s, CH_2Ph), 6.80 (1 H, br s, NH), 5.70 (1 H, br s, NH), 5.30 (1 H, br s, NH), 5.10 (3 H, br s + s, CH_2Ph + NH), 4.20—3.90 (1 H, m, CHN), 3.60—3.10 (6 H, m, 3 × CH_2NH), and 1.42 (18 H, s, 6 × CH_3) (Found: C, 58.05; H, 7.5; N, 12.05. $C_{23}H_{36}N_4O_7$ requires C, 57.49; H, 7.54; N, 11.66%).

N-*Benzyloxycarbonylaminoethyl*-4,5-*bis*(*t-butoxycarbonyl-amino*)*valeramide* (**3b**) (90%), m.p. 160—161 °C: $\delta_H(CDCl_3)$ 7.33 (5 H, s, Ph), 6.77—6.45 (1 H, br s, NH), 5.75—5.40 (1 H, br s, NH), 5.18—4.70 (2 H, 2 br s, 2 NH), 5.09 (2 H, s, CH_2Ph), 3.58—3.45 (1 H, m, CH), 3.50—2.95 (6 H, m, 3 CH_2NH), 2.24 (2 H, t, CH_2CO), and 1.42 and 1.38 (18 H, 2 s, 6 × CH_3) (Found: C, 59.35; H, 7.7; N, 10.91. $C_{25}H_{40}N_4O_7$ requires C, 59.04; H, 7.92; N, 11.01%).

N-*Benzyloxycarbonylaminopropyl*-4,5-*bis*(*t-butoxycarbonyl-amino*)*valeramide* (**3c**) (6.6 g, 72%), m.p. 141—142 °C: $\delta_H(CDCl_3)$ 7.33 (5 H, s, Ph), 6.75 (1 H, br s, NH), 5.55—5.20 (1 H, m, NH), 5.09 (2 H, s, CH_2Ph), 5.20—4.90 (2 H, br s, NH-Boc), 3.85—3.35 (1 H, m, CH), 3.45—2.95 [6 H, m, 3 × $CH_2(NH)$], 2.24 (2 H, t, *J* 7 Hz, CH_2CO), 1.85—1.50 (4 H, m, 2 × CH_2), and 1.42 (18 H, s, 6 × CH_3); $\delta_C(CDCl_3)$ 44.35, 51.39, 29.15, 33.02, 173.16, 36.15, 29.92, 37.88 (skeletal), 156.79 (CO), 79.47 (C-O), 28.38 (Bu^t) (Z), 156.79 (CO), 66.58 (C-O), 136.61, 128.00, and 128.44; $\nu_{max.}$(KBr) 3 358, 3 342 (NH,NH), 1 699 (CO, $NHCO_2Bz$), 1 685 (CO, $NHCO_2Bu^t$), and 1 641 cm^{-1} (CO, NHCO) (Found: C, 60.0; H, 7.95; N, 10.55. $C_{26}H_{42}N_4O_7$ requires C, 59.75; H, 8.10; N, 10.72%).

4,5-*Diamino*-N-(*benzyloxycarboxyaminopropyl*)*valeramide* (**4c**).—Compound (**3c**) (1.04 g, 2 mmol) was dissolved in absolute MeOH (32 ml), and 18% HCl in MeOH (32 ml) was added to give immediate evolution of CO_2. The mixture was stirred for 2 h, diluted with dry ether (600 ml), and then left overnight to allow precipitation of the product. The solvents were decanted and the solid residue was washed with dry ether (× 3) and dissolved in saturated brine containing KOH (pH ~ 13); it was then extracted with $CHCl_3$. The aqueous layer was washed with $CHCl_3$ (× 3) and the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to give an oil (0.51 g, 80%) which solidified with time. The ^1H n.m.r. signals at 6.80—6.40 and 5.60—5.25 disappeared when D_2O was added; the signal at 3.40—3.00 became a quartet and the peak for NH_2 disappeared: $\delta_H(CDCl_3)$ 7.34 (5 H, s, C_6H_5), 6.80—6.40 (1 H, br s, CONH), 5.60—5.25 (1 H, br t, CONH), 5.09 (2 H, s, CH_2Ph), 3.40—3.00 (4 H, m, 2 × CH_2NCHO), 2.90—2.40 (3 H, m, $CHNH_2$, CH_2NH_2), 2.30 (2 H, *J* 9 Hz, CH_2CO), and 1.95—1.35 (8 H, m, 2 × CH_2, 2 × NH_2); $\delta_C(CDCl_3$ +

CH_3OH) 48.09, 52.70, 30.87, 33.00, 173.87, 36.31, 29.71, 37.94 (skeletal), 157.08 (CO), 66.64 (C-O), 136.59, 128.05, 128.48, and 127.90 (Z); $\nu_{max.}$(KBr) 3 318 cm^{-1} (NH), 1 691 cm^{-1} (CO, $NHCO_2$-B_z), and 1 640 cm^{-1} (CO, NHCO) (Found: N, 14.05; Cl, 17.2. C_{16} $H_{28}Cl_2N_4O_3$ requires N, 14.17; Cl, 17.94%).

Compounds (**4a**) and (**4b**) were prepared in the same way as (**4c**) and were obtained as (**4a**)·2HCl and (**4b**)·2HCl.

2,3-*Diamino*-N-(*benzyloxycarbonylaminopropyl*)*propionamide* [(**4a**)·2HCl]: $\delta_H(D_2O)$ 7.50 (5 H, s, C_6H_5), 5.19 (2 H, s, CH_2Ph), 4.45—4.15 (1 H, m, CH), and 3.60—3.20 (6 H, m, CH_2NH_2, 2 CH_2NH) {Found: C, 44.3; H, 6.5; N, 15.8; Cl, 19.85. $C_{13}H_{22}Cl_2N_4O_3$ [for (**4a**)·2HCl] requires C 44.19; H, 6.23; N, 15.86, Cl, 20.11%}.

4,5-*Diamine*-N-(*benzyloxycarbonylaminoethyl*)*valeramide dihydrochloride* [(**4b**)·2HCl]: $\delta_H(D_2O)$ 7.61 (5 H, s, C_6H_5), 5.30 (2 H, s, CH_2Ph), 4.00—3.65 (1 H, m, CH), 3.65—3.10 (6 H, m, CH_2NH_2, 2 × CH_2NH), 2.62 (2 H, t, *J* 9 Hz, CH_2CO), and 2.22 (2 H, t, *J* 8 Hz, $CHCH_2CH_2$) {Found: C, 46.85; H, 6.5; N, 14.53. $C_{15}H_{26}Cl_2N_4O_3$ [for (**4b**)·2HCl] requires C, 47.25; H, 6.87; Cl, 18.6; N, 14.69%}.

4,5-*Bis*[*di*(*carboxymethyl*)*amino*]-N-(*benzylcarbonylaminoethyl*)*valeramide* (**5b**).—To a solution of bromoacetic acid (0.13 g, 0.93 mmol) dissolved in water (10 ml) and cooled in ice was slowly added KOH (1M) until pH 7.0 was reached. Compound (**4b**) (0.11 g, 0.2 mmol) dissolved in water (10 ml) was then added to the solution. The pH was adjusted to 11.0 after which the reaction mixture was warmed to 50 °C and kept at this temperature for 24 h. The pH was constantly measured and KOH added (1M) in order to keep the pH *ca.* 10.5—11.0. The mixture was then cooled to room temperature and stirring continued for 24 h. After this it was cooled with ice and the pH brought to 2.0 with HCl (6M). The water was removed by liophilization and the residue was triturated with methanol when the organic components dissolved. The product obtained by removal of the methanol had two new broad singlets: $\delta_H(D_2O)$ 7.44 (5 H, s, *Ph*), 5.10 (2 H, s, CH_2Ph), 4.23 (4 H, s, 2 × CH_2CO_2), 4.13 (4 H, s, 2 × CH_2CO_2), 3.90—3.55 (4 H, m, 2 × CH_2NHCO), 3.55—3.15 (3 H, m, NCH_2CHN), 2.60—2.10 (2 H, m, CH_2CO), and 2.20—1.65 (2 H, m, CH_2).

4,5-*Bis*[*di*(*methoxycarbonylmethyl*)*amino*]-N-(*benzylcarbonylaminopropyl*)*valeramide* (**6c**).—To a solution of the diamine (**4c**) (0.16 g, 0.49 mmol) dissolved in MeCN (0.9 ml) was added *N,N,N′,N′*-tetramethylnaphthalenediamine (0.57 g, 2.66 mmol) and NaI (0.05 g, 0.3 mmol). The solution was warmed to reflux and methyl 2-bromoacetate (0.3 ml, 3.17 mmol) added to give immediate precipitation of a white solid. After the reaction mixture had been stirred under N_2 for 17 h, it was cooled, diluted with $CHCl_3$, filtered to remove white solid, and evaporated under reduced pressure. The residue (oil) was washed with EtOAc–hexane (1:9; 3 × 35 ml) to remove excess of methyl 2-bromoacetate, the solvent being removed by decantation. The brown oil (0.43 g) was subjected to preparative t.l.c. (2 plates of 20 × 20 cm, 2 mm) using $CHCl_3$–MeOH (9:1) as eluant. The product (R_F 0.53) was extracted from the plates with $CHCl_3$–MeOH (9:1); removal of solvent gave the product as a yellowish oil (0.15 g, 48%); $\delta(CDCl_3)$ 7.33 (5 H, s, Ph), 6.80 (1 H, br s, NH), 5.50 (1 H, br s, NH), 5.08 (2 H, s, CH_2Ph), 3.67 and 3.66 (12 H, 2 × s, $4CO_2CH_3$), 3.52 and 3.51 (8 H, 2 × s, 4 × NCH_2CO_2), 3.40—3.00 (4 H, m, $2CONHCH_2$), 3.00—2.50 (3 H, m, CH_2N, CHN), 2.38 (2 H, t, CH_2CO), 1.90—1.40 (4 H, m, 2 × CH_2); $\delta_C(CDCl_3)$ 56.32, 60.43, 26.72, 33.26, 174.20, 36.16, 29.80, 37.81, 55.16, 172.53, (skeletal), 156.65 (CO), 66.48 (C-O), 136.72, 127.94, 128.42, (Z), and 51.77 (C-OMe); $\nu_{max.}$(neat) 3 326 (NH), 1 735 (CO_2), and 1 654 cm^{-1} (ν_{CO}, NHCO) (Found: C, 54.35; H, 6.75; N, 9.30. $C_{28}H_{42}N_4O_{11}$ requires C, 55.07; H, 6.93; N, 9.17%).

4,5-*Bis*[*di*(*benzyloxycarbonylmethyl*)*amino*]-N-(*benzylcarbonylaminopropyl*)*valeramide* (**6d**).—To a solution of the diamine (**4c**) (0.20 g, 0.62 mmol) dissolved in MeCN (1.7 ml) were added *N,N,N′,N′*-tetramethylnaphthalene-1,8-diamine (Sigma; 0.71 g, 3.29 mmol) and NaI (0.05 g, 0.3 mol) and the reaction mixture was heated to obtain a clear solution. Addition of benzyl 2-bromoacetate (0.53 ml, 3.29 mmol) gave precipitation of a white solid. The reaction mixture was refluxed under N_2 for 22 h after which it was cooled to room temperature, diluted with $CHCl_3$ and the solids were filtered off. Evaporation of the filtrate under reduced pressure gave an oil which was washed (× 3) with EtOAc–hexane (1:9) to remove excess of benzyl bromoacetate. The solvent was decanted and the residue dried under high vacuum to give a brown oil (0.56 g). This was flash chromatographed on silica gel ($CHCl_3$ ⟶ 1% MeOH in $CHCl_3$) to give the product as a yellow-brown oil (0.30 g, 56%); h.p.l.c. (THF–H_2O, 3:1) gave a single peak at 4 min; $\delta_H(CDCl_3)$ 7.30 (25 H, s, Ph), 6.70 (1 H, br s, NH), 5.60 (1 H, br s, NH), 5.07 and 5.06 (10 H, 2 × s, 5 × CH_2Ph), 3.51 (8 H, s, 4 × NCH_2CO_2), 3.30—2.95 (4 H, q, 2 × CH_2NHCO), 2.95—2.45 (3 H, m, NCH_2CHN), 2.40—2.10 (2 H, m, CH_2CO), and 1.85—1.35 (4 H, m, CH_2); $\delta_C(CDCl_3)$ 56.22, 60.57, 26.91, 33.26, 174.13, 35.97, 29.78, 37.69, 55.01, 171.89; (skeletal), 156.65 (CO), 136.69, 127.87, 128.37, 127.92 (N-Z), 66.36, 66.51 (C-O), 135.48, 135.54, 128.52, and 128.25 (O-CH_2PhZ); $\nu_{max.}$(neat) 3 340 (NH), 1 735 (CO_2), and 1 654 cm^{-1} (ν_{CO}, NHCO) (Found: C, 67.7; H, 6.25; N, 6.3. $C_{52}H_{58}N_4O_{11}$ requires C, 68.25; H, 6.39; N, 6.12%).

4,5-*Bis*[*di*(*methoxycarbonylmethyl*)*amino*]-N-(*aminopropyl*)-*valeramide* (**7c**).—To the tetraester (**6c**) (0.021 g, 0.034 mmol) dissolved in 95% methanol (3 ml) and glacial acetic acid (0.1 ml) was added Pd black (25 mg) and the mixture was hydrogenated in a Parr reactor for 20 h (hydrogen pressure 68 p.s.i., *ca.* 15 °C). The catalyst was filtered off and washed with methanol and combined filtrate and washings were evaporated to give the product (0.018 g, 88%) which was directly hydrolysed: $\delta(CDCl_3)$ 3.68 (12 H, s, 4 × CO_2CH_3), 3.52 (8 H, s, 4 × CH_2CO_2), 3.35—3.00 (3 H, m, CH_2NHCO,CHN), 3.00—2.55 (4 H, m, CH_2N,CH_2NH_2), 2.55—2.15 (2 H, m, CH_2CO), 2.05 (2 H, s, NH_2), and 1.85—1.45 (4 H, m, 2 × CH_2).

4,5-*Bis*[*di*(*carboxymethyl*)*amino*]-N-(*aminopropyl*)*valeramide* (**8c**) [*from* (**7c**)].—The tetraester (**7c**) (0.037 g, 0.31 mmol) was stirred for 20 h with 1M NaOH (0.47 ml) in 95% methanol (0.47 mmol). The reaction mixture was acidified to pH *ca.* 1 with conc. HCl after which the solvents were evaporated. The residue was dissolved in methanol, NaCl was filtered off, and the solvent was removed under reduced pressure to give the product (50 mg); $\delta_H(CDCl_3)$ 4.23 (4 H, s, 2 × CH_2CO_2), 3.89 (4 H, s, 2 × CH_2CO_2), 3.60—2.90 (7 H, m, CH_2N,CHN,CH_2NHCO,CH_2NH_2), 2.60—2.25 (2 H, m, CH_2CO), and 2.30—1.65 (4 H, m, 2 × CH_2); $\nu_{max.}$(KBr) 3 413 (ν_{OH},CO_2H), 3 270 (NH), 1 727 (CO_2H), 1 636 (ν_{CO},NHCO), and 1 440 cm^{-1} (CO_2) (Found: C, 44.6; H, 6.3; N, 12.5. $C_{16}H_{28}N_4O_9$ requires C, 45.7; H, 6.7; N, 13.3%).

4,5-*Bis*[*di*(*carboxymethyl*)*amino*]-N-(*aminoethyl*)*valeramide* (**8b**).—To the 2′-Z derivative (**5b**) (0.1 g, 0.185 mmol) dissolved in methanol (10 ml) were added cyclohexene (0.2 ml) and Pd black (0.025 g) and the mixture was refluxed under atmosphere of argon for 30 min; the catalyst was then filtered off. T.l.c. ($CHCl_3$–MeOH–HOAc, 9:1:1) indicated the absence of starting material. After removal of solvent, the residue was dissolved in methanol (2 ml) and precipitated by addition of

ethyl acetate. An n.m.r. spectrum (D_2O) of the solid residue (0.63 g, 84%) showed the absence of the Z peaks at δ 7.33 and 5.09; $\delta_H(D_2O)$ 3.93 (4 H, s, 2 × CH_2CO_2), 3.78 (4 H, s, 2 × CH_2CO_2), 3.70—3.20 (5 H, m, CH_2NH, NCH_2CHN), 3.20—2.90 (CH_2NH_2), 2.60—2.10 (2 H, m, CH_2CO), and 2.20—1.65 (2 H, m, CH_2).

(**8c**) [*From* (**6d**)].—The tetraester (**6d**) (0.036 g, 0.04 mmol) in 95% methanol (4 ml) and glacial acetic acid (0.1 ml), with Pd black (40 mg) was hydrogenated overnight in a Parr apparatus (70 p.s.i., *ca.* 15 °C). The reaction mixture was filtered and the residual palladium catalyst washed with methanol and water. The combined filtrate and washings were evaporated to give an oil which when treated with methanol (1 ml), solidified to give a white solid (0.014 g, 88%); $\delta_H(D_2O)$ 3.94 (4 H, s, 2 × CH_2CO_2), 3.78 (4 H, s, 2 × CH_2CO_2), 3.65—3.20 (5 H, m, CH_2NH, NCH_2CHN), 3.20—2.90 (CH_2NH_2), 2.60—2.25 (2 H, m, CH_2CO), and 2.20—1.70 (4 H, m, 2 × CH_2); $\delta_C(D_2O)$ 54.71, 59.50, 27.29, 32.49, 175.32, 36.76, 22.79, 37.68, 53.39, 56.56, 172.28, and 173.29 (skeletal); $\nu_{max.}$(KBr) 3 420 (NH, OH), 1 727 (CO_2), and 1 636 cm^{-1} (ν_{CO}, NHCO) (Found: C, 44.6; H, 6.3; N, 12.5. $C_{16}H_{28}N_4O_9$ requires C, 45.71; H, 6.71; N, 13.33%). H.p.l.c. of (**8c**) (THF–H_2O, 3:1) at pH 4 (fully protonated) showed a single peak at 2—6 min. Measurements at pH 10 and pH 14 show several ionic forms at 2.4 min and 3.3 min.

Acknowledgements

We thank Mrs. Hagit Hasson for developing the h.p.l.c. analyses. This work was supported in part by grants from the National Council for Research and Development, Israel Ministry of Science, 'YEDA' and Bioyeda.

References

1 T. G. Wensel and C. F. Meares, in 'Radioimmunoimaging and Radioimmunotherapy,' eds., S. W. Burchiel and B. A. Rhodes, Elsevier, 1983, p. 185.
2 D. A. Scheinberg, M. Strand, and O. A. Gansow, *Science*, 1982, **215**, 1511.
3 D. M. Goldenberg and F. H. Deland, *J. Biol. Response Modif.*, 1982, **1**, 121.
4 E. Soini and H. Kojola, *Clin. Chem.*, 1983, **29/1**, 65.
5 W. Layne, D. J. Hnatowich, P. W. Doherty, R. L. Childs, D. Lanteigne, and J. Ansell, *J. Nucl. Med.*, 1982, **23**, 627.
6 R. Lauffer, *Chem. Rev.*, 1987, **87**, 901.
7 J. Altman, N. Shoef, M. Wilchek, and A. Warshawsky, *J. Chem. Soc., Perkin Trans. 1*, 1984, 59.
8 J. Altman, N. Shoef, M. Wilchek, and A. Warshawsky, *J. Chem. Soc., Chem. Commun.*, 1985, 1133.
9 J. Altman, N. Shoef, M. Wilchek, and A. Warshawsky, *Isr. J. Chem.*, 1986, **27**, 29.
10 A. Warshawsky, N. Kahana, R. Arad-Yellin, A. Deshe, N. Kahana, N. Shoef, J. Altman, and M. Wilchek, Part 4, *Synthesis*, in press.
11 V. F. Pozdnev, *Khim. Prir. Soedin*, 1974, **6**, 764.
12 M. E. Grace, M. T. Loosemore, M. L. Semmel, and R. F. Pratt, *J. Am. Chem. Soc.*, 1980, **102**, 6784.
13 A. Morel and M. Delange, Fr. Demande FR 2 561 671, A1, 1985.
14 W. B. Lawson, M. D. Leafer, Jr., A. Tewes, and G. J. S. Rao, *Hoppe-Seyler's Z. Physiol. Chem.*, 1968, **349**, 251.
15 E. Wunsch and F. Drees, *Chem. Ber.*, 1966, **99**, 110.
16 G. W. Anderson, J. E. Zimmerman, and F. C. Callahan, *J. Am. Chem. Soc.*, 1964, **86**, 1839.
17 R. Y. Tsien and G. Grynkiewicz, U.S. P. 4 603 209, 1986.
18 M. W. Sundberg, C. F. Meares, D. A. Goodwin, and C. E. Diamanti, *J. Med. Chem.*, 1974, **17**, 1365.
19 S. M. Yeh, D. G. Sherman, and C. F. Meares, *Anal. Biochem.*, 1979, **100**, 152.
20 Y. Karube, T. Maeda, T. Imoto, M. Ohya, S. Sugata, A. Kono, H. Okano, and Y. Matsushima, *Chem. Pharm. Bull.*, 1982, **30**, 2529 and references 3—6 therein.
21 J. Altman, N. Shoef, M. Wilchek, and A. Warshawsky, *J. Chem. Soc., Perkin Trans. 1*, 1983, 365.
22 T. W. Greene, 'Protective Groups in Organic Synthesis,' Wiley, 1981, New York.
23 M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
24 W. H. Hartung and R. Simonoff, *Org. React. (N.Y.)*, 1953, **7**, 263.
25 C. Sergheraert, P. Maes, and A. Tartar, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1061.
26 W. C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
27 W. A. Kraft and R. M. Herbst, *J. Org. Chem.*, 1945, **10**, 483.

Received 16*th June* 1988; *Paper* 8/02414F