# Synthesis of δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine and δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valylglycine

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An efficient synthesis of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV; 1) and δ-(L-α-aminoadipyl)-L-cysteinyl-D-valylglycine (ACVG; 2) *via* the protected tripeptide *N*-benzyloxycarbonyl-1-(*p*-nitrobenzyl)-δ-(L-α-aminoadipyl)-*S*-benzyl-L-cysteinyl-D-valine, benzhydryl ester (3) is described.



In the course of studies on the biosynthesis of the penicillins and their acyclic precursor,[1] δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV; 1),† we required an efficient synthesis of the tripeptide which could be utilised for the preparation of $^{13}C$ labelled isotopomers. In addition we wished to prepare δ-(L-α-aminoadipyl)-L-cysteinyl-D-valylglycine (ACVG; 2), for biosynthetic studies. A tetrapeptide with this sequence of amino-acids but of undefined stereochemistry has been isolated from a β-lactam producing *Cephalosporium sp.*[2] but its significance in penicillin biosynthesis is as yet unknown.

Five syntheses of the ACV tripeptide have been reported previously in the literature [3-6] each of which utilise different strategies and/or protecting groups. Two distinct routes have been employed involving elaboration from either the valine carboxy [3-6] or the amino-terminus.[6] Since it was our intention to prepare isotopomers labelled in the valine residue the overall yield from D-valine was of paramount importance and necessitated an approach using the second strategy. We report here a synthesis of ACV (1) by a route employing benzyl-based and benzhydryl-based protecting groups which offers the advantage of removal of the protecting groups by reduction with sodium in liquid ammonia [6] or selective deprotection of the valine carboxy-group to afford, in a single step, the acid (4) as the intermediate for the synthesis of ACVG (2).

Treatment of *N*-benzyloxycarbonyl-L-α-aminoadipic acid [7] (6a) with *p*-nitrobenzyl bromide and one equivalent of triethylamine in dimethylformamide afforded the ester (6b) in 50% yield. Coupling of this derivative with *S*-benzyl-L-cysteine, benzhydryl ester [8] (7) was carried out with 1-hydroxybenzotriazole and dicyclohexylcarbodi-imide [9] to give the fully protected dipeptide (8a). Removal of the benzhydryl protecting group was achieved in virtually quantitative yield by mild acid hydrolysis and the resulting dipeptide acid (8b) coupled with D-valine benzhydryl ester using 1-hydroxybenzotriazole and dicyclohexylcarbodi-imide to afford the fully protected tripeptide (3) in 22% overall yield from (6a).

Reduction of the protected ACV (3) with sodium in liquid ammonia gave the unprotected tripeptide (1). Isolation as the corresponding sulphide followed by regeneration with hydrogen sulphide afforded ACV in apparently 77% yield. However elemental analysis of the freeze-dried product showed it to contain *ca.* 4% inorganic contaminants.[6] The product was shown to be a 3 : 1 mixture of (1) and the corresponding disulphide (9) by Ellman's procedure [10] and by paper electrophoresis. To circumvent the inhomogeneity introduced by the above procedure the crude product from the reduction was oxidised by passing air through an aqueous solution and the resultant disulphide [5] (9) isolated by ion exchange chromatography. The product obtained in this manner, in 96% yield from (3), was homogeneous by paper electrophoresis, h.p.l.c., and t.l.c. and proved free of inorganic contaminants. Confirmation that the product was the desired disulphide (9) was obtained from the $^{13}C$ n.m.r. spectrum which showed a resonance at 38.5 p.p.m. corresponding to C-3 of the cystine residue. In contrast the spectrum of the monomeric tripeptide showed a peak at 26.1 p.p.m.[6] for C-3 of cysteine. Oxidation of (9) with performic acid [11] afforded the sulphonic acid (11) which proved to be chromatographically and spectroscopically identical with material prepared from ACV isolated from *Cephalosporium acremonium* N-2.[1]

With the protected tripeptide (3) in hand, synthesis of ACVG (2) could now be carried out in a straightforward manner. Deprotection of the valine carboxy-group by mild acid hydrolysis afforded the acid (4). Coupling of (4) with glycine benzyl ester using dicyclohexylcarbodi-imide and 1-hydroxylbenzotriazole as above gave only poor yields of the desired product, the major product being the corresponding *N*-acyl urea.[12] In contrast, treatment of (4) with ethyl chloroformate in the presence of pyridine to afford a mixed anhydride and subsequent reaction of the anhydride with glycine benzyl ester gave the desired product (5) in 46% yield from (3). Sodium–liquid ammonia reduction of (5) followed by oxidation and purification of the product by ion-exchange chromatography as described for (9) above gave the tetrapeptide disulphide (10).

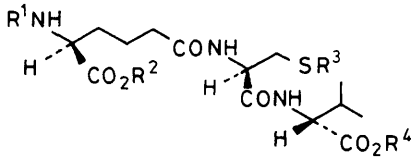
The disulphides (9) and (10) were quantitatively reduced to the respective monomers (1) and (2) with an excess of ethanethiol. The overall yields of ACV (ACVG) from D-valine and *S*-benzyl-L-cysteine by the route described were 68 (24%) and 43 (15%) respectively, which compare favourably with yields achieved in previous syntheses.[3-6]

## Experimental

Electrophoresis was carried out at 50 V/cm on Whatman 3MM paper using the apparatus described by Michl,[13] pH 2.1 and 3.5 buffers were prepared as described by Ambler.[14] Merck silica G60 (70—230 mesh) was used for column chromatography and preparative t.l.c. was carried out on 200 × 200 × 1 mm layers of Merck 60GF254 silica. Thiol determinations were carried out using Ellman's procedure.[10] L-α-Aminoadipic acid $[\alpha]_D^{20}$ +74° (*c* 2.0, 5M-HCl) (lit.,[15] $[\alpha]_D$ +25°) was purchased from Sigma Chemical Co. Inc. Solvents were purified and dried by standard procedures and organic extracts typically dried over $MgSO_4$ or $Na_2SO_4$. N.m.r. spectra were recorded on Varian EM360, Bruker WM300, or Bruker WM360 spectrometers. Mass spectra (e.i.) were recorded on an AEI MS901 and a Kratos MS50 using a FAB source.

*p-Nitrobenzyl N-Benzyloxycarbonyl-L-α-aminoadipate* (6b).—*N*-Benzyloxycarbonyl-L-α-aminoadipic acid [7] (2.065 g, 7.00 mmol) was dissolved in dimethylformamide (5 $cm^3$),

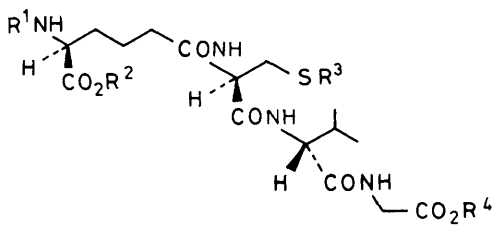
† α-Aminoadipyl = 5-amino-5-carboxypentanoyl



(1) $R^1 = R^2 = R^3 = R^4 = H$

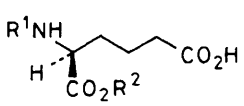
(3) $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$, $R^3 = PhCH_2$, $R^4 = Ph_2CH_2$

(4) $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$, $R^3 = PhCH_2$, $R^4 = H$

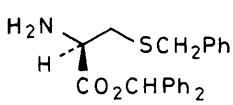


(2) $R^1 = R^2 = R^3 = R^4 = H$

(5) $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$, $R^3 = PhCH_2$, $R^4 = PhCH_2$



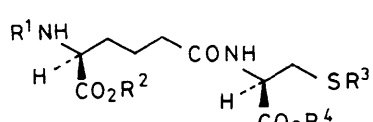
(6)



(7)

a; $R^1 = PhCH_2OCO$, $R^2 = H$

b; $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$

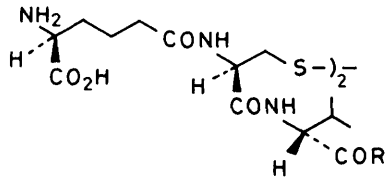


(8)

a; $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$, $R^3 = PhCH_2$, $R^4 = Ph_2CH$

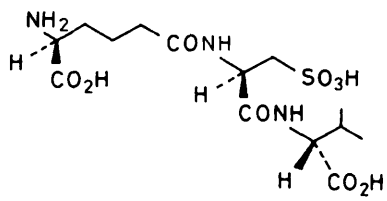
b; $R^1 = PhCH_2OCO$, $R^2 = p\text{-}NO_2C_6H_4CH_2$, $R^3 = PhCH_2$, $R^4 = H$

triethylamine (1.02 cm³, 7.35 mmol) added, and the olsution cooled to 0 °C. To the stirred solution, *p*-nitrobenzyl bromide (1.588 g, 7.35 mmol) was added in aliquots during 5 h. The reaction mixture was allowed to come to room temperature overnight, after which saturated aqueous NaCl (25 cm³) was added; the mixture was then adjusted to pH 1 with 5M-HCl and extracted with EtOAc (30 cm³ × 4). The extracts were evaporated to yield a yellow oil which was chromatographed on silica (200 g) with EtOAc–n-hexane (7 : 3) as eluant to afford (6b) which crystallised from EtOAc–n-hexane (1.22 g, 50%), m.p. 101—104 °C; $[\alpha]_D^{20}$ — 8.01° (*c* 2.0, acetone) (Found: C, 58.4; H, 5.1; N, 6.45. $C_{21}H_{22}N_2O_8$ requires C, 58.60; H, 5.15; N, 6.51%), δ(60 MHz, $CDCl_3$) 1.45—2.05 (4 H, m, 3, 4-H), 2.37 (2 H, t, *J* 5.5 Hz, 5-H), 4.18—4.70 (1 H, m, 2-H), 5.12 and 5.23 (4 H, 2s, benzyl-H), 5.4 (1 H, m, NH), 7.32 (5 H, s, ArH), 7.43 and 8.13 (4 H, 2 d, *J* 9 Hz, ArH),



(9) R = OH

(10) R = $NHCH_2CO_2H$



(11)

8.75 (1 H, s, $CO_2H$); *m/z* 430 ($M^+$), 306, and 250.1091 ($C_{13}H_{16}NO_4$ requires 250.1079), 206.1168 ($C_{12}H_{16}NO_2$ requires 206.1181).

N-*Benzyloxycarbonyl*-1-(p-*nitrobenzyl*)-δ-(L-α-*aminoadipyl*)-S-*benzyl*-L-*cysteine*, *Benzhydryl Ester* (8a).—*S*-Benzyl-L-cysteine (0.422 g, 2 mmol) was converted into the corresponding benzhydryl ester as previously described.[8] The crude product was added to a solution of (6b) (0.88 g, 2.05 mmol) and 1-hydroxybenzotriazole hydrate (0.284 g, 2.1 mmol) in EtOAc (10 cm³). To the stirred solution, dicyclohexycarbodi-imide (0.433 g, 2.1 mmol) in EtOAc (5 cm³) was added and the mixture stirred overnight. After addition of oxalic acid dihydrate (13 mg) the solution was stirred for 1 h, filtered, and the filtrate washed successively with aqueous HCl (1M; 30 cm³), water (30 cm³), and saturated aqueous $NaHCO_3$ (30 cm³), water (30 cm³) and saturated NaCl (30 cm³). Evaporation afforded an oil which was chromatographed on silica (150 g), with EtOAc–n-hexane (6 : 4) as eluant, to give (8a) which was crystallised from EtOAC–n-hexane (1.01 g, 64%), m.p. 122—127 °C; $[\alpha]_D^{20}$ —5.64° (*c* 1.0, $CH_2Cl_2$) (Found: C, 66.65; H, 5.6; N, 5.1. $C_{44}H_{43}N_3O_9S$ requires C, 66.90; H, 5.49; N, 5.32%), δ(360 MHz, $CDCl_3$) 1.68—1.90br (4 H, m, aminoadipyl 3,4-H), 2.15—2.26br (2 H, m, aminoadipyl 5-H), 2.85 (2 H, AB of ABX $\delta_A$ 2.91, $\delta_B$ 2.79, *J* 13.9, 4.7, 6.4 Hz, cys 3-H), 3.58 (2 H, AB; $\delta_A$ 3.62, $\delta_B$ 3.54, *J* 13.4 Hz, $SCH_2Ph$), 4.41 (1 H, m, aminoadipyl 2-H), 4.90 (1 H, M of ABMX, *J* 4.7, 6.4, 7.9 Hz, cys 2-H), 5.09 (2 H, AB; $\delta_A$ 5.12, $\delta_B$ 5.06, *J* 12.2 Hz; $OCH_2Ar$), 5.21 (2 H, AB, $\delta_A$ 5.24, $\delta_B$ 5.19, *J* 13.6 Hz, $OCH_2Ar$), 5.47 (1 H, d, *J* 7.9 Hz, NH), 6.19 (1 H, d, *J* 7.8 Hz, NH), 6.86 (1 H, s, $CHPh_2$), 7.18—7.41 (20 H, m, ArH), 7.46 and 8.17 (4 H, 2 d, *J* 8.5 Hz, ArH); *m/z* 698 (*M* — 91), 622 (*M* — 167), 167, and 91.

N-*Benzyloxycarbonyl*-1-(p-*nitrobenzyl*)-δ-(L-α-*aminoadipyl*)-S-*benzyl*-L-*cysteinyl*-D-*valine*, *Benzhydryl Ester* (3)—The protected dipeptide (8a) (0.59 g, 0.75 mmol) was dissolved in 0.2M HCl–nitromethane (19 cm³) and the solution stirred at room temperature for 5 h; it was then evaporated to give an oil which was subjected to column chromatography on silica (30 g), with EtOAc–n-hexane–HOAc (90 : 10 : 0.4) as eluant to afford the free acid (8b) (0.46 g, 98%) as a colourless foam.

The acid (8b) was added to a solution of 1-hydroxybenzotriazole hydrate (0.11 g, 0.83 mmol) and D-valine, benzhydryl ester [prepared from D-valine (97 mg, 0.83 mmol) as described [8]] in EtOAc (7.5 cm³). Dicyclohexylcarbodi-imide

(0.17 g) in EtOAc (5 $cm^3$) was added, the reaction mixture stirred overnight, treated with oxalic acid, and washed as described above. Evaporation of the organic fraction afforded crude (3) which was purified by column chromatography on silica (30 g), with EtOAc–n-hexane (6 : 4) as eluant, and crystallised from $CH_2Cl_2$–ether–n-hexane as colourless needles (0.47 g, 71%), m.p. 77—80 °C remelting at 133—135 °C; $[\alpha]_{436}^{20}$ −11.9° (*c* 1.0, acetone) (Found: C, 66.4; H, 5.8; N, 6.0. $C_{49}H_{52}N_4O_{10}S$ requires C, 66.20; H, 5.90; N, 6.30%); δ(300 MHz, $CDCl_3$) 0.75 (3 H, dd, *J* 6.9, 1.1 Hz, val 4-H), 0.87 (3 H, d, *J* 7.0 Hz, val 4-H), 1.68—1.85 (4 H, m, aminoadipyl 3, 4-H), 2.10—2.26 (3 H, m, aminoadipyl 5-H, val 3-H), 2.74 (2 H, AB of ABMX; $\delta_A$ 2.84, $\delta_B$ 2.68, *J* 14.0, 7.0, 6.1, 2.1, 1.8 Hz, cys 3-H), 3.72 (2 H, s, $SCH_2Ph$), 4.36—4.41 (1 H, m, aminoadipyl 2-H), 4.55—4.66 (2 H, m, cys 2-H, val 2-H), 5.09 (2 H, AB; $\delta_A$ 5.11, $\delta_B$ 5.06, *J* 11.9 Hz, $OCH_2Ar$), 5.20 (2 H, AB; $\delta_A$ 5.23, $\delta_B$ 5.17, *J* 13.7 Hz, $OCH_2$-Ar), 5.74 (1 H, dd, *J* 4.1, 7.8 Hz, NH), 6.50 (1 H, dd, *J* 7.3, 4.3 Hz, NH), 6.87 (1 H, s, $CHPh_2$), 6.95 (1 H, m, NH), 7.17—7.37br (20 H, m, ArH), 7.43 (2 H, m, ArH), and 8.16 (2 H, d, *J*, 8.9 Hz, ArH); *m/z* 797 (*M* − 91), 752, 721, 167, and 91.

N-*Benzyloxycarbonyl*-1-(p-*nitrobenzyl*)-δ-(L-α-*aminoadipyl*)-S-*benzyl*-L-*cysteinyl*-D-*valylglycine*, *Benzyl Ester* (5). The benzhydryl ester (3) (0.2 g, 0.23 mmol) was hydrolysed to the free acid (4) (0.16 g) as above and the product dissolved in dry THF (3 $cm^3$). Pyridine (20.0 μl, 0.25 mmol) was added, the solution cooled to 0 °C and a solution of ethyl chloroformate (23.8 μl, 0.25 mmol) in THF (2 $cm^3$) added in one portion. The solution was stirred for 15 min at 0 °C when a solution of glycine benzyl ester, toluene-*p*-sulphonic acid salt (83.6 mg) and pyridine (20.0 μl) in $CH_2Cl_2$ (3 $cm^3$) was added and the mixture allowed to warm to room temperature overnight. After evaporation of the solvent the residue was dissolved in EtOAc (15 $cm^3$), and the solution washed successively with aqueous HCl (1M; 10 $cm^3$), water (10 $cm^3$), and saturated aqueous NaCl (10 $cm^3$) and then dried, and evaporated. The residue was subjected to preparative t.l.c. on silica, with $CH_2Cl_2$–MeOH (19 : 1) as eluant, and the purified protected ACVG crystallised from $CH_2Cl_2$–EtOH–n-hexane as a microcrystalline solid (90 mg, 46%), m.p. 155—158 °C, $[\alpha]_D^{20}$ −7.4° [*c* 1.0, $CH_2Cl_2$–MeOH (1 : 1)] (Found: C, 61.85; H, 6.15; N, 7.8. $C_{45}H_{51}N_5O_{11}S$ requires C, 62.13; H, 5.91; N, 8.05%), δ[300 MHz, $CDCl_3$ : $CD_3OD$ (1 : 1)] 0.88 and 0.91 (6 H, 2 d, *J* 6.8 Hz, val 4-H), 1.66—1.85br (4 H, m, aminoadipyl 3, 4-H), 2.13—2.23br (3 H, m, aminoadipyl 5-H, val 3-H), 2.68 (2 H, AB of ABX; $\delta_A$ 2.74, $\delta_B$ 2.63, *J* 13.8, 7.4, 6.5 Hz, cys 3-H), 3.69 (2 H, s, $SCH_2Ph$), 4.24, 4.30, 4.46 (3 H, 3 m, 3 × 2-H), 5.06 (2 H, AB; $\delta_A$ 5.09, $\delta_B$ 5.03, *J* 12.2 Hz, $OCH_2Ar$), 5.11 (2 H, s, $OCH_2Ar$), 5.20 (2 H, AB; $\delta_A$ 5.23, $\delta_B$ 5.17, *J* 13.5 Hz, $OCH_2Ar$), 7.20—7.35 (15 H, m, ArH), and 7.45 and 8.15 (4 H, 2 d, *J* 8.6 Hz, ArH).

δ-(L-α-*Aminoadipyl*)-L-*cysteinyl*-D-*valine* (ACV; 1).—Typically the protected tripeptide (3) (100 mg, 0.12 mmol) was stirred in liquid $NH_3$ under $N_2$ and small pieces of freshly cut Na added until the blue colour persisted for 5 min. Solid $NH_4OAc$ (*ca.* 100 mg) was added, the $NH_3$ evaporated under a stream of dry $N_2$, and the residue desiccated over $H_2SO_4$. The product was isolated as follows.

*Procedure A*. The residue was dissolved in 5% (v/v) aqueous HOAc (5 $cm^3$), the solution filtered and a 10% (w/v) solution of $Hg(OAc)_2$ in 5% (v/v) aqueous HOAc added slowly to the filtrate to afford a precipitate of the tripeptide sulphide. The precipitate was separated by centrifugation and washed sequentially with degassed water (5 $cm^3$ × 4), MeOH (5 $cm^3$ × 2), and ether (5 $cm^3$). The dried residue was resuspended in degassed water (2 $cm^3$) and a stream of $H_2S$ passed through the suspension for 15 min. After separation by centrifugation the pellet of HgS was washed with water (1 $cm^3$) and the combined supernatant liquid and washings filtered through Celite. The filtrate was degassed *in vacuo* and lyophilized to afford a mixture of (1) and the corresponding disulphide (9) as a colourless powder (31.5 mg, 77%) (Found: C, 44.75; H, 6.85; N, 11.1; ash, 4%. $C_{14}H_{25}N_3O_6S$ requires C, 46.30; H, 6.87; N, 11.57%). Free thiol 75%, *m/z* (FABS) 364 [monomer (*M* + 1)].

*Procedure B*. The residue was dissolved in 5% (v/v) aqueous HOAc (5 $cm^3$), extracted with ether (2 $cm^3$ × 2), and the aqueous layer lyophilized. The residue was dissolved in water (5 $cm^3$), the pH adjusted to 8, and the solution aerated for 2 h. The solution was freeze dried, the residue dissolved in water (100 μl) and subjected to ion exchange chromatography on Biorad AG 50 × 2 resin (200—400 mesh, $H^+$, 18 × 1.5 cm) with a water–1M-pyridine gradient as eluant. Lyophilization of the ninhydrin positive fractions gave ACV disulphide as a colourless powder (39 mg, 96%), m.p. 200—203 °C (decomp.), $[\alpha]_D^{20}$ −9.5° (*c* 2.0, 2M-HCl) (lit.,[3] $[\alpha]_D$ −9.5°; lit.,[5] $[\alpha]_D$ −11.0°) (Found: C, 46.2; H, 6.35; N, 11.0. $C_{28}H_{48}N_6O_{12}S_2$ requires C, 46.40; H, 6.67; N, 11.59%), δ(300 MHz, $D_2O$) 0.27 (3 H, d, *J*, 6.9 Hz, val 4-H), 0.32 (3 H, d, *J*, 8.5 Hz, val 4-H), 1.06—1.27br (4 H, m, aminoadipyl 3,4-H), 1.54br (1 H, m, val 3-H), 1.76br (2 H, m, aminoadipyl 5-H), 2.40 (2 H, AB, cys 3-H), 3.18 (1 H, m, aminoadipyl 2-H), and 3.58 (1 H, m, val 2-H); $\delta_C$ (75 MHz, $D_2O$) 17.14 and 18.52 (val 4-C), 20.70 (aminoadipyl 4-C), 29.57 and 30.07 (val and aminoadipyl 3-C), 34.56 (aminoadipyl 5-C), 38.49 (cys 3-C), 52.43, 59.51, and 59.77 (3 × 2-C); *m/z* (FAB) 725 (*M* + 1) and 364.

δ-(L-α-*Aminoadipyl*)-L-*cysteinyl*-D-*valylglycine* (*ACVG*; 2).—Deprotection of the tetrapeptide derivative (5) (54 mg, 0.06 mmol) was carried out in a manner identical with that of (3) above. Isolation using procedure B afforded ACVG as its dimer (10), a colourless powder (19.4 mg, 74%), m.p. 230—235 °C (decomp.) (Found: C, 46.05; H, 6.2; N, 12.3. $C_{32}H_{54}N_8O_{14}S_2$ requires C, 45.8; H, 6.5; N, 13.35%), δ(300 MHz, $D_2O$) 0.25 and 0.28 (6 H, 2 d, *J* 7.5 Hz, val 4-H), 1.10br (4 H, m, aminoadipyl 3,4-H), 1.48br (1 H, m, val 3-H), 1.63—1.72 (2 H, m, aminoadipyl 5-H), 2.40 (2 H, AB of ABX; $\delta_A$ 2.48, $\delta_B$ 2.33, *J* 13.9, 7.4, 5.8 Hz, cys 3-H), 3.07 (1 H, t, *J* 5.6 Hz, aminoadipyl 2-H), 3.20 (2 H, s, gly 2-H), and 3.54 (1 H, m, val 2-H); *m/z* (FAB) 839 (*M* + 1) and 421.

Quantitative reduction of (10) to the monomer (2) was carried out by brief treatment with 5% (v/v) aqueous ethanethiol. The freeze-dried residue was homogeneous by electrophoresis and t.l.c. Reduction of (9) was carried out in a similar manner.

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