259. Synthesis of L-α-Aminoadipyl-L-serinyl[1])-D-valine, a Naturally Occurring Tripeptide from the fermentation of *Penicillium chrysogenum*

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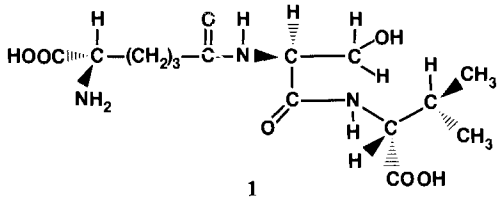
Summary

A new tripeptide, L-α-aminoadipyl-L-seryl-D-valine has been synthesized by coupling the protected L-seryl-D-valine dipeptide with an appropriately protected L-α-aminoadipic acid ester. The free tripeptide was obtained after treatment with liquid HF and purification by HPLC.

We have recently reported the isolation of a new tripeptide, L-α-aminoadipyl-L-seryl-D-valine (**1**), which was found to be present in small amounts in the fermentation broth of *Penicillium chrysogenum* [1]. As larger amounts of the compound were needed for cell-free [2] biosynthetic experiments, the tripeptide was synthesized as



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follows. D-Valine benzyl ester as *p*-toluene sulfonate (**2**) was coupled with BOC-(*O*-benzyl)-L-serine by the DCC method [3] to yield BOC-(*O*-benzyl)-L-seryl-D-valine benzyl ester (**3**). The protected dipeptide (**3**) was partially deblocked to *O*-benzyl-L-seryl-D-valine benzyl ester hydrochloride (**9**). In a second stage, an appropriately protected adipic acid derivative, the α-benzyl-*N*-carbobenzoxy-L-α-aminoadipate (**8**)

[1]) The conventional name for a serine residue is *seryl*. However, since *serinyl* was used in the previous paper, the term *serinyl* is retained in the title of this paper.

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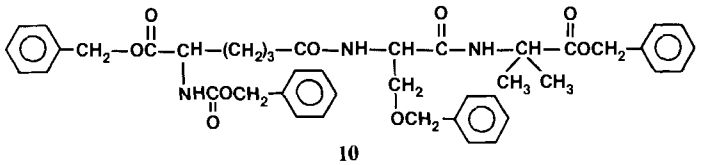
was prepared. The dipeptide salt (**9**) and the α-ester (**8**) were coupled by the DCC method to give δ-[α-benzyl, N^{α}-carbobenzoxy-L-aminoadipyl-]-L-seryl-D-valine benzyl ester (**10**). The FD./MS. and EI./MS. of this fully protected tripeptide are shown in *Figures 1a* and *1b*. The ^{1}H-NMR. spectrum was also in agreement with the structure of this compound and is shown in *Figure 2*.



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In the final stage of the synthesis, the protected tripeptide was treated with liquid HF and yielded impure tripeptide which was purified by HPLC. The pure tripeptide had ^{1}H-NMR. and FD./mass spectra identical to those of the naturally occurring L-α-aminoadipyl-L-seryl-D-valine [1] (**1**).

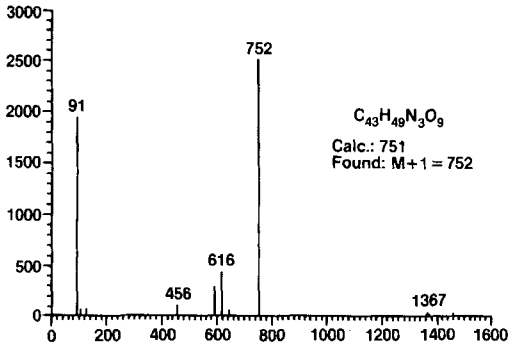

Fig. 1a. *FD.-Mass spectrum of the fully protected tripeptide* **10**

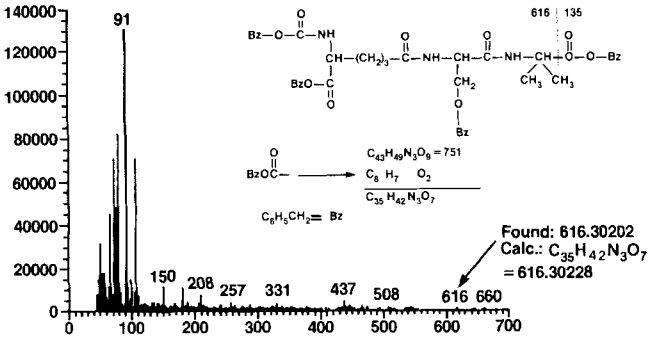

Fig. 1b. *EI.-Mass spectrum of the fully protected tripeptide* **10**

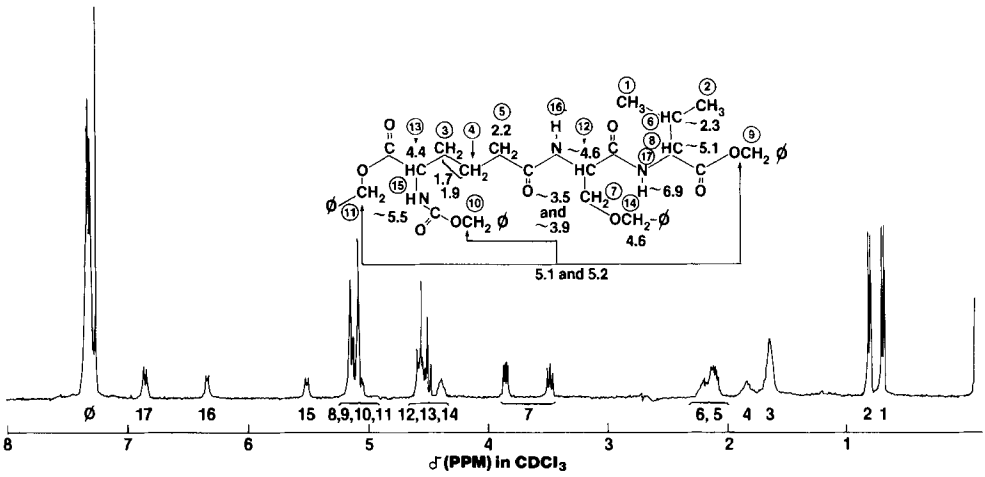

Fig. 2. *^{1}H-NMR. spectrum of the fully protected tripeptide* **10**

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Experimental Part

General Remarks. NMR. spectra were recorded using a *BRUKER* WH 360 NMR. Spectrometer. *Optical rotations* were recorded using a *Perkin Elmer* Model 241 Polarimeter. *Mass spectra* were recorded using *Varian-MAT* Model 731 Mass Spectrometer. *HPCL chromatographies* were performed using a *Waters* M6000 A Pump, V6K Septumless injector (*Waters Assoc.*, Milford, Mass.) with a *Shoeffel* Model 770 UV Detector (*Schoeffel Inst.* Westwood, N.J.) and *Fisher* Ominscribe Recorder (*Fisher Scientific,* Cincinnati, Ohio) and *Waters* Refractive Index Detector. Only spectrally pure solvents and deionized water were used in HPLC. Abbreviations: i.V. = in vacuum, RT. = room temperature.

D-*Valine benzyl ester (as* p-*toluenesulfonate)* (**2**) was prepared by the azeotropic distillation method [5] [6] in CCl_4. The reflux condensate was dried by passage through a bed of *Drierite.* The product was crystallized from ethanol by additon of ether. M.p. 159–161°; $[\alpha]_D^{25} = +7.6°$ (c = 1, $CHCl_3$).

$C_{19}H_{25}NO_5S$	Calc.	C 60.14	H 6.64	N 3.69	S 8.45%
(379.475)	Found	„ 60.08	„ 6.74	„ 4.00	„ 8.29%

*BOC-(*O*-benzyl)-*L*-seryl-*D*-valine benzyl ester* (**3**) was synthesized from BOC-(*O*-benzyl)-L-serine and (**2**) by the DCC method [3] [4] in the presence of one equivalent of diisopropylethylamine in methylene chloride (50 mmol in 200 ml). The product was crystallized from ethyl acetate/hexane and recrystallized from aqueous ethyl alcohol. Yield, 20.6 g (85%), m.p. 96–97°; $[\alpha]_D^{25} = +6°$ (c = 1, CF_3CH_2OH). The compound was shown to be pure by TLC. in toluene/acetic acid 9:1, Rf 0,27 and in chloroform/methanol/acetic acid 135:15:1, Rf 0.67.

$C_{27}H_{36}N_2O_6$ (484.593) Calc. C 66.92 H 7.49 N 5.78% Found C 66.03 H 6.22 N 5.34%

N-*Carbobenzoxy-*L*-α-aminoadipic acid* (**4**) was synthesized from a commercial sample of L-α-aminoadipic acid and carbobenzoxy chloride in dioxane/water and sodium hydroxide at 0° in the usual

manner. The product was crystallized from ethyl acetate and hexane. M.p. 132–135°; $[\alpha]_D^{25} = -11.4°$ (c = 1, dimethylformamide).

$C_{14}H_{17}N_1O_6$ (295.291) Calc. C 56.95 H 5.80 N 4.74% Found C 56.79 H 6.04 N 4.51%

N-*Carbobenzoxy*-L-*α*-*aminoadipic acid bis-(*p-*nitrobenzyl) ester* (**5**) was prepared by treating **4** (0.89 g, 3 mmol) with *p*-nitrobenzyl bromide (1.43 g, 6.6 mmol) and excess dicyclohexylamine (1.8 ml) in 30 ml of DMF at RT. After *ca.* 3 h, the reaction mixture was diluted to 120 ml with ethyl acetate and filtered. The filtrate was washed three times with 5% solution of citric acid, twice with water, thrice with 2N $KHCO_3$, and twice again with water. The organic phase was dried with anhydrous $MgSO_4$, filtered, and evaporated i.V. The residual syrup was crystallized from ethyl acetate/hexane. Yield, 1.2 g (70%) M.p. 69–71°; $[\alpha]_D^{25} = -9.90$ (c = 1, CF_3CH_2OH). The product gave a single spot on TCL. in chloroform/methanol/acetic acid 135:15:1, Rf 0.71.

$C_{28}H_{27}O_{10}$ (565.535) Calc. C 59.47 H 4.81 N 7.43% Found C 59.60 H 5.06 N 7.47%

*δ-(*p-*Nitrobenzyl)* N-*carbobenzoxy*-L-*α*-*aminoadipate* (**6**): Partial saponification [7] of **5** was performed on 1.10 g (1.95 mmol) in 25 ml acetone and 5 ml water. A solution of 10 ml of 0.5N LiOH in acetone/water 1:1 was added dropwise with stirring over *ca.* 30 min, followed by an equal period of standing. The mixture was then diluted with water and washed twice with ethyl acetate. The aqueous phase emulsified and was separated by addition of solid KCl. After acidification to pH < 2 with 6N HCl, the product was extracted into ethyl acetate. The organic phase was washed three times with water, dried over $MgSO_4$, filtered, and evaporated i.V. The residual syrup contained three components as determined by TLC. Isolation of pure product by preparative reverse-phase HPLC. [8] afforded seed cystals. Subsequent syntheses did not require HPLC. purification. The entire syrupy product from above was dissolved in a little acetonitrile, and added to a *ca.* 10 volumes of ammonium formate (*ca.* 1M) at pH 7–8. This was about 10 ml in volume and was applied from a sample injection loop to a glass column (*ca.* 950 ml overall volume, 59 cm long) packed with C_{18} LP-1 [8] equilibrated with 20% acetonitrile, 0.08M ammonium formate/formic acid, pH 4.25. The sample was eluted with a linear gradient (4000 ml total) from 20% to 60% acetonitrile, 0.08M to 0.05M ammonium formate/formic acid, pH 4.25. The major product eluted between *ca.* 50 to 53% acetonitrile, from 3635 ml to 3883 ml. The fractions located in this region were pooled and evaporated to a small volume i.V. An oil separated on cooling; it was dissolved in a small volume of warm acetonitrile and ammonium formate (pH 7). A solid separated on cooling and was filtered, washed with water, and dried; yield, 0.3 g. The synthesis was subsequently carried out on 4.0 g of **5** as above, but the crude syrup was crystallized directly from acetonitrile/aqueous buffer and recrystallized from aqueous methanol. Yield, 1.32 g (42%), m.p. 128–129° $[\alpha]_D^{25} + 0.8°$ (c = 1, CF_3CH_2OH). The product was homogeneous by TLC. in chloroform/methanol/acetic acid 135:15:1, Rf 0.47.

$C_{21}H_{22}N_2O_8$ (430.414) Calc. C 58.60 H 5.15 N 6.51% Found C 58.59 H 5.06 N 7.69%

*α-Benzyl δ-(*p-*nitrobenzyl)* N-*carbobenzoxy*-L-*α*-*aminoadipate* (7) was prepared by treatment of **6** (1.56 g, 3.5 mmol) in 40 ml ethyl acetate/DMF 1:2 with 0.7 ml (3.5 mmol) dicyclohexylamine and 0.42 ml (3.5 mmol) benzyl bromide at RT. The reaction was allowed to proceed for four days although it may well have been complete sooner. Finally, it was diluted with ethyl acetate to 100 ml, filtered to remove crystallized salts, and the filtrate washed successively with several portions each of 1N HCl, water, 2N $KHCO_3$, and water. The organic phase was dried over $MgSO_4$, filtered, and evaporated i.V. The syrupy residue was crystallized from ethyl acetate/hexane. Yield, 1.44 g (79%). M.p. 68.5–70.5°; $[\alpha]_D^{25} = -13.4°$ (c = 1, CF_3CH_2OH). The product was homogeneous on TCL., and behaved indistinguishably from **5**.

$C_{28}H_{28}N_2O_8$ (520.538) Calc. C 64.61 H 5.42 N 5.38% Found C 64.79 H 5.35 N 5.08%

α-Benzyl N-*carbobenzoxy*-L-*α*-*aminoadipate* ***(8)***. Diester **7** (1.0 g, 1.9 mmol) was cleaved with 4 g Zn in 50 ml 85% aqueous solution of acetic acid at RT. for 4 h. The reaction mixture was diluted with 1N HCl, filtered and evaporated. The residue was distilled azeotropically with toluene to remove more of the acetic acid, then dissolved in a mixture of water and ethyl acetate. After addition of 6N HCl to pH < 1, the organic phase was separated and washed twice with 1N HCl and three times with water. It was dried over $MgSO_4$, filtered, and evaporated i.V. The product was crystallized twice from

ethyl acetate by addition of hexane. Yield, 0.6 g (80%), m.p. 88.5–90.5°; $[\alpha]_D^{25} = -19.4°$ (c = 1, CF_3CH_2OH).

$C_{21}H_{23}NO_6$ (385.416) Calc. C 65.44 H 6.02 N 3.63% Found C 65.22 H 6.23 N 2.96%

O-Benzyl-L-seryl-D-valine benzyl ester hydrochloride (9). The protected dipeptide **3** (7.0 g, 14.4 mmol) was treated with HCl in acetic acid for 10 min at RT. The reaction mixture was concentrated i.V., and the product was crystallized from methanol/ether. Yield, 5.87 g (96%), m.p. 178–180° (softened at 172°). This material was used without further purification.

δ-[α-Benzyl, N$^{\alpha}$-carbobenzoxy-L-α-aminoadipyl]-O-benzyl-L-seryl-D-valine benzyl ester (**10**). Dipeptide hydrochloride (**9**) (1.1 g, 2.6 mmol) and α-ester **8** (1.0 g, 2.6 mmol) were coupled by 0.6 g dicyclohexylcarbodiimide in 50 ml DMF and 0.46 ml dicyclohexylamine. The reaction mixture was stirred overnight at RT., then cooled to 0° and filtered. The filtrate and ethyl acetate washes were evaporated i.V., and the residue was dissolved in ethyl acetate and filtered. The organic solution was worked up as for compound **7**, above, and recrystallized from 95% ethanol until homogeneous by TLC. (chloroform/methanol/acetic acid 135:15:1). Yield, 0.33 g (17%), m.p. 139–142°, $[\alpha]_D^{25} = -4.4°$ (c = 1, DMF). Additional pure product was recovered by extensive recrystallization of the residues from the mother liquors of the first crop, yield 733 mg (37%), m.p. 137–141°. ^{1}H-NMR.: see *Figure 2*.

$C_{43}H_{49}N_3O_9$ (751.877) Calc. C 68.69 H 6.57 N 5.59% Found C 68.89 H 6.57 N 5.51%

δ-[L-α-aminoadipyl]-L-seryl-D-valine (**1**). The protected tripeptide **10** was treated with liquid HF at 0° in the presence of 3% anisole. After 2 h, the HF was distilled under vacuum. The residue was treated with ether to precipitate the peptide, which was filtered, washed with ether, and air-dried briefly. The residual solid was then dissolved in 60 ml 1M CH_3COOH and lyophilized. The lyophilized peptide was purified by reverse phase HPLC. on a C_{18} column *(Microbondapak)* with formic acid/methanol/water 0.5:0.5:99. A yield of 73 mg was obtained, which was characterized by high resolution mass spectrometry and by amino acid analysis. Amino acid analysis showed the solids to be 91.6% peptide by weight. Based on this composition, $[\alpha]_D^{25} = -16.4°$ (c = 0.5, 1M CH_3COOH). Amino acid analysis found (ratios to average of αAA + Val): $\alpha AA_{1.02}$ $Ser_{0.83}$ $Val_{0.98}$. The ^{1}H-NMR. and mass spectra were identical with those of the naturally occurring peptide.

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