AN EFFICIENT SYNTHESIS OF δ-(L-α-AMINOADIPYL)-L-SERYL--D-VALINE (LLD ASV), A NATURALLY OCCURING TRIPEPTIDE FROM THE FERMENTATION OF *Penicillium chrysogenum**

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Dedicated to the memory of Dr Karel Bláha.

The synthesis of δ-(L-α-aminoadipyl)-L-seryl-D-valine (LLD ASV), a naturally occuring congener of the well known tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (LLD ACV) which is the linear precursor of isopenicillin N, penicillin N cephalosporin C is described. An efficient method for producing the requisite α-monobenzyl ester of N-benzyloxycarbonyl-L-homoglutamic acid for subsequent condensation at the side chain δ-carboxy group is presented.

There is a constant demand for preparative methods for δ-(L-α-aminoadipyl)-L--cysteinyl-D-valine (LLD ACV) the well known linear precursor of isopenicillin N, penicillin N and cephalosporin C (refs[1-5]). The same accounts for various congeners of this tripeptide.

One of such compounds is the δ-(L-α-aminoadipyl)-L-seryl-D-valine (LLD ASV) which was recently isolated from the fermentation broth of *Penicillium chrysogenum* by Neuss et al.[6]. Since larger quantities of the compound were needed for cell-free biosynthetic experiments the tripeptide was synthetized in the Eli Lilly laboratory by the following route[7]:

$$\text{H-L-Aad-OH} \xrightarrow{} \underset{I}{\text{Z-L-Aad-OH}} \xrightarrow{} \underset{II}{\text{Z-L-Aad(ONb)-ONb}} \xrightarrow{} \underset{III}{}$$

$$\xrightarrow{} \underset{IV}{\text{Z-L-Aad(ONb)-OH}} \xrightarrow{} \underset{V}{\text{Z-L-Aad(ONb)-OBzl}} \xrightarrow{} \underset{VI}{\text{Z-L-Aad-OBzl}}\,.$$

Thus, the [N, C^{α}]-diprotected intermediate *VI* for subsequent condensation at the δ-carboxy group was prepared in a roundabout way[7]. The overall yield in this multistep, very ineffective procedure starting from the commercially available but

* Symbols and abbreviations are in accordance with the recommendations of IUPAC-IUB Commision on Biochemical Nomenclature. In addition the following abbreviation is used: Aad, α-aminoadipic acid.

rather expensive L-α-aminoadipic acid ($ 52 per gram, Sigma 1987 Price List) was under 19·5% (yield of one synthetic step not disclosed)[7]. The α-monobenzyl ester *VI* was then coupled by dicyclohexylcarbodiimide with benzyl ester of O-benzyl-L--seryl-D-valine. Final deprotection was achieved in liquid hydrogen fluoride[7].

We have repeated the synthesis of the ASV tripeptide according to the strategy of the Eli Lilly group[7]. The intermediate α-monobenzyl ester Z-L-Aad-OBzl *VI* was, however, prepared in two steps from Z-L-Aad(NHZ)-OH(*VII*) (ref.[8]). Compound *VII* according to our recently published procedure is produced by the permanganate oxidation of Z-L-Lys(Z)-OH (*VIII*). (ref.[8]). Thus, the readily available and inexpensive L-lysine is the starting material for peptide synthesis involving homoglutamic acid and homoglutamine[9]. In the first step the versatile compound *VII* was transformed into the benzyl ester Z-L-Aad(NHZ)-OBzl (*IX*) by the Williamson ester synthesis with benzyl bromide[9]. In the second stage the fully protected compound *IX* was partially deprotected by the acid-catalysed hydrolysis to the δ-carboxy function, thus affording the α-monobenzyl ester Z-L-Aad-OBzl (*VI*) in two steps with 60% overall yield. The protected ASV Z-L-Aad[L-Ser(Bzl)-D-Val-OBzl]-OBzl was reached in the next stage by the 1 + 2 coupling with dicyclohexylcarbodiimide as described by Shields et al.[7]. Final deprotection was, however, achieved by the action of sodium in liquid ammonia instead of the liquid hydrogen fluoride treatment. The overall yield of our synthesis of the LLD ASV tripeptide compares favourably with the global yield achieved in the previous synthesis by the Eli Lilly group[7].

EXPERIMENTAL

Capillary melting points were determined and are reported without correction. Optical rotations were measured using a Hilger and Watts polarimeter. ^{1}H NMR spectra were recorded on a Tesla BS 487C (80 MHz) instrument, chemical shifts were determined by using the signal hexamethyldisiloxane as standard with the value of 0·055 ppm assigned to it. Infrared spectra were recorded on a Specord 71 IR instrument (Zeiss, Jena).

For thin-layer chromatography precoated "TLC aluminium sheets silica gel 60, thickness 0·2 mm" (Merck, Darmstadt) were used. The following mixtures (v/v) of solvents were used as eluents for the TLC: A (benzene–acetone 2 : 1), B (benzene–acetone 3 : 1), C (benzene–acetone–acetic acid 2 : 1 : 0·05), D (isopropanol–water 7 : 3), E (1-butanol–acetic acid–water 4 : 1 : 4). Preparative chromatography separations were performed on columns packed with silica gel (MN-Kieselgel 60, minus 0·08 mm, Macherey, Nagel).

Z-L-Aad(NHZ)-OH (*VII*)

The synthesis of this compound from Z-L-Lys(Z)-OH was described in our previous paper[8].

Z-L-Aad(NHZ)-OBzl (*IX*)

The synthesis of this compound was described in our previous paper[9].

Z-L-Aad-OBzl (*VI*)

A solution of Z-L-Aad(NHZ)-OBzl (*IX*) (5·19 g, 10 mmol) in acetone (25 ml) was treated portionwise with 2M-HCl (100 ml) and left at room temperature with occasional shaking and TLC monitoring in the solvent A. The solvents were removed in vacuo and the residue was purified by silica gel column chromatography using the solvent B for elution. Yield 2·73 g (71%). TLC (A) R_F 0·70, (C) R_F 0·58; m.p. 90–92°C (ethyl acetate–diisopropyl ether–hexane), $[\alpha]_D^{20}$ −13·8° (*c* 2, acetone), ref.[7] m.p. 88·5–90·5°C, $[\alpha]_D^{20}$ −19·4° (*c* 1, CF_3CH_2OH); ref.[10] m.p. 90–92°C, $[\alpha]_D^{20}$ −13·3° (*c* 2, acetone); ref.[11] m.p. 87–89°C, $[\alpha]_D^{20}$ −13·3° (*c* 2, acetone). For $C_{21}H_{23}NO_6$ (385·4) calculated: 65·44% C, 6·02% H, 3·63% N; found: 65·19% C, 6·17% H, 3·88% N. Satisfactory IR and ^{1}H NMR spectra were obtained.

L-Aad(L-Ser-D-Val-OH)-OH (LLD ASV)

Ammonia (200 ml) was distilled onto Z-L-Aad(L-Ser(Bzl)-D-Val-OBzl)-OBzl (1·1 g, 2·6 mmol), prepared according to Shields et al.[7]. Sodium metal was added in small lumps until a permanent blue colour developed in the solution. Acetic acid was added until the blue colour faded and ammonia was evaporated. The residue was dissolved in 20% acetic acid (10 ml) and the solvents evaporated. The residual solid dissolved in the solvent D was subjected to Sephadex G-25 column chromatography using the same solvent system for elution. Yield of lyophilizate 0·312 g (90%). TLC (E) R_F 0·20, $[\alpha]_D^{20}$ −16·0° (*c* 1, 1M-acetic acid). For $C_{14}H_{25}N_3O_7$ (347·4) calculated: 48·40% C, 7·25% H, 12·09% N; found: 48·32% C, 7·10% H, 12·21% N. IR (Nujol), cm^{-1}: 3 280, 3 100–2 500, 1 700, 1 660, 1 580, 1 550, 1 530. ^{1}H NMR (D_2O + CF_3COOH): 1·30 d, 3 H (CH_3^{Val}); 1·38 d, 3 H (CH_3^{Val}); 2·30 m, 4 H ($CH_2CH_2CH^{Aad}$); 2·60 m, 1 H ($NHCHCH^{Val}$); 2·78 t, 2 H ($COCH_2^{Aad}$); 4·40 m, 2 H (CH_2^{Ser}); 4·25–4·60 m, m, m, 3 H (3 × $NHCH^{Aad,Ser,Val}$). The tripeptide gave satisfactory amino acid analysis.

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