EFFICIENT ROUTES TO THE ARNSTEIN'S TRIPEPTIDE δ-(L-α-AMINOADIPYL)-L-CYSTEINYL-D-VALINE (LLD ACV) FROM THE OXIDATION PRODUCTS OF L-LYSINE DERIVATIVES*

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Received February 2nd, 1988
Accepted March 1st, 1988

Dedicated to the memory of Dr Karel Bláha.

Two efficient syntheses of the linear tripeptide precursor of penam and cephem antibiotics are presented. The routes are characterised by the use of L-lysine derivatives, Z-L-Lys(Z)-OH and H-L-Lys(Z)-OH as starting materials. By the permanganate oxidation the protected side chain amino grouping in L-lysine derivatives is transformed into the benzyloxycarbonylcarbamoyl substituent with formation of Z-L-Aad(NHZ)-OH and H-L-Aad(NHZ)-OH compounds. Both oxidation products are easily transformed into [N, C^{α}]-diprotected derivatives. Subsequent condensation at the δ-carboxy group afforded protected LLD ACV tripeptide.

All organisms that produce penicillin and cephalosporin synthesize and utilize the common precursor δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine, LLD ACV (refs[1-5]). This tripeptide is an acyclic precursor of isopenicillin N, penicillin N and cephalosporin C (refs[1-5]). Studies on the biosynthesis of β-lactam antibiotics from the ACV tripeptide led to isolation of four enzymes which are associated with the transformation of this tripeptide into the desacetylcephalosporin C (refs[2-5]).

There is a constant demand for the linear LLD ACV tripeptide for studies on the enzymatic approach to the synthesis of unnatural β-lactam antibiotics with cell free extracts, isolated enzymes or even cloned and recombinant enzymes[2-5].

A number of syntheses of the ACV tripeptide precursor have been reported[6-12]. The majority of these routes are characterised by low overall yields of the incorporation of the δ-Aad residue. This accounts not only for the syntheses commencing with the Rudinger's approach involving the homologation of glutamic acid[6-8] or the total malonic ester syntheses of racemic homoglutamic acid followed by its resolution

* Symbols and abbreviations are in accordance with the recommendations of IUPAC-IUB Commision on Biochemical Nomenclature. In addition the following abbreviations are used: Aad, α-aminoadipic acid; DCHA, dicyclohexylamine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; FD-MS, field desorption mass spectrometry; NMM, N-methylmorpholine.

into enantiomers[8-9] but also for the syntheses starting from the commercially available L-α-aminoadipic acid[10-12].

Many novel synthetic routes for producing L-α-aminoadipic acid have been published recently[13-23]. In spite of all these new syntheses the price of L-enantiomer of this uncoded monoaminodicarboxylic acid remains fairly expensive ($ 52 per gram, Sigma 1987 Price List). Some people found the published routes to indirect and unsuitable for large scale preparation[23]; Sklavounos made even enantiomeric α-aminoadipic acid in large quantities by hydrolysis of cephalosporin C (ref.[24]).

Our first ACV tripeptide synthesis started with Boc-L-Aad(ONSu)-OBu^t. The synthesis of this compound from the permanganate oxidation product H-L-Aad-(NHZ)-OH was already reported as one of the model routes to the starting materials for subsequent formation of the δ-peptide bond[18]. This first synthetic approach is characterised by hydroxysuccinimide active ester couplings with carboxyl unprotected amino components. The final two stage deprotection involved acidolytic cleavage with hydrogen chloride in dioxane followed by reduction with sodium in liquid ammonia. The tripeptide was isolated as the disulfide. The overall yield in this approach is comparable to the global yields of the two most efficient syntheses of the ACV tripeptide by Baldwin[11] and Thomson[12].

Our second synthetic approach to the ACV tripeptide reduces considerably the number of synthetic steps and is characterised by the use of benzyl type protection. As in the ASV tripeptide synthesis[25] the easily prepared, in two steps from the oxidation product Z-L-Aad(NHZ)-OH, α-monobenzyl ester Z-L-Aad-OBzl was used for the incorporation of the Aad residue by the delta peptide linkage. Coupling with amino dipeptide component H-L-Cys(Bzl)-D-Val-OBzl was achieved by soluble carbodiimide. The final one-stage deprotection of the all benzyl type protecting groups was achieved by the action of sodium in liquid ammonia. The global yield of the disulfide form in this ACV tripeptide synthesis compares favourably with the yields achieved in all ACV syntheses published until now.

Methods for [N, C$^\alpha$]-diprotection of L-α-aminoadipic acid are very inefficient[23,26]. Our approach based on the permanganate oxidation of protected L-lysine derivatives[17] avoids the intermediacy of L-α-aminoadipic acid and is characterised by the ease of subsequent formation of [N, C$^\alpha$]-diprotected derivatives suitable for condensation at the δ-carboxy group[18,26]. The presented routes contribute effectively to the removal of a bottleneck which has heretofore made access to the ACV tripeptide a rather tedious and troublesome process. The only other procedure which avoids also the intermediacy of L-α-aminoadipic acid for [N, C$^\alpha$]-diprotection and subsequent condensation at the δ-carboxy group is that of Baldwin[11,23].

To shed more light on the problem of [N, C$^\alpha$]-diprotection of L-α-aminoadipic acid the recent work of Olsen should be mentioned[27]. He applied the Rudinger's approach[28] with formaldehyde and through the oxazolidone intermediate carried out condensations at the δ-carboxy group[27].

spot of Z-L-Aad-OBzl disappeared on the TLC(A, R_F 0·58) Ethyl acetate was added and the organic phase was washed with 1M-HCl, brine, 5% $NaHCO_3$ solution, water, dried and rotary evaporated. The residue crystallized on trituration with ether–hexane. Yield 2·18 g (86%) and 1·90 g (75%) after recrystallization from ethanol, m.p. 114—115°C, TLC(A) R_F 0·79. $[\alpha]_D^{20}$ —17·9° (*c* 1, acetone). Ref.[11] m.p. 114—115°C; $[\alpha]_D^{20}$ —18·2° (*c* 1, acetone). For $C_{43}H_{49}N_3O_8S$ (767·95) calculated: 67·25% C, 6·43% H, 5·47% N; found: 67·32% C, 6·47% H, 5·41% N. IR spectrum (Nujol), cm^{-1}: 3 220, 1 730, 1 685, 1 650; ^1H NMR ($CDCl_3$): 0·83 d, 3 H (CH_3^{Val}); 0·89 d, 3 H (CH_3^{Val}); 1·73 m, 4 H ($CH_2CH_2CH^{Aad}$); 1·88 m, 1 H ($NHCHCH^{Val}$); 2·13 t 2 H (CH_2CO^{Aad}); 2·76 m, 2 H (CH_2^{Cys}); 3·75 s, 2 H (SCH_2Ar); 4·35—4·62 m, m, m, 3 H ($NHCH^{Aad\ Cys\ Val}$); 5·12—5·16 s, s, s, 6 H (3 × OCH_2Ar); 5·65 m, 6·32 m, 6·80 m, 3 H (3 × × NH); 7·12—7·40 s, s, s, s, 20 H, (4 × C_6H_5).

H-L-Aad(-L-Cys-D-Val-OH)-OH (LLD ACV)

Z-L-Aad(-L-Cys(Bzl)-D-Val-OBzl)-OBzl (1·54 g, 2·0 mmol) was dissolved in liquid ammonia. Sodium metal was added until a permanent blue colour developed in solution. Acetic acid was added until the blue colour faded and ammonia was evaporated. The residue was dried in a vacuum desiccator over P_2O_5 and dissolved in 5% acetic acid. The solution was washed with ether, its pH adjusted to 8 by addition of ammonia and aerated for 3 h. After freeze-drying the lyophilizate was subjected to ion-exchange chromatography on a column packed with Dowex 50Wx8 (200—400 mesh, H^+ cycle) with water — 1M-aqueous pyridine gradient as eluant. Ninhydrine positive fractions were collected and lyophilized affording the ACV tripeptide as disulfide in form of a colourless powder. Yield 0·65 g (90%); TLC(B) R_F 0·18, m.p. 199—202°C (dec); $[\alpha]_D^{20}$ —9·6° (*c* 2, 2M-HCl), (ref.[12] m.p. 200—203°C (dec); $[\alpha]_D^{20}$ —9·5° (*c* 2, 2M-HCl)). FD-MS: *m/z* 725 (M + 1). For $C_{28}H_{48}N_6O_{12}S_2$ (724·9) calculated: 46·40% C, 6·67% H, 11·59% N; found: 45·99% C, 6·72% H, 11·50% N. IR spectrum (Nujol), cm^{-1}: 3 100—2 600, 1 710, 1 665, 1 550, 1 530; ^1H NMR (D_2O): 0·82 d, 0·88 d, 6 H (2 × CH_3^{Val}); 1·79 m, 4 H ($CH_2CH_3CH^{Aad}$); 2·04 m, 1 H ($NHCHCH^{Val}$); 2·37 t, 2 H (CH_2CO^{Aad}); 2·92 m, 2 H (CH_2^{Cys}); 3·69—4·04 m, m, m, 3 H (3 × $NHCH^{Aad,Cys,Val}$).

This work was supported by the Polish Academy of Sciences Grant CPBP-01.13.2.9. We thank Dr N. Leo Benoiton (University of Ottawa) for a generous gift of soluble carbodiimide and di-tert-butyldicarbonate.

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