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TETRAHEDRON

Synthesis of δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-allothreonine a Substrate for Isopenicillin-N Synthase and its O-Methyl-D-threonine Epimer

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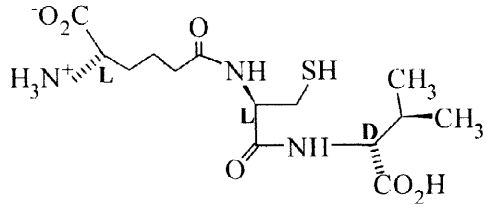
Abstract:
The paper describes the synthesis of two epimeric tripeptides δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-threonine (13) and δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-allothreonine (14), modified substrates for the isopenicillin-N synthase enzyme. The D-allothreonine tripeptide (14) has been shown to be an excellent substrate for the enzyme whereas the D-threonine epimer did not react at all. The compound formed by the enzyme with the D-allothreonine tripeptide is a new 2-α-methoxypenicillin.

INTRODUCTION

Isopenicillin-N synthase (IPNS) is an enzyme which catalyses the formation of the penicillin nucleus, the bicyclic β-lactam-thiazolidine unit. This enzyme has been found in *Penicillium Chrysogenum*, in *Streptomyces* species and in *Cephalosporium Acremonium*. After the enzyme from *C. Acremonium* was cloned and became available in large amounts in a cell free system a large research effort has been devoted to the study of the substrate specificity of the enzyme and to its mechanism of action.[1] The natural substrate of the enzyme is δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (LLD-ACV) (1).[2]



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It has been shown that the enzyme will tolerate certain modifications of any of the three amino acid residues of the tripeptide. Thus, by substituting either phenylacetic acid or phenoxyacetic acid for the L-α-aminoadipoic acid residue, *Penicillin G* and *Penicillin V* were formed respectively, albeit with reduced efficiency.[3] Modifications of the central cysteine moiety have also been shown to be tolerated by the enzyme. Thus, tripeptides with both α-S-methylcysteine and β-S-methylcysteine as the central amino acid residue, were

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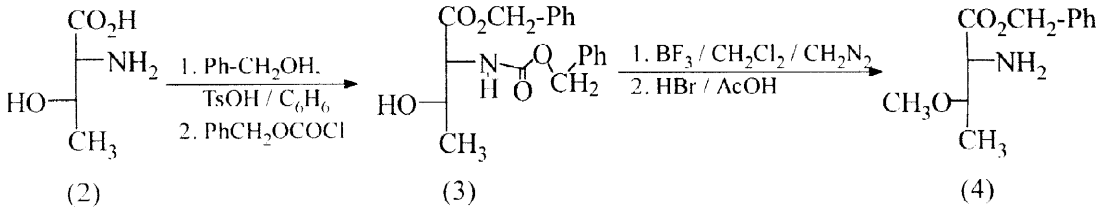
good substrates for the enzyme whereas the β-R-methylcysteine was not.[4] Modifications of the terminal D-valine residue give the most interesting possibilities and a large number of such tripeptides have been made and many have been shown to be converted to β-lactam compounds.[5] This paper describes the synthesis of the tripeptides containing the epimeric O-methyl-D-threonine and O-methyl-D-allothreonine as the terminal residue in place of D-valine.

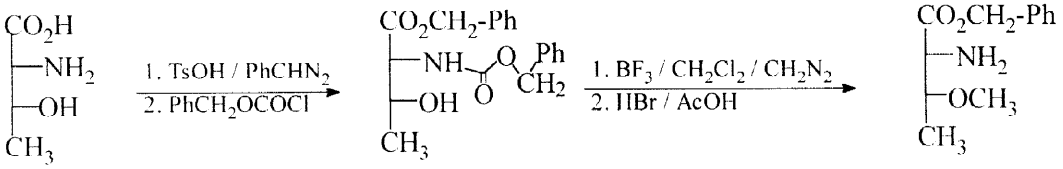
DISCUSSION

Several authors have reported the synthesis of O-alkyl derivatives of the hydroxyamino acids. Slitz and Carter prepared racemic O-methyl serine via mercuration of methyl acrylate and methoxylation. Resolution of the enantiomers was done by acylase catalysed hydrolysis of the N-acetyl derivative.[6] The mercuration methods to obtain both serine and threonine via the O-methyl compounds were later reported by Carter in Organic Synthesis.[7] Chimiak and Rudinger prepared N-benzyloxycarbonyl-O-methyl-L-threonine methyl ester by methylation with methyl iodide using silver oxide as a catalyst.[8] Hodges and Merrifield, used diazomethane to methylate the alcohol group in serine with fluoroboric acid as a catalyst. Their synthesis was done in six steps in about 53% overall yield, using phthalimido protection of the amino group and *p*-nitrobenzyl protection of the carboxyl function.[9] Chen and Benoiton also used direct base catalysed O-methylation with methyl iodide to prepare both O-methyl-L-serine and O-methyl-L-threonine. The amine was protected as N-*tert*-butyloxycarbonyl. The yields from the methylation steps were 40-50%.[10] Probably the most efficient general synthesis of O-alkyl derivatives of hydroxyamino acids is that reported by Barlos and coworkers who synthesized both O-methyl and O-ethyl derivatives of L-serine, L-homoserine and L-threonine in good yields using N-trityl protection and sodium hydride to produce the disodium salt and subsequent alkylation with methyl and ethyl iodides.[11] In the present work the N-benzyloxycarbonyl benzyl esters of both D-threonine and D-allothreonine were methylated with diazomethane in dichloromethane at low temperature with boron trifluoride etherate as a catalyst. Both compounds were isolated pure as oils off a column of silica gel. The yield of N-benzyloxycarbonyl-O-methyl-D-allothreonine benzyl ester (3) was 50% and N-benzyloxycarbonyl-O-methyl-D-threonine benzyl ester (6) was obtained in 68% yield. The yield of the N-deprotected compounds after treatment with hydrobromic acid in acetic acid was 75 and 68% respectively (Scheme 1). The benzyl ester of threonine (2) has been made in modest yield by acid catalysed esterification with benzyl alcohol in benzene.[12] This method was used here to give the benzyl ester in 45% yield which was N-protected with benzyloxycarbonyl chloride. Improved yield was obtained in the case of allothreonine (5) by using excess phenyldiazomethane.[13,14] The N-benzyloxycarbonyl derivative (3) was prepared directly from the chromatographically homogeneous benzyl ester and isolated in 68% yield overall.

Threonine series

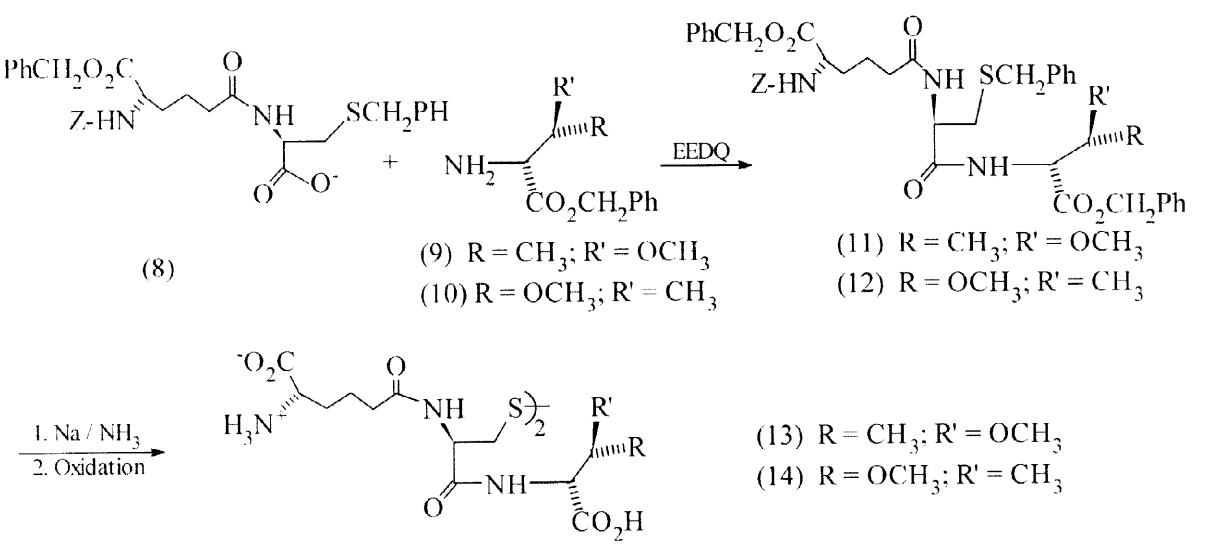


Allothreonine series



Scheme 1. Preparation of the 3-epimeric O-methyl D-threonine and D-allothreonine.

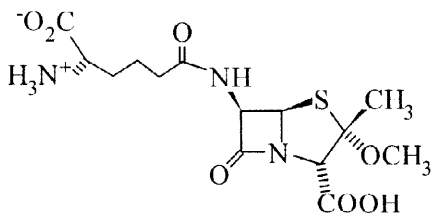
The protected tripeptides were made by 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) coupling to N-benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteine.[15] The threonine isomer (11) was obtained analytically pure after chromatography on silica gel in 77% yield and the allothreonine (12) isomer was crystallised from acetonitrile after purification on silica gel. Both compounds were deprotected in one step by sodium in liquid ammonia (Scheme 2). For testing the tripeptides as substrates for the enzyme a portion of the isolated material was purified by preparative electrophoresis and isolated as the disulphide after oxidation with oxygen.



Scheme 2. Preparation of δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-threonine (13) and of δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-allothreonine (14).

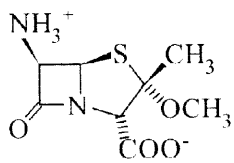
TESTING THE TRIPEPTIDES (13) AND (14) AS SUBSTRATES FOR IPNS AND THE FORMATION OF A NEW ANTIBIOTIC

When the tripeptides were incubated with the IPNS enzyme a striking difference was observed between the two isomers. δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-threonine (13) was shown not be a substrate at all and the tripeptide was recovered unchanged from the incubation mixture. In sharp contrast δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-allothreonine (14) was an excellent substrate for the enzyme producing a single penicillin with antibiotic activity against *S. aureus*. Mass spectra and NMR studies showed the new penicillin to be (2S, 3S, 5R, 6R)-6-(5S-5-amino-5-carboxypentamido)-3-methoxy-3-methyl-7-oxo-1-aza-4-thiabicyclo[3.2.0]heptane-2-carboxylic acid or 2-α-methoxyisopenicillin N (15).[16]



(15)

The α-aminoadipoyl sidechain has been removed chemically from the 2-α-methoxyisopenicillin-N to give a compound equivalent to 6-amino-2-α-methoxypenicilloic acid, a compound similar to 6-aminopenicilloic acid (6-APA) (16).[17] When phenylacetyl and penoxyacetyl sidechains were introduced into this compund to give 2-α-methoxypenicillins equivalent to Penicillin G (17) and Penicillin V (18) respectively. Comparison of the antimicrobial activity of the phenoxyacetyl-2-α-methoxypenicillin with Penicillin V showed it to have similar and better activities against a number of bacteria[16] as shown in Table 1.



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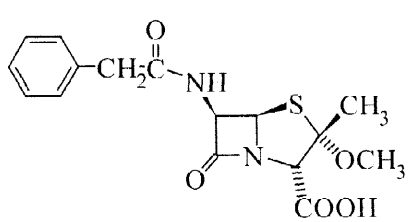

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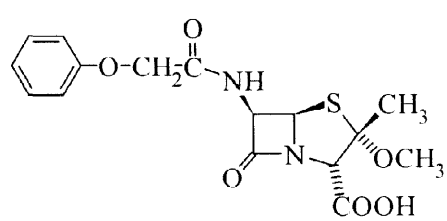

(18)

Table 1. Comparison of antibacterial activity of phenoxyacetyl-2-α-methoxypenicillin compared with Penicillin V.[16]

	Relative Antimicrobial Activity	
Organism	**Penicillin V**	**Phenoxyacetyl-2-α-methoxypenicillin**
Staphylococcus aureus	1	0.95
Bacillus subtilis	1	1.05
Echerischia coli (+)	-	-
Echerischia coli (-)	1	1.2
Salmonella typhi (-)	-	-
Pseudomonas aerugenosa	-	-
Sarchina lutea	1	1.2
Alcaligenes faecalis	-	-
Acinetobacter Sp	-	-
Klebsiella aerogenes	-	-

EXPERIMENTAL

Melting points were determined in capilliary tubes and are uncorrected. Optical rotations were determined on a Perkin Elmer 241 Polarimeter. Proton nuclear magnetic resonance spectra at 300 MHz were recorded on a Bruker WH 300 spectrometer. All chemical shifts, δ, are expressed in parts per million. Spectra run in $CDCl_3$ use residual $C\underline{H}Cl_3$ shift of 7.27 ppm as an internal reference and those run in D_2O use TSP as an internal reference.

D-Threonine benzyl ester (Based on Gutmann and Chang[11]).

D-Threonine (2.00 g, 16.8 mmol) and *p*-toluenesulfonic acid monohydrate (3.55 g, 18.5 mmol) were dissolved in benzyl alcohol (25 mL) and dry benzene (150 mL). The flask was fitted with a Dean and Stark collector and the solution refluxed for 25 h. At the end of this time the benzene was evaporated on a rotary evaporator and the residue partitioned between water (100 mL) and ethyl acetate (100 mL). The layers were separated and the organic layer extracted with more water (4 × 100 mL). Sodium bicarbonate (3.5 g, 42 mmol)

was added to the combined aqueous extracts and the free amine back-extracted into ethyl acetate 5 x 100 mL, dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in ether (50 mL) and on addition of some light petroleum (bp. 40/60) crystallisation started which was left to proceed for 3 h at 4°C. The white crystalline material was isolated by filtration and dried. Yield 1.6 g, 45%, mp 65-66°C, $[\alpha]_D^{20}$ = +4.0° (c 0.98, methanol). ^{1}H-NMR ($CDCl_3$): δ 1.22, 3H d, $J_{CH3\text{-}CH}$ 6.3 Hz, C$\underline{H}_3$; 2.0, 3H broad, N$\underline{H}_2$ and O$\underline{H}$; 3.29, 1H d, $J_{\alpha H\text{-}\beta H}$ 6.2 Hz, α-$\underline{H}$; 3.89, 1H multiplet, β-$\underline{H}$; 5.19, 2H s, Ph-C$\underline{H}_2$; 7.37, 5H s, aromatic. Signal at 2.0 disappears on D_2O shake. M^+ 210. Found: C 63.26, H 7.13, N 6.77; $C_{11}H_{15}NO_3$ requires: C 63.14, H 7.23, N 6.70.

N-Benzyloxycarbonyl-D-allothreonine benzyl ester (6)

D-Allothreonine (180 mg, 1.50 mmol) and *p*-toluenesulfonic acid monohydrate (285 mg, 1.50 mmol) were dissolved in water (15 mL). Acetone (22 mL) was added and the solution cooled on ice. Phenyldiazomethane (prepared[3] from benzaldehyde *p*-toluenesulfonyl hydrazone[4], 3.3 g 12 mmol) was dissolve in acetone (7.5 mL) and added portionwise to the above solution while cooling on ice. The mixture was allowed to reach room temperature and then most of the acetone was evaporated on a rotary evaporator, more water (15 mL) was added and the aqueous solution washed with ether. Sodium bicarbonate (495 mg, 6 mmol) was added and the free amine extracted into ethyl acetate (6 x 30 mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated to dryness. The yellowish oily product was purified on a column of silica gel ($CHCl_3$ / CH_3OH 9:1, R_f 0.35). The chromatographically homogeneous product was dissolved in dry THF (10 mL, triethylamine (0.200 mL, 1.40 mmol) added followed by benzyl chloroformate (0.210 mL, 1.40 mmol). A precipitate of $Et_3N{\cdot}HCl$ formed almost instantly and after 30 minutes the rection mixture was filtered and evaporated. The product was purified on a column of silica gel giving an oil (350 mg, 68%) which crystallized on standing, mp 75-76°C; $[\alpha]_D^{20}$ = -12.3° (c 2.0, $CHCl_3$); M^+ 344; ^{1}H-NMR ($CDCl_3$): δ 1.16, 3H d, $J_{CH3\text{-}CH}$ 6.5 Hz, C-C$\underline{H}_3$; 2.8, 1H b, O$\underline{H}$; 4.17, 1H m, α-$\underline{H}$; 4.49, 1H m, β-$\underline{H}$; 5.12, 2H s, C$\underline{H}_2$-Ph; 5.21, 2H AB q, J_{AB} 12.3 Hz, ester C$\underline{H}_2$-Ph; 5.71, 1H d, $J_{NH\text{-}\alpha}$ 6.5 Hz, N$\underline{H}$; 7.36, 10H m, aromatic. Found: C 66.26; H 5.96; N 4.01. $C_{19}H_{21}NO_5$ requires: C 66.46; H 6.17; N 4.08.

N-Benzyloxycarbonyl-O-methyl-D-allothreonine benzyl ester

N-Benzyloxycarbonyl-D-allothreonine benzyl ester (250 mg, 0.728 mmol) was dissolved in dry dichloromethane (10 mL), cooled on a dry ice/acetone bath and boron trifluoride etherate (50 microliters) was added. Diazomethane (15 mmol) in 10 mL dichloromethane was added slowly. After a few minutes t.l.c. (CH_2Cl_2/EtOAc, 95:5) showed that most of the starting material had reacted giving a single product (R_f 0.55). The solvent was evaporated under reduced pressure and the product purified on a column of silica gel giving an

oily compound, 130 mg, 50%, $[\alpha]_D^{20}$ = -3.8° (c 2.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.17, 3H d, $J_{CH3-\beta}$ 6.3 Hz, C-C$\underline{H}_3$; 3.35, 3H s, OC$\underline{H}_3$; 3.68, 1H m, β-$\underline{H}$; 4.59, 1H dd, $J_{\alpha H-\beta H}$ 3.7, $J_{\alpha H-NH}$ 8.8 Hz, α-$\underline{H}$; 5.12, 2H s, C$\underline{H}_2$-Ph; 5.21, 2H AB q, J_{AB} 12.4 Hz, ester C$\underline{H}_2$-Ph; 5.54, 1H d, $J_{NH-\alpha H}$ 8.3 Hz, N$\underline{H}$; 7.3, 10H s, aromatic.

O-Methyl-D-allothreonine benzyl ester (7)

N-Benzyloxycarbonyl-O-methyl-D-allothreonine benzyl ester (100 mg, 0.28 mmol) was treated with hydrogen bromide/acetic acid (0.5 mL) and dichloromethane (0.5 mL) for 30 minutes at room temperature. Toluene (3 mL) was added and the reaction mixture evaporated to dryness under high vacuum. The solid residue was dissolved in dichloromethane (3 mL) and extracted with saturated sodium bicarbonate (3 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The free amine was isolated as a yellowish oil, 48 mg, 75%, after chromatography on silica gel, $[\alpha]_D^{20}$ = -10.5° (c 2.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.03, 3H d, J_{CH3-CH} 6.3 Hz, C-C$\underline{H}_3$; 1.60, 3H s, N$\underline{H}_2$ + residual H_2O; 3.26, 3H s, OC$\underline{H}_3$; 3.54, 1H m, β-$\underline{H}$; 3.67, 1H d, $J_{\alpha-\beta}$ 4.4 Hz, α-$\underline{H}$; 5.11, 2H s, C$\underline{H}_2$-Ph; 7.3, 5H m, aromatic.

N-Benzyloxycarbonyl-O-methyl-D-threonine benzyl ester

N-Benzyloxycarbonyl-D-threonine benzyl ester (687 mg, 2.00 mmol) (prepared from the benzyl ester as described for the allothreonine derivative, m.p. 75-76°C, $[\alpha]_D^{20}$ = +10.3 (c 2.0, EtOH), Gutman & Chang[11], L-enantiomer: m.p. 79-80°C, $[\alpha]_D^{20}$ = -10.5 (c 2.0, EtOH)) was dissolved in dry dichloromethane (20 mL) cooled on dry ice/acetone and boron trifluoride etherate (0.100 mL) added. Diazomethane (30 mmol in 20 mL CH_2Cl_2) was added portionwise. After about 30 min. the reaction mixture was filtered and evaporated to give an oily product which was purified on a column of silica gel, 486 mg, 68%; R_f 0.55 (CH_2Cl_2, 95:5); $[\alpha]_D^{20}$ +19.4° (c 4.0, $CHCl_3$); M^+ 358; ^{1}H-NMR ($CDCl_3$): δ 1.2, 3H d, J_{CH3-CH} 6.2 Hz, C-C$\underline{H}_3$; 3.18, 3H s, OC$\underline{H}_3$; 3.94, 1H dq, $J_{\beta-\alpha}$ 2.3, $J_{\beta-CH3}$ 6.3 Hz, β-H; 4.39, 1H dd, $J_{\alpha-\beta}$ 2.3, $J_{\alpha-NH}$ 9.5 Hz, α-$\underline{H}$; 5.14, 2H s, C$\underline{H}_2$-Ph; 5.22, 2H AB q, J_{AB} 12.3 Hz, ester C$\underline{H}_2$-Ph; 5.49, 1H d, $J_{NH-\alpha}$ 9.6 Hz, N$\underline{H}$; 7.36, 10H m, aromatic.

O-Methyl-D-threonine benzyl ester (4)

N-Benzyloxycarbonyl-O-methyl-D-threonine benzyl ester (480 mg, 1.3 mmol) was treated with 45% hydrogen bromide in glacial acetic acid (1 mL) in dichloromethane (1 mL) at room temperature for 30 minutes. At the end of this period the mixture was evaporated on the rotary evaporator, toluene (10 mL) added and reevaporated. Xylene was then added and reevaporated using an oil pump (2 × 10 mL). The residue was dissolved in dichloromethane (5 mL and triethylamine (0.5 mL) was added and evaporated. More

dichloromethane was added and reevaporated (2 x 5 mL). Ether (10 mL) was added and the solution filtered. After evaporation of the ether the product was purified on a coulumn of silica gel ($CHCl_3/CH_3OH$, 95:5) to give a brownish oil, 205 mg, 68%; $[\alpha]_D^{20}$ = +23.4° (c 2.0, $CHCl_3$); M^+ 224; ^{1}H-NMR ($CDCl_3$): δ 1.23, 3H d, $J_{CH3-\beta}$ 6.2 Hz, C-C$\underline{H}_3$; 1.64, 3H s, N$\underline{H}_2$/$\underline{H}_2$O; 3.24, 3H s, OC$\underline{H}_3$; 3.42, 1H d, $J_{\alpha-\beta}$ 3.4 Hz, α-$\underline{H}$; 3.76, 1H dq, $J_{\beta-\alpha}$ 3.6, $J_{\beta-CH3}$ 6.2 Hz, β-$\underline{H}$; 5.20, 2H AB q, J_{AB} 12.3 Hz, C$\underline{H}_2$-Ph; 7.4, 5H m, aromatic.

N-Benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteinyl-(O-methyl)-D-threonine benzyl ester (11)

N-Benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteine (332 mg, 0.574 mmol) and O-methyl-D-threonine benzyl ester (127 mg, 0.568 mmol) were placed in a flask and dissolved in dry dichloromethane (4 mL). 2-Ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (140 mg, 0.574 mmol) was added and the reaction left at room temperature overnight. The solvent was evaporated to dryness and the crude product redissolved in chloroform (20 mL) and washed with 0.10 M hydrochloric acid (20 mL) and saturated sodium bicarbonate solution (20 mL), dried ($MgSO_4$) and evaporated to dryness. The product was purified on a column of silica gel to give the chromatographically homogeneous product, 345 mg, 77%; $[\alpha]_D^{20}$ = 0.00° (c 4.0, $CHCl_3$); M^+ 783/4; ^{1}H-NMR ($CDCl_3$): δ 1.13, 3H d, J_{CH3-CH} 6.2 Hz, C-C$\underline{H}_3$; 1.6-2.3, 7H m β- and γ-protons of αAA + residual H_2O; 2.8, 2H o, AB part of ABX systems, J_{AX} 6.8, J_{AB} 14.0, J_{BX} 6.0 Hz, Cys β-protons; 3.18, 1H s, O-C$\underline{H}_3$; 3.75, 2H s, S-C$\underline{H}_2$Ph; 3.93, 1H dq, $J_{\beta-\alpha}$ 2.3, $J_{\beta-CH3}$ 6.3 Hz, Thr β-$\underline{H}$; 4.41, 4.51, 4.61, 3 x 1H m, α-protons; 5.04-5.27, 6H m, 3 x O-C$\underline{H}_2$-Ph; 5.6, 6.3, 6.8, 3 x 1H d, J 8.1, 7.4, 9.0 Hz, 3 x N$\underline{H}$; 7.3, 20 H m, aromatic. Found: C 65.70, H 6.14, N 5.37; $C_{43}H_{49}N_3O_9S$ requires: C 65.88, H 6.30, N 5.36.

δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-threonine (13)

N-Benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteinyl-(O-methyl)-D-threonine benzyl ester (230 mg, 0.294 mmol) was dissoved in about 1 mL THF and treated with sodium in dry liquid ammonia until the blue colour persisted. The excess sodium was destroyed with ammonium sulphate and the ammonia allowed to evaporate at room temperature. The product was dissolved in water (20 mL), the pH adjusted to 8 and a stream of oxygen passed through the solution for about 1 h to convert it to the disulphide. The solvent was freeze dried to give a crude yield of 250 mg containing some salt. A portion of the crude material (10 mg) was purified by electrophoresis on a 3 MM electrophoresis paper at pH 3.5, 3 KV for 2 hrs. The tripeptide was located by ninhydrin, eluted off the paper with water, the volume reduced on the rotary evaporator and then freeze dried to give 5.3 mg of pure material, M^+ 757 (disulfide); ^{1}H-NMR (D_2O): δ 0.96, 3H d, $J_{CH3-\beta H}$ 6.3 Hz, Thr C-C$\underline{H}_3$; 1.5-1.8, 4H m, α-AA β- and γ-protons; 2.23, 2H t, $J_{\delta-\gamma}$ 7.0 Hz, α-AA δ-

protons: 2.94, 2H o, AB part of ABX, J_{AX} 9.1, J_{AB} 14.1, J_{BX} 5.1 Hz, Cys β-protons; 3.14, 3H s, $OC\underline{H}_3$; 3.56, 1H t, $J_{\alpha\text{-}\beta}$ 6.0 Hz, Cys α-protons; 3.77, 1H m, Thr-β-protons; 4.09, 1H d, $J_{\alpha\text{-}\beta}$ 3.2 Hz, Thr β-proton.

N-Benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteinyl-(O-methyl)-D-allothreonine benzyl ester (12)

The protected tripeptide was made by EEDQ activation of the protected dipeptide in 68% yield as described for the threonine tripeptide and crystallized from acetonitrile, mp 116-118°C; $[\alpha]_D^{20}$ = -11.7° (c 1.0, $CHCl_3$): M^+ 783/4; ^{1}H-NMR ($CDCl_3$): δ 1.15, 3H d, $J_{CH3\text{-}CH}$ 6.4 Hz, C-C$\underline{H}_3$; 1.6-2.3, 8H m β- to δ-protons of αAA + residual H_2O; 2.77, 2H o, AB part of ABX systems, J_{AX} 7.1, J_{AB} 14.0, J_{BX} 5.7 Hz, Cys β-protons; 3.30, 3H s, O-C$\underline{H}_3$; 3.64, 1H m, allothr β-$\underline{H}$; 3.76, 2H s, S-C$\underline{H}_2$Ph; 4.4, 4.5, 4.6, 3 × 1H m, α-protons; 5.1, 6H m, 3 × O-C$\underline{H}_2$-Ph; 5.59, 6.24, 7.00, 3 × 1H d, J 8.1, 7.2, 8.3 Hz, 3 × N$\underline{H}$; 7.3, 20 H m, aromatic. Found: C 65.80, H 6.16, N 5.38; $C_{43}H_{49}N_3O_9S$ requires: C 65.88, H 6.30, N 5.36.

δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-(O-methyl)-D-allothreonine (14)

N-Benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-S-benzyl-L-cysteinyl-(O-methyl)-D-allothreonine benzyl ester was deprotected by sodium in liquid ammonia as described for the threonine tripeptide. After converting the free thiol to the disulfide, the product was purified by preparative electrophoresis in 48% yield; ^{1}H-NMR (D_2O): δ 0.99, 3H d, $J_{CH3\text{-}\beta H}$ 6.5 Hz, allothr C-C$\underline{H}_3$; 1.5-1.7, 4H m, α-AA β- and γ-protons; 2.24, 2H t, $J_{\delta\text{-}\gamma}$ 6.9 Hz, α-AA δ-protons; 2.94, 2H o, AB part of ABX, J_{AX} 9.8, J_{AB} 14.1, J_{BX} 5.3 Hz, Cys β-protons; 3.20, 3H s, $OC\underline{H}_3$; 3.62, 1H t, $J_{\alpha\text{-}\beta}$ 6.0 Hz, Cys α-protons; 3.70, 1H m, allothr-β-protons; 4.50, 1H d, $J_{\alpha\text{-}\beta}$ 4.3 Hz, allothreonine α-proton.

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