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PEPTIDES–XXXVIII

SYNTHESIS OF THE 38–49 FRAGMENT OF A LYSOZYME ANALOGUE

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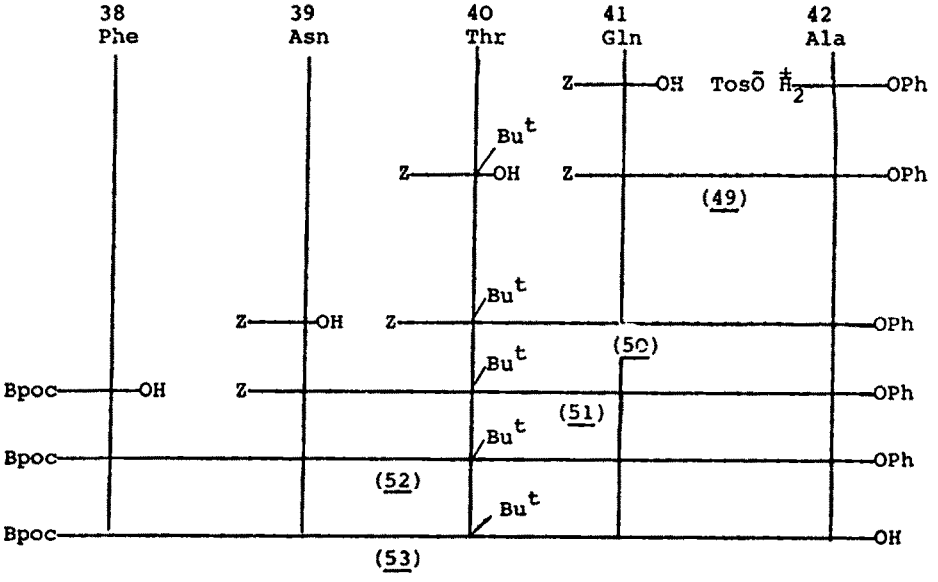
Abstract—The synthesis of the fully protected (38–49) fragment of a lysozyme analogue was successfully achieved. The two protected subfragments (38–42) and (43–49) were assembled by a stepwise approach in which the α-amino protection was afforded by the benzyloxycarbonyl function and the terminal carboxyl protection by the phenyl ester. These two subfragments were coupled by the DCCI/HONSu method and purification was accomplished by gel filtration on Sephadex LH20.

The preceding five papers in this series [1–5] have described the general strategy for the synthesis of a lysozyme analogue and the assembly of the 1–37 fragment of this sequence. This work represents the initial phase in the preparation of the fragments constituting the second major portion (38–75) of the molecule. Construction of the 38–49 subfragment of the analogue represented a challenging synthetic objective in its own right.

Phe.Asn.Thr.Gln.Ala.Thr.Asn.Orn.Asn.Thr.Glu.Gly
38 42 45 49

Although from inspection of the fragment sequence it may be seen that there are no points at which racemisation free coupling might be carried out, it was nevertheless, decided to construct the protected dodecapeptide by the fragment condensation approach. The less hindered alanine-42 was chosen as the coupling point with the remaining heptapeptide ultimately being assembled by the stepwise method after a tri-plus tetrapeptide approach had been investigated.

The protected 38–42 pentapeptide was synthesised by the route outlined in Scheme 1 in which the dipeptide **49** was prepared by the pivalic mixed anhydride method, using an equivalent of pyridine[6] in addition to the one equivalent of triethylamine required to form the carboxylate anion. The compound numbering sequence follows that established in earlier papers in this series.[2–5] The amino-protecting group was removed by hydrogenolysis and the resulting *p*-toluenesulphonate coupled to *N*-benzyloxycarbonyl-*O*-tert-butylthreonine by the pivalic mixed anhydride method. The resulting tripeptide **50** was subjected to hydrogenolysis in order to remove the benzyloxycarbonyl function and the



Scheme 1. Synthesis of the protected (38–42) pentapeptide **52**

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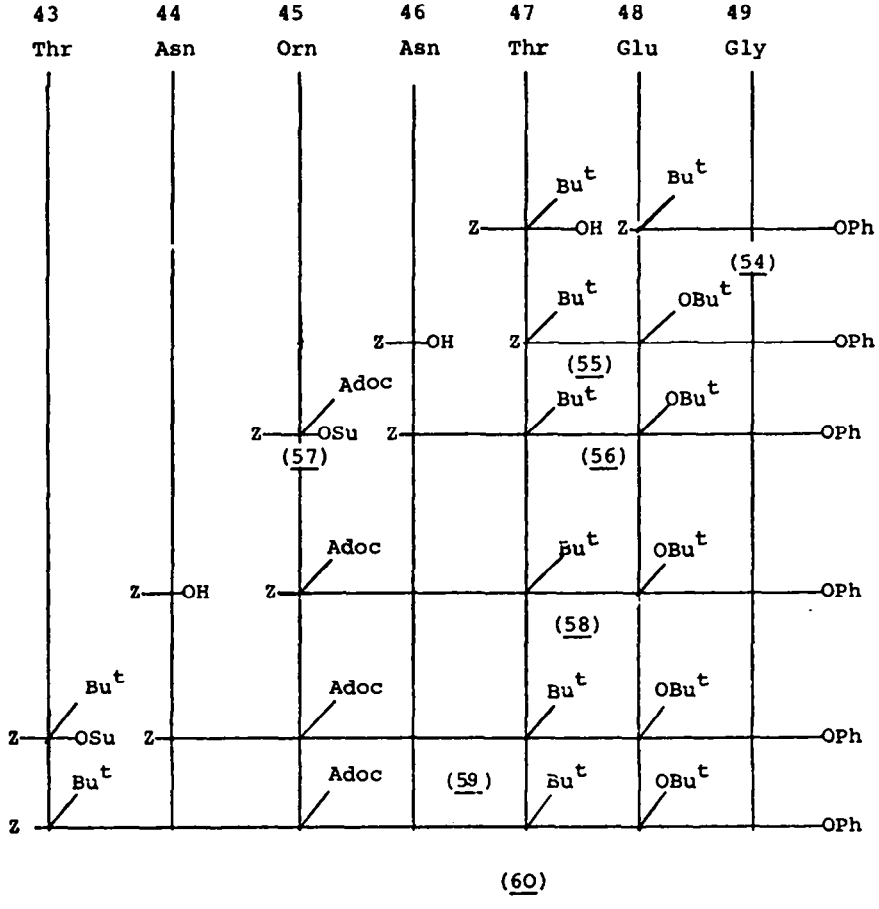
resulting salt again coupled using the pivalic mixed anhydride method. The rather insoluble tetrapeptide **51** which was obtained in high yield was readily purified by reprecipitation. Hydrogenolysis of this material gave a *p*-toluene-sulphonate salt which was coupled with *N*-*p*-biphenylisopropoxycarbonyl-phenylalanine. Two methods of coupling were examined. The first, Bates reagent[7] gave a reasonable yield (51 %) of pure material, however, the pentapeptide **52** was best prepared in 60 % yield by the pivalic anhydride method followed by purification of the product first by precipitation and subsequently by chromatography on Sephadex LH20 eluting with DMF.

The synthesis of the 43–49 heptapeptide was initially planned as a 3 + 4 fragment coupling in which the 43–45 fragment would be synthesised by a salt coupling approach using *N*-hydroxysuccinimide esters and the fully protected 46–49 fragment would be assembled by a stepwise approach. However, low yields and impurities were encountered in the salt coupling preparation of the tripeptide and ultimately the total stepwise synthesis of the heptapeptide shown in Scheme 2, was adopted.

The protected dipeptide **54**[8] was converted into the corresponding *p*-toluenesulphonate by hydrogenolysis and coupled with *N*-benzyloxycarbonyl-*O*-t-butyl-threonine *via* the pivalic mixed anhydride method to give the crystalline tripeptide **55**. In an analoguous manner, the peptide chain was elongated using *N*-benzyloxycarbonylasparagine to give the tetrapeptide **56** after purification by careful reprecipitation from DMF with water. The protected tetrapeptide **56** was then converted to its *p*-toluene-sulphonate in the usual way, however coupling with N^{δ}-adamantyloxy-carbonyl-N^{α}-benzyloxycarbonylornithine[3] by the pivalic mixed anhydride method gave a low yield of the required product **58**. It appeared that this coupling was rather slow and thus allowed time for partial decomposition of the mixed anhydride. An alternative approach using the corresponding *N*-hydroxy-succinimide active ester **57** was considerably more successful yielding a 67 % yield of the product **58** after gel filtration on Sephadex LH20 eluting with DMF. Unfortunately the active ester **57** could only be obtained as a dry foam although the starting carboxylic acid had been obtained in analytically pure form.[3] Hydrogenolysis of the protected peptide **58** gave the corresponding *p*-toluene-sulphonate which was successfully coupled with benzyloxycarbonyl-asparagine by the pivalic mixed anhydride method giving an excellent yield of the required hexapeptide **59**. After removal of the benzyloxycarbonyl protecting group by hydrogenolysis the hexapeptide was coupled to the threonine derivative shown in Scheme 2, using



Scheme 2. Synthesis of the projected 43–49 heptapeptide **60**

the *N*-hydroxysuccinimide ester[9] rather than a mixed anhydride as the reaction appeared to be quite slow. Under these acylation conditions an 80% yield of the required heptapeptide **60** was obtained after gel filtration on Sephadex LH20 eluting with DMF.

The efficient synthesis of the penta **52** and hepta **60** peptides thus provided the subfragments required for completion of the protected 38–49 fragment **61**. The phenyl ester function was cleaved from the fully protected pentapeptide **52** by the usual hydrogen peroxide catalysed reaction[8] at pH 10.5, between 25 and 30 minutes being required for total cleavage. In this particular example the cleavage to the pentapeptide acid **53** was carried out in aqueous DMF solution without the addition of a scavenger as all the amino acids in the sequence were known to be unaffected by the cleavage conditions. The *p*-toluene-sulphonate of the heptapeptide **60** was obtained by hydrogenolysis. A number of trial couplings to give the fully protected (38–49) sequence **61** were carried out using *N*-*N*′-dicyclohexylcarbodiimide (DCCI) in the presence of three different *N*-hydroxy compounds commonly used for the suppression of racemisation in DCCI coupling in peptide synthesis. In this fragment coupling the best yield obtained using *N*-hydroxybenzotriazole[10] was 25% and using *N*-hydroxy-5-norbornene-2, 3-dicarboxamide[11] it was 20%, whereas when *N*-hydroxysuccinimide[12] was employed a yield of up to 75% was obtained on one occasion with a yield of around 60% being typical.

The optimum coupling conditions adopted for the preparation of the dodecapeptide **61** utilised slight excesses of the carboxyl compound **53**, DCCI and *N*-hydroxysuccinimide (all 20% excess). The reaction mixture was precipitated after 4 days and subjected to gel filtration in DMF using LH20 as the matrix gave a good recovery of the required peptide. The homogeneity of the product was confirmed by TLC and electrophoresis of the deprotected peptide, by standard amino acid analysis, combustion analysis and substantiated by ^{1}H NMR at 220 MHz although a full assignment could not quite be achieved. The synthesis described therefore provides a route to the fully protected fragment **61** which was subsequently to be used in the preparation of the 38–75 portion of the enzyme analogue.

Bpoc.Phe.Asn.Thr(Bu^t).Gln.Ala.Thr(Bu^t).Asn.Orn(Adoc).Asn.Thr(Bu^t).Glu(OBu^t).Gly.OPh.

61

EXPERIMENTAL

The abbreviations, TLC systems and general experimental methods are those detailed in an earlier paper in this series[2] excepting the TLC systems: (32) BuOH/acetone/H_2O 2:2:1 and (33) BuOH/AcOH/H_2O 10:1:3.

Z–Gln–Ala–OPh **49**. A solution of Z–Gln–OH (50.8 g, 180 mM) in DMF (300 ml) was cooled to −10°, TEA (25.2 ml, 180 mM) and pyridine (14.4 ml, 180 mM) were added followed by pivaloyl chloride (21.7 ml, 180 mM). After allowing 15 min for activation a precooled solution of $ToSO^-H_3^+$-Ala–OPh (62.2 g, 183 mM) and TEA (25.2 ml, 180 mM) in DMF (100 ml) was added and the reaction mixture allowed to attain room temperature overnight. The solution was concentrated and the product precipitated with water. After washing with 1 M $NaHCO_3$, 1 M AcOH and water the white solid product was reprecipitated first from DMF/water and subsequently from DMF/Et_2O yielding the pure protected dipeptide **49** (43.0 g, 56%), m.p. 185–188°, $[\alpha]_D^{25}$ − 39.2° (c = 2, DMF), $R_f(2)$ − 0.4, $R_f(32)$ − 0.8, $Glu_{1.03}Ala_{0.97}$. (Found: C, 61.63; H, 5.98; N, 9.76. $C_{22}H_{25}N_3O_6$ requires: C, 61.82; H, 5.90; N, 9.83%).

Z–Thr(Bu^t)–Gln–Ala–OPh **50**: The protected dipeptide **49** (34.2 g, 80 mM) and Tos.OH.H_2O (15.3 g, 80 mM) were dissolved in DMF (200 ml), 10% Pd/C (4.0 g) was added and hydrogen passed through the solution for 8 hours. The catalyst was removed by filtration and the solution volume reduced to a minimum. Z–Thr(Bu^t)O$^-$DCHA$^+$ (49.0 g, 100 mM) was converted to the corresponding acid by treatment with 10% citric acid and extraction into EtOAc. After backwashing with water and drying, the solution was evaporated and the residue dissolved in CH_2Cl_2 (150 ml). The mixed anhydride was formed in the usual way using pivaloyl chloride (11.0 g, 92 mM) and TEA (10.1 g, 100 mM) with a 15 min activation. The DMF solution from the hydrogenolysis was cooled and added along with TEA (8.1 g, 80 mM) and the reaction mixture stirred overnight. The reaction mixture was filtered and the filtrate carefully reduced in volume, water was then added to precipitate the product. Washing with acid and base followed by reprecipitation from DMF/water and DMF/Et_2O gave the pure product **50** (21.8 g, 47%), m.p. 180–183°, $[\alpha]_D^{25}$ − 7.9° (c = 2, DMF), $R_f(2)$ − 0.3, $R_f(32)$ − 0.8, $Thr_{0.91}Glu_{1.03}Ala_{0.95}$. (Found: C, 61.37; H, 6.83; N, 9.64. $C_{30}H_{40}N_4O_8$ requires: C, 61, 63; H, 6.89; N, 9.58%.)

Z–Asn–Thr(Bu^t)–Gln–Ala–OPh **51**. A solution of compound **50** (16.7 g, 37 mM) in DMF (30 ml) was hydrogenolysed for 4 hours in the presence of Tos.OH.H_2O (5.5 g, 37 mM) over 10% Pd/C (1.75 g). After filtration the solution was set on one side for use in the mixed anhydride coupling. The anhydride was formed from Z–Asn–OH (7.6 g, 35 mM), TEA (2.8 g, 35 mM), pyridine (2. 3 g, 35 mM) and pivaloyl chloride (3.4 g, 35 mM) in the usual way allowing 20 minutes for activation. The cooled solution from the hydrogenolysis was then added followed by TEA (2.8 g, 35 mM) and the reaction mixture stirred overnight. The solution volume was reduced and water added to precipitate the product, washing with acid and base in the usual way and precipitation from DMF/water followed by washing with Et_2O gave the pure product **51** (20.8 g, 85%), m.p. 216–219°, $[\alpha]_D^{25}$ − 23.1° (c = 2, DMF), $R_f(33)$ − 0.1, $R_f(4)$ − 0.8, $Asp_{0.99}Thr_{0.90}Glu_{1.05}Ala_{0.95}$. (Found: C, 58.15; H, 6.59; N, 11.73. $C_{34}H_{46}N_6O_{10}$ requires: C, 58.44; H, 6.63; N, 12.02%).

Bpoc–Phe–Asn–Thr(Bu^t)–Gln–Ala–OPh **52**. A solution of compound **51** (6.98 g, 10 mM) and Tos.OH.H_2O (1.90 g, 10 mM) in DMF (80 ml) was then hydrogenolysed for 6 hours in the presence of 10% Pd/C (0.5 g). The reaction mixture was filtered and the filtrate used directly in the subsequent coupling reaction.

Bpoc–Phe–OH (4.83 g, 12 mM) was dissolved in CH_2Cl_2 (40 ml) and the mixed anhydride formed in the usual way from pivaloyl chloride (1.44 g, 11.9 mM) and TEA (1.21 ml, 12 mM). After 20 minutes activation the solution of the amino-component was added followed by TEA (1.01 g, 10 mM). The reaction mixture was stirred at room temperature overnight then reduced in volume and precipitated with 1 M $NaHCO_3$ solution. The precipitated crude product was washed alternately with 1 M AcOH and 1 M $NaHCO_3$ solutions then dried and chromatographed on Sephadex LH 20 eluting with DMF. The purified product **52** had Ve/Vt = 0.46 and was isolated by evaporation of the appropriate fractions followed by precipitation with water giving (5.68 g, 60%), m.p. 212–214°, $[\alpha]_D^{25}$ − 19.2° (c = 1, DMF), $R_f(23)$ − 0.75, $R_f(28)$ − 0.9, $Asp_{0.99}Thr_{0.91}Glu_{1.04}Ala_{0.97}$. (Found: C, 63.08, H, 6.67; N, 10.14. $C_{51}H_{63}N_7O_{11}.H_2O$ requires: C, 63.27; H, 6.78; N, 10.13%).

Bpoc–Phe–Asn–Thr(Bu^t)–Gln–Ala–OH **53**. The fully protected peptide phenyl ester **52** (0.95 g, 1 mM) was dissolved in a mixture of DMF (20 ml) and HMPA (3 ml) and water added until the solution became slightly turbid (1.5 ml). 100 Volume H_2O_2 (0.1 ml, 1 mM) was then added and the pH brought to 10.5 with 1 M NaOH solution. The pH was maintained at 10.5 for 25 minutes by the addition of 1 M NaOH solution (total volume added 1.2 ml) also water (5 ml) was gradually added as cleavage was more rapid in the presence of up to 20% water. The pale brown solution (due to phenolic oxidation products) was acidified to pH 3 with ice cold 10% citric acid, precipitating the acid **53** as a white solid. Filtration followed by washing with water and Et_2O gave the pure free acid **53** (0.81 g, 93%), m.p. 235–238°, $[\alpha]_D^{25}$ – 26.1° (c = 0.8, DMF) $R_f(23)$ – 0.65, $R_f(14)$ – 0.35, (Found: C, 59.36; H, 6.67; N, 11.10. $C_{45}H_{59}N_7O_{11}.2H_2O$ requires: C, 59.36; H, 6.97; N, 10.77%).

Z–Glu(OBu^t)–Gly–OPh **54**. The preparation of this compound in 86% yield by the trichlorophenyl active ester method has been described in an earlier paper in this series.[8]

Z–Thr(Bu^t)–Glu(OBu^t)–Gly–OPh **55**. The protected dipeptide **53** (14.2 g, 30.2 mM) was subjected to hydrogenolysis in the usual way in the presence of Tos.OH.H_2O (5.75 g, 30.2 mM) and 10% Pd/C (1.5 g) with DMF (350 ml) as solvent. After hydrogenolysis (16 h) the solution was filtered and evaporated to give an oil, which was solidified by the addition of Et_2O giving the dipeptide *p*-toluene-sulphonate salt in quantitative yield. Z–Thr(Bu^t)O$^-$DCHA$^+$ (19.2 g, 39 mM) was converted to the free acid in the usual way and dissolved in CH_2Cl_2 (100 ml). The mixed anhydride was generated by the established method using pivaloyl chloride (4.50 g, 37.5 mM) and TEA (3.75 g, 37.5 mM) allowing 15 minutes for activation. The salt from the hydrogenolysis was dissolved in DMF (100 ml) and added to the mixed anhydride solution followed by TEA (3.03 g, 30 mM). After stirring overnight at room temperature the reaction mixture was evaporated to give an oil which was dissolved in EtOAc. This solution was washed with water and dried. Evaporation produced an oil which was crystallised from Et_2O/petroleum ether giving the required product **55** (13.4 g, 71%), m.p. 118–120°, $[\alpha]_D^{25}$ + 9.6° (c = 2, DMF), $R_f(2)$ – 0.75, $R_f(26)$ – 0.5, $Thr_{0.89}Glu_{1.05}Gly_{1.00}$. (Found: C, 63.02; H, 7.16; N, 6.70. $C_{33}H_{45}N_3O_9$ requires: C, 63.14; H, 7.23; N, 6.69%).

Z–Asn–Thr(Bu^t)–Glu(OBu^t)–Gly–OPh **56**. Compound **55** (12.54 g, 20 mM) and Tos.OH.H_2O (3.8 g, 20 mM) were dissolved in DMF (100 ml) 10% Pd/C (1.0 g) was added and hydrogenolysis carried out in the usual way. After hydrogenolysis (4 h) the reaction mixture was filtered and used directly in the coupling reaction. Z–Asn–OH (6.65 g, 25 mM) was dissolved in DMF (50 ml) and the mixed anhydride formed in the usual way using pivaloyl chloride (3.0 g, 25 mM), TEA (2.53 g, 25 mM) and pyridine (1.97 g, 25 mM) allowing 15 minutes for activation. The solution of the amino-component was added followed by TEA (2.02 g, 20 mM) and the reaction mixture stirred overnight to complete reaction. The solvent was evaporated and the residual oil solidified by addition of 1 M $NaHCO_3$ solution, alternate washing with 1 M $NaHCO_3$ and 1 M AcOH and reprecipitation from DMF/H_2O gave the homogenous product **56** (13.2 g, 89%), m.p. 156–159°, $[\alpha]_D^{25}$ – 1.9° (c = 2, DMF), $R_f(2)$ – 0.4, $R_f(23)$ – 0.7, $Asp_{0.99}Thr_{0.80}Glu_{1.00}Gly_{1.03}$. (Found: C, 59.98; H, 6.93; N, 9.23. $C_{37}H_{51}N_5O_{11}$ requires C, 59.91; H, 6.93; N, 9.44%).

Z–Orn(Adoc)–Asn Thr(Bu^t)–Glu(OBu^t)–Gly–OPh **58**. The addition of the ornithine residue was achieved by the *N*-hydroxysuccinimide active ester method. The active ester **57**, which could not be obtained in analytically pure form was prepared as follows. Z–Orn(Adoc)–OH (31.8 g, 72 mM) obtained from the DCHA salt **28**[3] in the usual way and HONSu (9.94 g, 86 mM) were dissolved in dimethoxyethane (80 ml); after cooling to – 10° a solution of DCCI (17.8 g, 86 mM) in dimethoxyethane (40 ml) was added and the reaction mixture stirred overnight. The reaction mixture was then cooled to – 20° for 1 h and filtered to remove DCU, evaporation gave a pale yellow oil. This was dissolved in EtOAc and rapidly washed with 1 M $NaHCO_3$ solution, the organic phase was dried and evaporated giving the active ester **57** as a white dry foam (28.4 g, 88%). The IR spectrum showed the characteristic peaks associated with *N*-hydroxysuccinimide esters and TLC showed one major spot in addition to several minor impurities which could not readily be removed. The dry foam was consequently used directly in the formation of the pentapeptide.

The protected tetrapeptide **56** (8.75 g, 11.8 mM) and Tos.OH.H_2O (2.24 g, 11.8 mM) were dissolved in DMF (100 ml), 10% Pd/C (0.6 g) was added and the reaction mixture hydrogenolysed overnight. Filtration gave a clear colourless solution to which was added Z–Orn(Adoc)ONSu (7.35 g, 13.8 mM) and NMM (1.2 g, 11.8 mM). The solution was stirred for three days until fluoroescamine-negative and then evaporated to a small volume and precipitated with 1 M $NaHCO_3$ solution. The resulting white solid (12.01 g, 97% crude yield) was washed with Et_2O and dried, further purification being achieved by gel filtration on Sephadex LH20 eluting with DMF, Ve/Vt = 0.46. After precipitation with water the homogeneous product **58** was obtained (8.2 g, 67%), m.p. 175–178°, $[\alpha]_D^{25}$ – 4.9° (c = 1, DMF), $R_f(2)$ – 0.4, $R_f(7)$ – 0.6, $Orn_{0.98}Asp_{1.02}Thr_{0.95}Glu_{1.03}Gly_{1.00}$. (Found: C, 60.26; H, 7.15; N, 9.06. $C_{35}H_{75}N_7O_{14}.H_2O$ requires: C, 60.40; H, 7.30; N, 9.34%).

Z Asn–Orn(Adoc)–Asn–Thr(Bu^t)–Glu(OBu^t)–Gly–OPh **59**. Hydrogenolysis overnight of a solution of the protected pentapeptide **58** (4.84 g, 4.7 mM), Tos.OH.H_2O (0.89 g, 4.7 mM) and 10% Pd/C (0.24 g) in DMF (25 ml) by the usual method gave a solution of the amino-component which was used directly in the coupling reaction. The mixed anhydride of Z–Asn–OH (1.56 g, 5.9 mM) with pivaloyl chloride (0.65, 5.4 mM) was formed in 20 minutes under standard conditions using TEA (0.59 g, 5.8 mM) and pyridine (0.46 g, 5.8 mM) employing DMF (10 ml) as solvent. The amino-component from the hydrogenolysis was then added followed by TEA (0.47 g, 4.7 mM) and the reaction mixture stirred overnight. 1 M $NaHCO_3$ solution was then added to precipitate the product which was washed alternately with 1 M AcOH and 1 M $NaHCO_3$ solutions. The crude product was reprecipitated from DMF/H_2O and DMF/Et_2O giving the required amorphous product **59** (4.87 g, 91%), m.p. 210–223°, $[\alpha]_D^{25}$ – 20.6° (c = 1.4, DMF), $R_f(25)$ – 0.6, $R_f(23)$ – 0.8, $Orn_{0.98}Asp_{1.99}Thr_{0.96}Glu_{1.01}Gly_{1.00}$. (Found: C, 58.91; H, 7.20; N, 10.71. $C_{57}H_{81}N_9O_{16}.H_2O$ requires: C, 58.70; H, 7.17; N, 10.81%).

1–Thr(Bu^t)–Asn–Orn(Adoc)–Asn–Thr(Bu^t)–Glu(OBu^t)–Gly–OPh **60**. The hexapeptide **59** (2.01 g, 1.75 mM) and Tos.OH.H_2O (0.34 g, 1.75 mM) were dissolved in DMF (10 ml), 10% Pd/C (88 mg) was added and the reaction mixture hydrogenolysed for 18 hours. Filtration gave a clear solution of the corresponding *p*-toluene-sulphonate to which was added Z–Thr(Bu^t)–ONSu[9] (0.93 g, 2.3 mM) and NMM (0.18 g, 1.75 mM). The solution was stirred for 5 days at room temperature then concentrated to a small volume and the crude product (2.18 g) precipitated by the addition of 1 M $NaHCO_3$ solution. Gel filtration on Sephadex LH 20 eluting with DMF was then carried out, the product **60** eluted at Ve/Vt = 0.43 and was precipitated after concentration of the fractions, by the addition of water giving (1.74 g, 80%), m.p. 233–235°, $[\alpha]_D^{25}$ – 4.0° (c = 1.5, DMF), $R_f(17)$ – 0.7, $R_f(23)$ – 0.8, $Orn_{0.95}Asp_{2.03}Thr_{1.91}Glu_{1.04}Gly_{1.02}$. (Found: C, 58.41; H, 7.20; N, 10.51. $C_{65}H_{96}N_{10}O_{18}.2H_2O$ requires: C, 58.18; H, 7.15; N, 10.44%).

Bpoc–Phe–Asn–Thr(Bu^t) Gln–Ala–Thr(Bu^t)–Asn–Orn(Adoc)–Asn–Thr(Bu^t)–Glu(OBu^t) Gly OPh **61**. *Bpoc*(38–49)OPh. The heptapeptide **60** (0.65 g, 0.5 mM) and Tos.OH.H_2O (95 mg, 0.5 mM) were dissolved in DMF (5 ml) and 10% Pd/C (25 mg) added. Hydrogenolysis was carried out overnight giving a product which showed a single fluorescamine-positive spot on TLC in two solvent systems. Filtration and evaporation gave a clear oil which was

dissolved in DMF (20 ml) along with Bpoc(38–42)OH **53** (0.52 g, 0.6 mM).

After cooling to −10° HONSu (0.14 g, 1.2 mM), DCCI (0.14 g, 0.66 mM) and NMM (0.5 ml of a 1 mM/ml DMF solution, 0.5 mM) were added and the solution stirred for 24 h. It was then cooled to −10° and HONSu (69 mg, 0.6 mM) and DCCI (68 mg, 0.3 mM) added, stirring being continued for a further 2 days. The total reaction mixture was then applied to Sephadex LH 20 and eluted with DMF, the required product **61** eluting with Ve/Vt = 0.36. The appropriate fractions were combined and evaporated; the homogeneous fully protected amorphous dodecapeptide **61** being obtained in 63% yield, (0.64 g), m.p. 350° (dec), $[\alpha]_D^{25}$ − 6.0° (c = 1.1, DMF), $R_f(7)$ − 0.7, $R_f(23)$ − 0.75, $R_f(3)$ − 0.7, $Orn_{0.98}Asp_{2.92}Thr_{2.76}Glu_{2.07}Gly_{1.01}Ala_{0.98}Phe_{0.99}$. Enzyme digest (pronase/AP M) on deprotected peptide: $Orn_{0.95}$ Thr + Asn + Gln (overlapping)$_{5.71}$ $Glu_{1.03}$ $Gly_{0.94}Ala_{1.11}Phe_{1.00}$. Material isolated from electrophoresis pH 2.1 of deprotected material $E^{2.1}_{Dnp.Lys}1.8$, $Orn_{0.97}$ $Asp_{2.87}Thr_{2.57}Glu_{2.07}Gly_{1.03}Ala_{1.05}Phe_{1.03}$. (Found: C, 58.68; H, 7.31; N, 11.19. $C_{102}H_{147}N_{17}O_{26}\cdot 3H_2O$ requires: C, 58.85; H, 7.41; N, 11.44%).

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