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SYNTHESIS OF THE 27–37 FRAGMENT OF A LYSOZYME ANALOGUE

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Abstract—Two syntheses of the fully protected 27–37 fragment have been carried out. The results indicate that coupling of the 27–32 and 33–37 fragments is superior to the alternative 27–29 plus 30–37 approach.

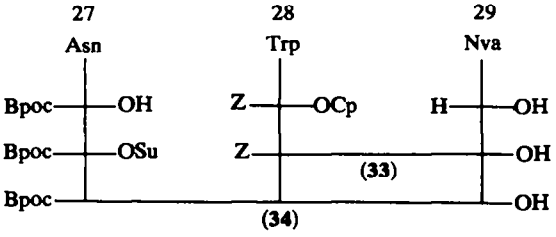
Here we describe the synthesis of the (27–37) fragment of our lysozyme analogue,[1] the sequence of this fragment being given below.

Asn.Trp.Nva.Cys.Ala.Ala.Lys.Phe.Glu.Ser.Gly.
27 30 33 37

The strategy of maximal protection has been used and the overall methodology follows that outlined in a previous paper.[1]

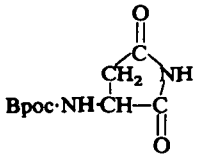
By inspection of the fragment it is clear that there are no racemisation free coupling points for fragment condensation and in the initial mode of synthesis it was decided to construct the molecule by fragment coupling at norvaline. This tri- plus octapeptide route could be criticised since it involved the stepwise build up of the (30–37) octapeptide. Whilst this is by no means impossible, in general we have found it best to restrict stepwise syntheses to approximately the hexapeptide, level, as yields and reaction times are often unacceptable when working on fragments over this size.

The tripeptide fragment was synthesised by the route outlined in Scheme 1.



Scheme 1. Synthesis of the protected (27–29) tripeptide (**34**).

N-Benzyloxycarbonyltryptophan 2,3,5-trichlorophenylester was coupled with norvaline by a salt coupling, the protected dipeptide acid (**33**) was obtained in virtually quantitative yield although a trace of the phenol remained after work up. A small sample was purified further for total characterisation and the remainder taken through to the next step in the slightly impure form. Hydrogenolysis for 7 hr in a mixture of methanol and 10% aqueous acetic acid over 5% Pd/C catalyst gave the crystalline dipeptide acetate in 95% yield. If the corresponding 10% catalyst was used in this reaction, it was found that several minor impurities were observed; this may be attributed to side-reactions involving tryptophan. The dipeptide acetate was coupled to *p*-biphenylylisopropoxycarbonylasparagine N-hydroxy-succinimide ester in the presence of triethylamine, and the protected tripeptide acid (**34**) was obtained in 57% yield. The active ester used in this reaction was prepared, as a dry foam, by a 3 hr reaction at 0° immediately before use. This was done in order to minimise any decomposition of the active ester as we found that during a longer reaction time, or prolonged storage, significant quantities of the cyclic imide shown below were formed. The route to the protected



(30–37) sequence is shown in Scheme 2 starting with the coupling of α-2,4,5-tri-chlorophenyl γ-t-butyl benzyloxycarbonylglutamate to the serine derivative illustrated. As the resulting protected dipeptide (**35**) was contaminated with 2,4,5-trichlorphenol it was converted to the hydrazide (**35a**) without further purification. The hydrazide (**35a**) was treated with t-butyl nitrite under standard conditions and the resulting azide coupled to glycine phenyl ester hydrobromide to yield the protected tripeptide (**36**). Residues 31 to 34 were then introduced as their 2,4,5-trichlorophenyl esters; the

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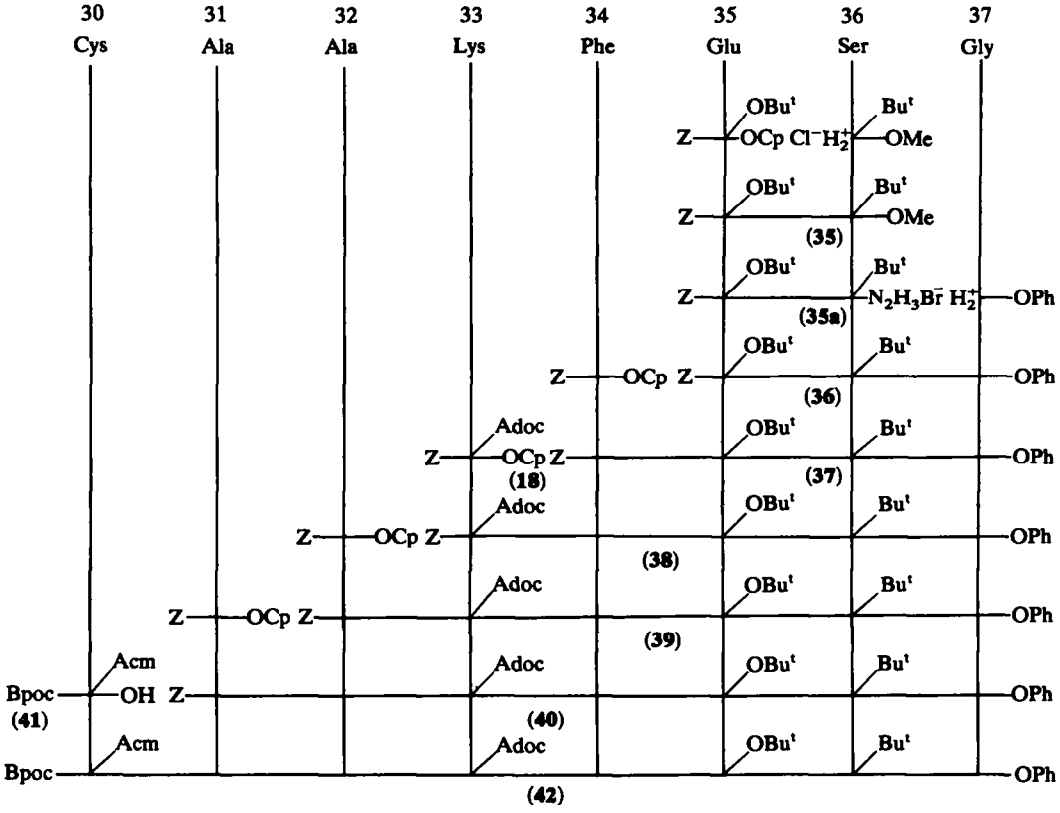


Scheme 2. Synthesis of the protected (30–37) octapeptide (**42**).

yield at each step being in the 80–90% region. acetamidometh-N-*p*-Biphenylylisopropoxycarbonyl-S-yl-cysteine (**41**) was prepared by treatment of S-acetamidomethyl cysteine hydrochloride with *p*-biphenylyloxycarbonyl azide in the presence of tetramethylguanidine. The cysteine derivative (**41**) was then coupled to the *p*-toluenesulphonate of the dydrogenolysed heptapeptide (**40**) using the N,N'-dicyclohexylcarbodiimide method incorporating N-hydroxysuccinimide as an additive. The final product (**42**) being obtained as a white glassy solid after purification by chromatography on silica gel eluting with chloroform/isopropanol (9/1).

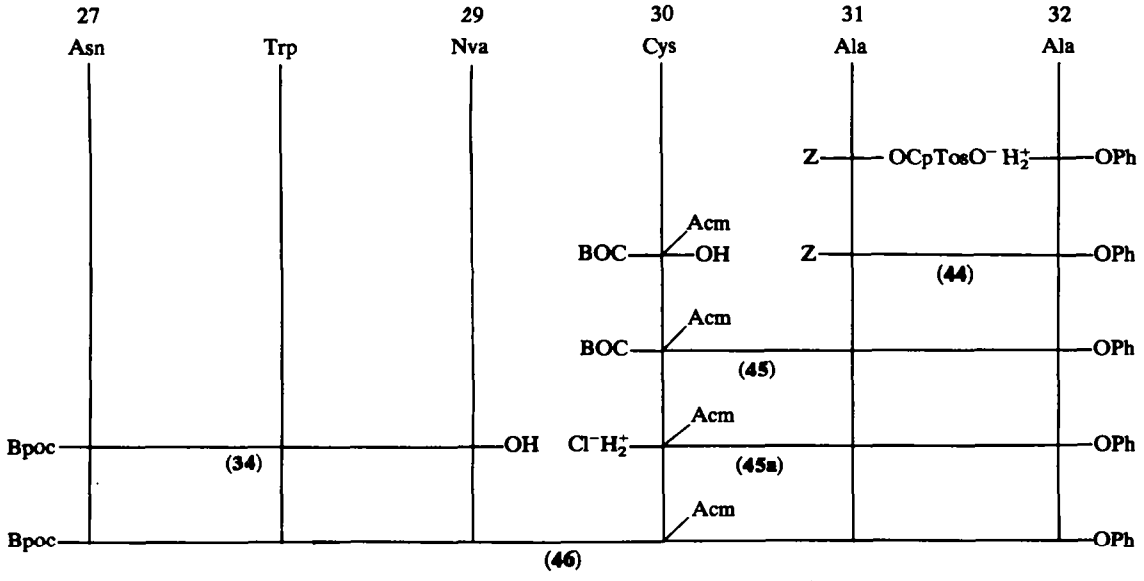
The N^{α}-protecting group was then removed from the fragment (**42**) by a 2 hr treatment with a mixture of formic acid, acetic acid and water (7:1:2). The resulting formate was converted to the corresponding hydrochloride by treatment with hydrogen chloride in DMF in order to avoid any possibility of formylation in the subsequent coupling step. This hydrochloride was then coupled with the protected tripeptide acid (**34**) by the N,N'-dicyclohexylcarbodiimide method with N-hydroxysuccinimide as additive. The crude undecapeptide (**43**) was obtained in 97% yield but could not readily be purified by gel filtration on Sephadex LH20 eluting with DMF. The only successful purification was achieved by chromatography on silica gel eluting with chloroform/isopropanol (5/1), unfortunately heavy losses were encountered and an overall yield of only 33% was achieved.

In order to circumvent this problem we reconsidered the strategy of synthesis, reasoning that a hexa- plus pentapeptide coupling might result in a product which could be purified more easily. The marginal disadvantage of this route was that, as envisaged, it involved two fragment couplings at points which were susceptible to racemisation whereas the former route only involved one such coupling.

The protected (27–32) fragment was synthesised by the route shown in Scheme 3 below, utilising the previously synthesised fragment (**34**).

The protected dipeptide (**44**), prepared by the active ester shown in 86% yield, was hydrogenolysed in the usual way and coupled with N-t-butyloxycarbonyl-S-acetamido-methylcysteine[4] by the pivaloyl mixed anhyride. The resulting protected tripeptide (**45**) was obtained in 85% yield by crystallisation from ethyl acetate/petroleum ether after removal of non-neutral materials by washing. The t-butyloxycarbonyl group was then removed by treatment with 2 M hydrogen chloride in dioxan using anisole as a scavenger- the hydrochloride (**45a**) crystallising from the reaction mixture. A slight excess of the protected tripeptide acid (**34**) was then coupled with the hydrochloride (**45a**) by the N,N'-dicyclohexylcarbodiimide/N-hydroxysuccinimide method using N-methylmorpholine as base. After 2 days the mixture was filtered and the filtrate applied directly to Sephadex LH20 eluting with DMF. The product (**46**) eluted with Ve/Vt = 0.44 and was obtained in 53% yield by precipitation with water.

The phenyl ester was removed by treating an aqueous DMF solution of (**46**) with 1 M NaOH in the



Scheme 3. Synthesis of the protected (27–32) fragment (**45**).

Bpoc.Asn.Trp.Nva.Cys(Acm).Ala.Ala.Lys(Adoc).Phe.Glu(OBu^t).Ser(Bu^t).Gly.OPh

(**43**)

presence of hydrogen peroxide.[8] At pH 10.5 the cleavage was complete in 15 min as evidenced by tlc and base uptake. During the cleavage dimethylsulphide was present in order to prevent the possibility of oxidation of the cysteinyl peptide. The crystalline protected hexapeptide acid was then coupled to the *p*-toluenesulphonate obtained from (**38**) (see Scheme 2) by the N,N′-dicyclohexylcarbodiimide/N-hydroxysuccinimide method using N-methylmorpholine as base. After 2 days the mixture was filtered and the filtrate applied directly to a column of Sephadex LH20 eluting with DMF, the required undecapeptide (**43**) being obtained in 68% yield.

If the two routes are compared it may be calculated that the second route gives approximately double the yield found in the first instance. The major contribution to this improvement comes from the improved yield in the final fragment coupling. As may be seen from the experimental section, the physical properties of the products from the two routes are identical. An important indicator of optical purity is provided by the close agreement of the optical rotations (−24.6° and −25.3° for the two routes respectively) as it is unlikely that such agreement would be obtained if significant racemisation has occurred during any of the fragment couplings. The optical purity of the protected undecapeptide (**43**) synthesised by the second route was confirmed by enzymic digestion of the S-acetamidomethyl fully deprotected peptide. The homogeneity of the deprotected peptide was also confirmed by paper electrophoresis at pH 2.8 and 6.5 and by isoelectric focussing on polyacrylamide gels.

EXPERIMENTAL

The abbreviations, tlc systems and general experimental methods are detailed in a preceding paper.[9]

Scheme 1

Z-Trp-Nva-OH (**33**). A soln of Z-Trp-OCp (50.6 g, 98 mM) in DMF (75 ml) was added to a soln of H-Nva-OH (11.0 g, 93 mM) in water (25 ml) and the resulting soln stirred for 4 days. The solvent was evaporated and the residue dissolved in EtOAc (200 ml), this soln was treated with 3-dimethylaminopropylamine (2.0 ml, 16.7 mM) for 30 min. This soln was washed with 10% citric acid, water and sat NaCl aq; drying and evaporation gave a foam which was crystallised from EtOAc-petroleum ether. A quantitative yield was achieved, however tlc indicated the presence of very slight traces of HOCp $R_f(17)-0.7$. The bulk of this material was used directly in the subsequent coupling and a purified sample, obtained by repeated recrystallisation from EtOAc petroleum–ether, was used for the determination of analytical data; m.p. 116–117°, $[\alpha]_D^{21}-21.4°$ ($c=2$, DMF), $R_f(17)-0.6$, $R_f(23)-0.8$, $R_f(24)-0.6$, (Found; C, 64.66; H, 6.44; N, 9.06. $C_{24}H_{27}N_3O_5.0.5H_2O$ requires: C, 64.56; H, 6.32; N, 9.41%).

Bpoc-Asn-Trp-Nva-OH (**34**). The slightly impure protected dipeptide acid (**33**) (10.0 g, 23 mM) was dissolved in MeOH (45 ml), 10% aqueous AcOH (30 ml) and 5% Pd/C (1.5 g) were added and the solution hydrogenolysed for 7 hr. Filtration and evaporation yielded an oil which crystallised on trituration with Et_2O giving (8.0 g, 95%), m.p. 262° (dec), $R_f(23)-0.55$. The dipeptide acetate was dissolved in DMF (100 ml) and added to Bpoc-Asn-ONSu (11.7 g, 25 mM), TEA (6.2 ml, 44 mM) was then added and the soln stirred for 20 hr. Bpoc-Asn-ONSu was prepared as a dry foam by reacting Bpoc-Asn-OH (13.9 g, 37.5 mM) with HONSu (4.6 g, 40 mM) and DCCl (9.1 g, 44 mM) in DMF (60 ml) at 0° for 3 hr. The mixture was filtered and evaporated, the resulting oil being dissolved in EtOAc (60 ml) and cooled to −60° for 10 min. Filtration and evaporation yielded the rather unstable active ester (17.5 g, 100%). The solvent was evaported and the residue dissolved in EtOAc, this soln was partitioned with 5% citric acid, washed with water and sat NaCl aq then dried. Evaporation gave a crystalline product which was recrystallised from EtOAc petroleum-ether yielding (8.2 g, 57%), m.p. 139°, $[\alpha]_D^{21}-28.9°$ ($c=2$, DMF), $R_f(17)-0.4$, $R_f(14)-0.55$, APM digest (3 days) $Asn_{1.01}Trp_{1.20}Nva_{0.99}$ a blank accounted for the

observed high Trp, (Found: C, 65.10; H, 6.60; N, 10.35. $C_{36}H_{41}N_5O_7.0.5H_2O$ requires: C, 65.04; 6.37; N, 10.54%).

Scheme 2

Z-Glu(OBu^t)-Ser(Bu^t)-N_2H_3 (**35a**). Z-Glu(OBu^t)-OCp (28.5 g, 55 mM), $Cl^-H_2^+$-Ser(Bu^t)-OMe (11.6 g, 55 mM) and TEA (7.7 ml, 55 mM) were dissolved in DMF (200 ml) and stirred for 4 days. Evaporation gave an oil which was dissolved in EtOAc (500 ml), this soln was washed in the usual way, dried and evaporated to yield a yellow oil (59.0 g). The nmr spectrum and tlc $R_f(26)-0.65$, 0.57 showed this oil to be a mixture of HOCp and (**35**) in the approximate ratio 2:5. This mixture, *ca* 5 mM of (**35**) in MeOH (25 ml) was treated with hydrazine hydrate (1.0 ml, 21 mM) for 12 hr. Evaporation of the solvent and excess hydrazine gave a gum which was crystallised from a mixture of EtOAc and petroleum–ether giving (**35a**) (1.7 g, 69% based on $Cl^-H_2^+$-Ser(Bu^t)-OMe), m.p. 133–134°, $[\alpha]_D^{21}+5.3°$ ($c=3$, DMF), $R_f(25)-0.6$, (Found: C, 58.21; H, 7.68; N, 11.31. $C_{24}H_{38}N_4O_7$ requires: C, 58.28; H, 7.74; N, 11.33%).

Z-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**36**). A soln of 8.6 M HCl in dioxan (0.93 ml, 8 mM) was added to a soln of (**35a**) (0.99 g, 2 mM) in DMF (8 ml) at −30°. The temp was raised to −25° and t-butyl nitrite (0.93 ml, 8.1 mM) added, after stirring for 15 min the temp was lowered to −30° prior to the addition of TEA (1.1 ml, 8 mM). A precooled soln of $Br^-H_2^+$-Gly-OPh (0.49 g, 2.1 mM) and TEA (0.3 ml, 2.1 mM) in DMF (7 ml) was added and the reaction stirred at 0° for 3 days. After filtration, evaporation gave a residue which was dissolved in EtOAc. Washing in the usual way, drying and evaporation give a solid which was crystallised from EtOAc and petroleum–ether yielding **36** (1.0 g, 82%), m.p. 121–122°, $[\alpha]_D^{22}-1.1°$ ($c=3$, DMF), $R_f(26)-0.4$, $Ser_{0.79}Glu_{0.98}$-$Gly_{1.00}$, (Found: C, 62.67; H, 6.87; N, 6.98. $C_{32}H_{43}N_3O_9$ requires: C, 62.62; H, 7.06; N, 6.85%).

Z-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**37**). A soln of (**37**) (14.1 g, 23 mM) in DMF (400 ml) was hydrogenolysed for 12 hr in the presence of 8.1 M HCl) in dioxan (3.4 ml), 27.5 mM) and 10% Pd/C (2.0 g). Work up in the usual way yielded the corresponding hydrochloride $R_f(25)-0.6$. The hydrochloride was dissolved in DMF (100 ml), Z-Phe-OCp (12.2 g, 25.5 mM) and TEA (3.22 ml, 23 mM) added, and the mixture stirred for 3 days. Evaporation gave a residue which was dissolved in $CHCl_3$ (50 ml). Washing in the usual way, drying and evaporation produced a residue which was crystallised from EtOAc and petroleum–ether giving (**37**) (16.0 g, 91%), m.p. 160–161°, $[\alpha]_D^{22}-5.9°$ ($c=3$, DMF), $R_f(26)-0.3$, $R_f(27)-0.8$, $Ser_{0.97}Glu_{1.00}Gly_{1.00}Phe_{1.00}$ +corrected for decomposition by extrapolation, (Found: C, 64.36; H, 6.97; N, 7.53. $C_{41}H_{52}N_4O_{10}$ requires: C, 64.72; H, 6.89; N, 7.36%).

Z-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**38**). The tetrapeptide derivative (**37**) (14.5 g, 18 mM) dissolved in DMF (400 ml) was hydrogenolysed for 14 hr in the presence of 8.8 M HCl in dioxan (2.5 ml, 22 mM) and 10% Pd/C (1.8 g). The corresponding hydrochloride, $R_f(25)-0.75$, was obtained by work up in the usual way. This salt (**18**) (16.0 g, 25 mM) and TEA (2.66 ml, 19 mM) were dissolved in DMF (100 ml) and the resulting soln stirred for 4 days. Evaporation gave a residue which was dissolved in $CHCl_3$ (400 ml), this soln was washed and dried in the usual way. A solid was obtained by evaporation, and this was recrystallised from EtOAc and petroleum–ether affording (**38**) (18.3 g, 90%), m.p. 190–191°, $[\alpha]_D^{21}-17.2°$ ($c=2$ DMF), $R_f(2)-0.7$, $R_f(27)-0.7$, $Lys_{0.93}Ser_{0.85}Glu_{1.02}Gly_{1.04}Phe_{1.01}$ (Found: C, 65.21; H, 7.30; N, 8.09. $C_{58}H_{78}N_6O_{13}$ requires: C, 65.27; H, 7.37; N, 7.88%).

Z-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**39**). The protected pentapeptide (**38**) (17.1 g, 16 mM) was dissolved in DMF (350 ml) containing 5.0 M HCl in dioxan (3.8 ml, 19 mM) and hydrogenolysed for 12 hr over 10% Pd/C catalyst (1.6 g). Filtration and evaporation yielded the corresponding hydrochloride as a white solid, this was dissolved in DMF (80 ml) and Z-Ala-OCp (7.7 g, 19 mM) added along with TEA (2.24 ml), 16 mM). After stirring at 20° for 3 days the solvent was evaporated and the residue disssolved in $CHCl_3$, this soln was subjected to an acidic and basic washing in the usual way then dried. Evaporation, followed by trituration with Et_2O gave (**39**) as an amorphous solid (16.1 g, 88%), m.p. 195–197°, $[\alpha]_D^{21}-19.3°$ ($c=2$, DMF), $R_f(2)-0.8$, $Lys_{0.93}Ser_{0.98}Glu_{1.04}Gly_{1.03}Ala_{1.02}Phe_{1.02}$ +corrected for decomposition by extrapolation, (Found, C, 64.15; H, 7.43; N, 8.74. $C_{61}H_{83}O_{14}N_7$ requires: C, 64.35; H, 7.35; N, 8.61%).

Z-Ala-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**40**). A soln of (**39**) (15.4 g, 13.5 mM) in DMF (300 ml) was hydrogenolysed for 16 hr in the presence of 5.0 M HCl in dioxan (3.2 ml, 16 mM) and 10% Pd/C catalyst (1.4 g). Work up in the usual way gave a homogeneous white solid $R_f(2)-0.42$ which was dissolved in DMF (100 ml). Z-Ala-OCp (6.4 g, 16 mM) and TEA (1.89 ml, 13.5 mM) were added and the soln stirred for 4 days. At this stage the mixture still showed a ninhydrin positive spot on tlc; the volume of the soln was reduced to 60 ml and additional quantities of Z-Ala-OCp (3.12 g, 8 mM) and TEA (0.38 ml, 2.7 mM) added. After a further 2 days the reaction was complete. Work up in a similar manner to compound (**39**) gave a white amorphous solid which was slowly reprecipitated from MeOH with Et_2O yielding (**40**) (13.6 g, 83%) m.p. 226–228°, $[\alpha]_D^{21}-11.7°$ ($c=1.5$, DMF), $R_f(2)-0.7$, $R_f(25)-0.7$, $Lys_{0.94}Ser_{0.95}Glu_{1.08}Gly_{1.00}$-$Ala_{2.01}Phe_{0.98}$ +corrected for decomposition by extrapolation, (Found: C, 63.48; H, 7.25; N, 9.25. $C_{64}H_{88}O_{15}N_8$ requires: C, 63.56; H, 7.33, N, 9.27%).

N-p-Biphenylylisopropoxycarbonyl-S-acetamidomethyl-cysteine (**41**). *p*-Biphenylyloxycarbonyl azide (15.4 g, 55 mM) and tetramethylguanidine (9.6 g, 84 mM) were added to a soln of S-acetamidomethylcysteine hydrochloride (9.6 g, 42 mM) in DMF (150 ml) and stirred for 2 days. Evaporation of the solvent gave an oil which was dissolved in water and extracted with Et_2O. The aqueous phase was cooled to 0° and acidified with ice-cold 10% citric acid. The resulting oil was extracted into EtOAc, this soln was then washed with water, dried and evaporated to yield a dry foam (13.8 g, 76%), (this dry foam may be converted into an amorphous powder by dissolving in EtOAc and slowly adding this soln to rapidly stirred petroleum–ether) m.p. 39–40°, $[\alpha]_D^{23}-36.0°$ ($c=2$, DMF), $R_f(3)-0.3$, $R_f(17)-0.3$. The compound was further characterised as the dicyclohexyl-ammonium salt: compound (**41**) (4.4 g, 10 mM) was dissolved in MeOH (25 ml) and DCHA (1.9 g, 10 mM) added. After 20 min the solvent was evaporated and the salt crystallised by trituration with petroleum–ether giving (5.5 g, 86%), m.p. 93–98°, (Found: C, 66.70; H, 8.12; N, 6.60. $C_{34}H_{49}N_3O_5S$ requires: C, 66.74; H, 8.07; N, 6.87%).

Bpoc-Cys(Acm)-Ala-Ala-Lys(Adoc)-Phe-Glu(OBu^t)-Ser(Bu^t)-Gly-OPh (**42**). The derivative (**40**) (5.2 g, 4.3 mM) was dissolved in DMF (120 ml) containing 4.7 M HCl in dioxan (1 ml, 4.7 mM) and hydrogenolysed for 15 hr in the presence of 10% Pd/C (0.25 g). Work up in the normal way gave the hydrochloride in quantitative yield. This hydrochloride, Bpoc-Cys(Acm)-OH (**41**) (2.3 g, 5.3 mM) and NMM (0.48 g, 4.3 mM) were dissolved in DMF (25 ml) and cooled to −20°. HONSu (1.2 g, 106 mM) and DCCl (1.2 g, 5.6 mM) were added and the mixture stirred for 5 days after attaining room temp during the first 2 hr. Filtration followed by evaporation yielded an oil which on trituration with EtOAc gave a

pale yellow solid. This was purified by chromatography on Silica gel 60 eluting with $CHCl_3$/^{i}PrOH (9/1), evaporation of appropriate fractions and reprecipitation from DMF/H_2O gave the protected octapeptide (**42**) (2.8 g, 45%), m.p. 234° (dec), $[\alpha]_D^{22}$ − 17.3° (*c* = 1, DMF), $R_f(2)$ − 0.6, $R_f(25)$ − 0.7, $R_f(27)$ − 0.5,. $Lys_{0.91}Ser_{0.85}Glu_{1.04}$-$Gly_{1.00}Ala_{2.00}Phe_{1.01}$, (Found: C, 62.24; H, 7.27; N, 9.44; S, 2.20. $C_{78}H_{106}O_{17}N_{10}S.H_2O$ requires: C, 62.21; H, 7.23; N, 9.30; S, 2.15%).

Scheme 3

Z-Ala-Ala-OPh (**44**). A soln of Z-Ala-OCp (64.4 g, 160 mM), $TosO^-H_2^+$-Ala-OPh (54.0 g, 160 mM) and TEA (22.4 ml, 160 mM) in DMF (300 ml) was stirred for 3 days. The solvent was evaporated and the residue dissolved in EtOAc (500 ml). This soln was washed in the usual way, dried and evaporated to yield the crude product. Recrystallisation from EtOAc petroleum–ether gave (**44**) (51.2 g, 86%), m.p. 126–127°, $[\alpha]_D^{21}$ − 45.2° (*c* = 4, DMF), $R_f(25)$ − 0.7, $R_f(26)$ − 0.4, (Found: C, 64.73; H, 5.65; N, 7.79. $C_{20}H_{22}N_2O_5$ requires: C, 64.85; H, 5.99; N, 7.56%).

BOC-Cys(Acm)-Ala-Ala-OPh (**45**). Z-Ala_2-OPh (**44**) (12.9 g, 35 mM) in DMF (350 ml) was hydrogenolysed overnight in the presence of Tos.OH.H_2O (6.7 g, 35 mM) and 10% Pd/C (1.7 g). After checking that the cleavage was complete by the R_f(23)–0.6 the soln was filtered and the filtrate reduced to 200 ml. BOC-Cys(Acm)-OH (11.8 g, 40 mM) was dissolved in 50% CH_2Cl_2/DMF (80 ml) and cooled to −10°, Piv. Cl (4.6 g, 40 mM) and TEA (5.7 ml, 40 mM) were added and 15 min allowed for activation. The DMF soln from the hydrogenolysis was added together with TEA (4.9 ml, 35 mM) and the reaction allowed to attain room temp overnight. Evaporation yielded a residue which was dissolved in EtOAc; the soln was washed with 5% $NaHCO_3$, 5% citric acid and sat NaClaq. After drying, an equal volume of petroleum–ether was added to bring about crystallisation, this yielded (**45**) (15.1 g, 85%), m.p. 173–174°, $[\alpha]_D^{21}$ −48.5° (*c* = 2, DMF), $R_f(25)$ − 0.6, $R_f(27)$ − 0.50, (Found: C, 54.06; H, 6.67; N, 10.67. $C_{23}H_{34}N_4O_7S$ requires: C, 54.10; H, 6.71; N, 10.97%).

Bpoc-Asn-Trp-Nva-Cys(Acm)-Ala-Ala-OPh (**46**). The protected tripeptide (**45**) (2.6 g, 5 mM) was dissolved in a 2 M HCl in dioxan soln (25 ml) containing anisole (2.5 ml). After stirring for 1 hr, (**45a**) had crystallised out, the soln was filtered and the product washed with Et_2O yielding (2.3 g, 100%), $R_f(17)$ − 0.3, $R_f(23)$ − 0.3, $R_f(25)$–0.2. A soln of (**45a**) (2.3 g, 5 mM), the tripeptide acid (**34**) (3.6 g, 5.4 mM) and NMM (0.55 ml, 5 mM) was cooled to −20°; HONSu (1.3 g, 10.8 mM) and DCCI (1.3 g, 6.3 mM) were added and the soln stirred for 2 days. After filtration the mixture was applied directly to Sephadex LH20 eluting with DMF. The product, having Ve/Vt = 0.44, was obtained by precipitation with water and washing with Et_2O yielding (**46**) (2.8 g, 53%) m.p. 180–182°, $[\alpha]_D^{21}$ − 4.2° (*c* = 2, DMF), $R_f(17)$ − 0.65, $R_f(25)$ − 0.5, $R_f(27)$ − 0.2, $Asp_{0.98}Ala_{2.07}Nva_{1.00}$, (Found: C, 59.95; H, 6.24; N, 11.51. $C_{54}H_{65}N_9O_{11}S.2H_2O$ requires: C, 59.81; H, 6.41; N, 11.63%).

Bpoc(27–37)*OPh*

First route—$Cl^-H_2^+$*(30–37)OPh* (**42a**). The fully protected octapeptide (**42**) (1.5 g, 1 mM) was treated for 2 hr with AcOH/H.CO_2H/H_2O (7/1/2) (25 ml). The solvent was evaporated and the residue thoroughly triturated with EtOAc and Et_2O. The resulting white solid was treated with 0.046 M HCl in DMF (50 ml, 2.3 mM) for 10 min then evaporated and the exchange process repeated. Finally trituration with Et_2O yielded (**42a**) as a white amorphous solid (1.2 g, 92%), $R_f(25)$ − 0.4.

Bpoc(27–37)*OPh* (**43**). A mixture of (**42a**) (0.44 g, 0.34 mM), the protected tripeptide acid (**34**) (0.33 g, 0.5 mM) and TEA (0.5 ml, 0.35 mM) in DMF (8 ml) was treated with HONSu (0.12 g, 1 mM) and DCCI (0.11 g, 0.55 mM) at −20° for 1 hr, then at 20° for 3 days. Further portions of (**34**) (0.11 g, 0.16 mM), HONSu (30 mg, 0.35 mM) and DCCI (35 mg, 0.17 mM) had to be added in order to ensure complete reaction. Filtration and evaporation gave a residue which was triturated with Et_2O and EtOAc, giving the crude (**43**) (0.63 g, 97%) as a pale yellow solid. Purification by chromatography on silica gel 60 with $CHCl_3$/^{i}PrOH (5/1) gave the pure (**43**) (0.165 g, 33%), m.p. 255° dec, $[\alpha]_D^{22}$ − 24.6° (*c* = 1, DMSO), $R_f(25)$ − 0.6, $R_f(27)$ − 0.25, $Lys_{0.97}Asp_{0.93}Ser_{0.89}Glu_{0.99}$-$Gly_{1.02}Ala_{2.00}Nva_{0.91}Phe_{1.01}$, (Found: C, 61.58; H, 6.90; N, 10.74. $C_{98}H_{131}O_{21}N_{15}S.H_2O$ requires: C, 61.78; H, 7.04; N, 11.03%).

Second route—Bpoc(27–32)*OH* (**46a**). Compound **46** (0.79 g, 0.75 mM) was dissolved in DMF (24 ml) and water (6 ml); DMS (1.5 ml) was added, and the pH brought to 10.5 with 1 M NaOH. 100 Volume H_2O_2 (0.08 ml, 0.8 mM) was added and the pH maintained at the above value for 15 min with 1 M NaOH. The pH was then brought to 3.5–4.0 by the addition of ice cold 5% citric acid and the solon volume reduced by evaporation. Pouring into sat NaCl aq gave a ppt which was washed with water and ether, then dried giving (**46a**) (0.64 g, 88%), m.p. 148–150°, $[\alpha]_D^{24}$ − 14.7° (*c* = 1, HMPA), $R_f(17)$ − 0.1, (Found: C, 57.36; H, 6.33; N, 12.37. $C_{48}H_{61}N_9O_{11}S.2H_2O$ requires: C, 57.18; H, 6.50; N, 12.51%).

Tos.O$^-H_2^+$*(33–37)OPh* (**38a**). A soln containing (**38**) (5.3 g, 5 mM), Tos.OH.H_2O (1 g, 5.3 mM) and 10% Pd/C (0.5 g), was hydrogenolysed for 12 hr. Filtration and evaporation gave a residue which was solidified by trituration with Et_2O, filtration and drying gave (**38a**) (5.6 g, 100%), m.p. 173–174°, $[\alpha]_D^{21}$ + 6.3° (*c* = 2, DMF), $R_f(25)$ − 0.7.

Bpoc(27–37)*OPh* (**43**). The protected hexapeptide acid (**46a**) (0.99 g, 1 mM), the *p*-toluenesulphonate (**38a**) 1.11 g, 1 mM) and NMM (0.11 ml, 1 mM) were dissolved in DMF (14 ml) and cooled to −20°. HONSu (0.25 g, 1.2 mM) and DCCI (0.25 g, 1.2 mM) were added and the mixture stirred for 2 days. After filtration the crude mixture was applied directly to a column of Sephadex LH20 eluting with DMF. The product, having Ve/Vt = 0.38, was obtained by precipitation with water and washing with Et_2O, yielding (**43**) (1.3 g, 68%), m.p. amorph chars at 255°, $[\alpha]_D^{22}$ − 25.3° (*c* = 1, DMSO), $R_f(25)$ − 0.6, $R_f(27)$ − 0.25, $Lys_{0.96}Asp_{0.99}Ser_{0.88}Glu_{1.05}Gly_{1.03}Ala_{1.97}$-$Nva_{0.96}Phe_{1.02}$, (Pronase/APM) $Trp_{0.87}Lys_{0.94}$-$Cys(Acm)_{0.91}Asn/Ser_{2.07}Glu_{1.11}Gly_{1.00}Ala_{1.96}Nva_{1.00}$-$Phe_{1.04}$, (Found: C, 61.25; H, 7.17; N, 10.68. $C_{98}H_{131}N_{15}O_{21}S.2H_2O$ requires: C, 61.20; H, 7.08; N, 10.92%).

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REFERENCES

[1]G. W. Kenner, R. Ramage and R. C. Sheppard, *Tetrahedron* **35**, 2767 (1979).

[2]J. Pless and R. A. Boissonnas, *Helv. Chim. Acta* **46**, 1609 (1963).

[3]D. Hudson, G. W. Kenner, B. Mason, B. A. Morgan, R. Ramage, B. Singh and R. Tyson, *Proceedings of the 12th Europ. Peptide Symp.*, Reinhardsbrunn, GDR, (1972); 'Peptides 1972', p 70, Ed. H. Hanson and H. D. Jakubke, North Holland (1973).

[4]D. F. Veber, J. D. Milkowski, S. L. Varga, R. G. Denkewalter and R. Hirschmann, *J. Am. Chem. Soc.* **94**, 5456 (1972).
[5]P. Sieber and B. Iselin, *Helv. Chim. Acta* **51**, 622 (1968).
[6]P. Sieber and Б. Iselin, *Ibid.* **52**, 1525 (1969).
[7]G. A. Fletcher, M. Low and G. T. Young, *J. Chem. Soc.* Perkin I, 1162 (1973): G. P. Schwartz and P. Katsoyannis, *Ibid.* Perkin I, 2890 (1973).
[8]I. J. Galpin, P. M. Hardy, G. W. Kenner, J. R. McDermott, R. Ramage, J. H. Seely and R. G. Tyson, *Tetrahedron* **35**, 2577 (1979).
[9]I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage and B. Singh, *Ibid.*, **35**, 2771 (1979).