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PEPTIDES—XXXXV

SYNTHESIS OF THE 118–129 FRAGMENT OF A LYSOZYME ANALOGUE

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Abstract—The synthesis of the (118–129) fragment of a Lysozyme analogue was achieved by the fragment coupling approach. The fragments were assembled using the DCCI/HONSu method and the (118–122), (123–126) and (127–129) subfragments were each built up in a stepwise manner. At several stages the diphenylphosphinic mixed anhydride method was found to be superior to the pivalic mixed anhydride method.

The (118–129) fragment represents the *C*-terminal dodecapeptide of the target lysozyme analogue.[1] The sequence of this dodecapeptide is shown below:

Ser.$_{118}$ Asp. Val. Ser. Ala.$_{122}$ Trp. Val. Orn. Gly.$_{126}$ Cys. Gly. Leu.$_{129}$

In accordance with our general tactics[2] fragments have been assembled by combination of smaller subfragments. On inspection it may be seen that glycine-126 is a convenient point of fragmentation and that there are no other obvious fragmentation points. In order to have the least hindered carboxyl component in a fragment coupling, the (118–126) sequence was severed at alanine-122 in the retrosynthetic planning so that the protected peptides (**141**), (**144**) and (**148**) were the initial synthetic targets required for the final assembly of (**150**). The protecting groups were those that we have routinely employed with the exception of t-butyl-ester protection on the *C*-terminal leucine residue. This protection has been used in this case since the terminal carboxyl protecting group has to remain intact until the final deprotection, unlike the intermediate ester protections which are removed at various stages during the synthesis.

The protected (127–129) fragment was synthesised by the route shown in Scheme 1. Z.Leu.OBu^t[3] was hydrogenolysed in the presence of *p*-toluenesulphonic acid to give the corresponding salt which was coupled with Z.Gly.OH by the pivalic mixed anhydride method to give the dipeptide (**140**). The dipeptide produced was obtained as a white solid but required further purification by chromatography on silica gel. Hydrogenolysis of the amino protecting group in the presence of *p*-toluenesulphonic acid gave the dipeptide salt as an oil. This was coupled with the cysteine derivative (**41**)[4] again using the pivalic mixed anhydride method. On isolation of the product, thin layer chromatography showed several impurities which were removed by gel filtration on Sephadex LH20 eluting with DMF. The required tripeptide (**141**) eluted with (Ve/Vt) ratio of 0.49 in a yield of 44%.

The (123–126) tetrapeptide was assembled in a stepwise manner using the general route shown in Scheme 2. In the initial synthesis the dipeptide (**142**) was prepared by pivalic mixed anhydride coupling between the ornithine derivative (**28**)[5] and glycine phenyl ester hydrobromide.[6] The product required silica gel chromatography for purification giving a relatively low yield. The coupling was then repeated using the diphenylphosphinic

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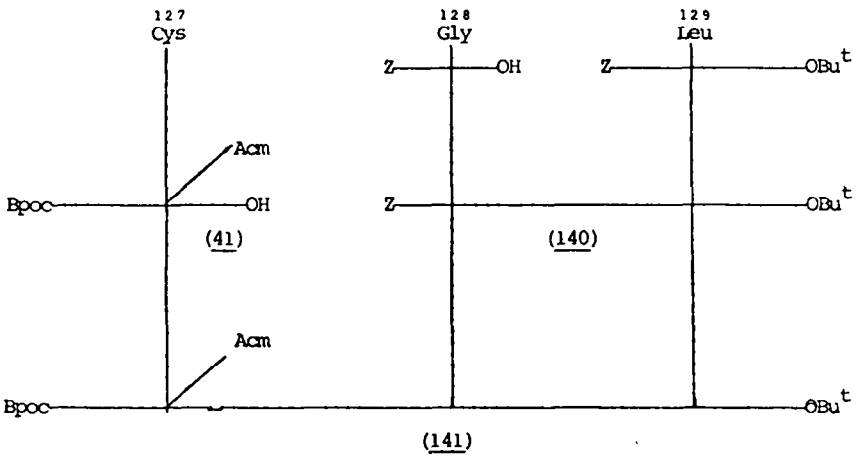
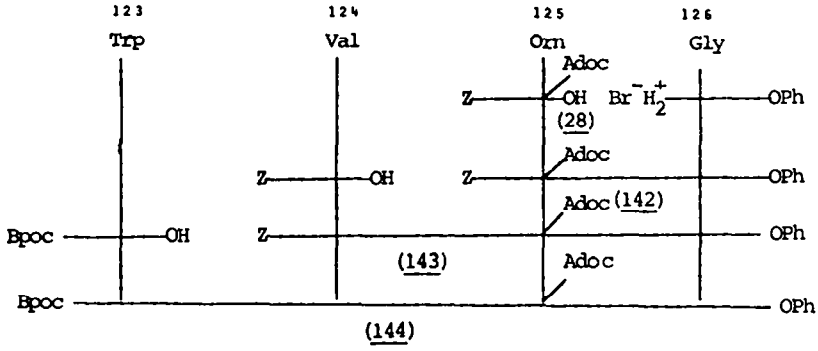


Scheme 1. Synthesis of the (127–129) tripeptide.



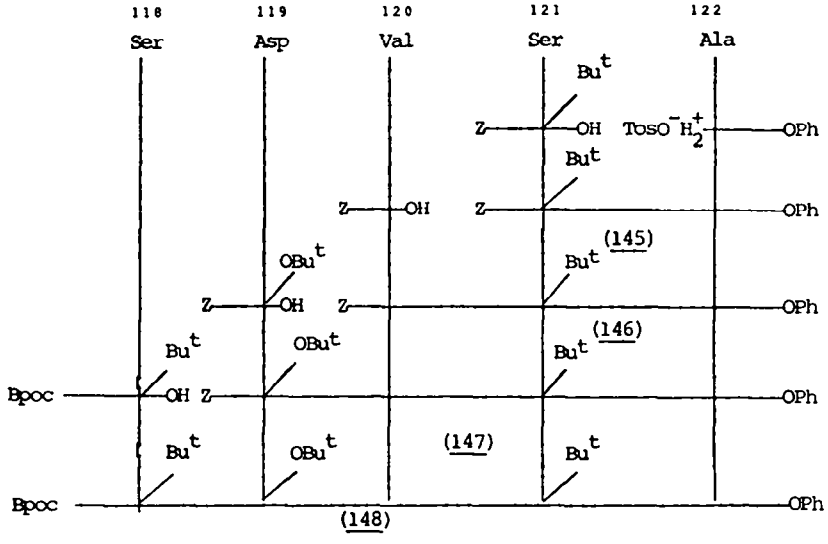
Scheme 2. Synthesis of the (123–126) tetrapeptide.

mixed anhydride method.[7] Using a slight excess of the amino component and *N*-methylmorpholine as a base a 58% yield of the dipeptide (**142**) was obtained after crystallisation. The unusual approach of using an excess of the amino component was chosen as removal of unreacted Z.Orn(Adoc).OH is difficult since the sodium salt is insoluble in water.

A trial overnight hydrogenolysis in the pesence of *p*-toluenesulphonic acid showed the presence of a low running ninhydrin negative spot on TLC in addition to the required product. Careful hydrogenolysis over 4 hr, however, gave only the required material. It is believed that the low running spot on this occasion was due to the dioxopiperazine which may be particularly readily formed in this case due to the steric constraints imposed by the bulky Adoc group permitting the amino terminus to come into close proximity with the phenyl ester. This reaction is unusual in that it proceeds even in the presence of *p*-toluenesulphonic acid which would normally be expected to protonate the free α-amino function and thus prevent any side reaction. The addition of Z.Val.OH was initially attempted by the pivalic mixed anhydride method, however using the diphenylphosphinic mixed anhydride method a higher yield was achieved. This coupling gave the tripeptide (**143**) in 64% yield after silica gel chromatography. The benzyloxycarbonyl function was then removed by hydrogenolysis in the presence of *p*-toluenesulphonic acid over 5.5 hr to afford the required salt as a colourless oil. Bpoc.Trp.OH[8] was then added, but on this occasion the pivalic mixed anhydride was the method of choice giving the required fully protected tetrapeptide (**144**) in 57% yield after gel filtration on Sephadex LH20 eluting with DMF.

The pentapeptide (118–122) was synthesised in a stepwise manner using the route shown in Scheme 3. In the assembly of this fragment the diphenylphosphinic mixed anhydride method again proved to be valuable. Both the dipeptide (**145**) and tripeptide (**146**) were initially synthesised by the pivalic mixed anhydride method. The yields for the two peptides being 52 and 64% respectively. However, when the diphenylphosphinic mixed anhydride method was used the yield improved to 75% for the dipeptide and 72% for the tripeptide. On this occasion the hydrogenolysis of the benzyloxycarbonyl function from the protected dipeptide (**145**) in the presence of *p*-toluenesulphonic acid gave rise to no dioxopiperazine. Catalytic hydrogenolysis of the tripeptide for 5.5 hr in the usual way gave the corresponding salt which was then coupled with the aspartic acid derivative shown by the pivalic mixed anhydride method giving the tetrapeptide (**147**) as a white crystalline solid in excellent yield.

A 5 hr hydrogenolysis in the presence of *p*-



Scheme 3. Synthesis of the (118–122) pentapeptide.

toluenesulphonic acid gave the tetrapeptide salt which was initially coupled with Bpoc. Ser(Bu^t).OH by the pivalic mixed anhydride method giving the pentapeptide (**148**) in 22% yield after purification on Sephadex LH20 eluting with DMF. The coupling was then repeated using Bates' reagent[9] as the activating reagent in the presence of HONSu to give a yield of 78% after crystallisation.

The assembly of the (118–129) dodecapeptide was then commenced using the three constituent subfragments (see Scheme 4). The Bpoc protection was removed from the peptide (**141**) by treatment with 0.05 M HCl in dichloromethane,[10] although later experiments used 0.05 M HCl in 90% trifluoroethanol[11] with equal effectiveness. In both cases, however, a 50-fold excess of dimethylsulphide was added to act as a carbonium ion scavenger thus protecting the Cys(Acm) from electrophilic attack. Cleavage was achieved and after 45 min the protected tripeptide hydrochloride (**141a**) was isolated in 79% yield. The phenyl ester protection was removed from the (123–126) tripeptide (**144**) using standard cleavage conditions[6] and DMF/water (6:1) as the solvent. Again a 50-fold excess of dimethylsulphide was added in water to protect the tryptophan from oxidation. The cleavage was complete within 1 hr giving the pure tripeptide acid (**144a**) in 74% yield.

Coupling of the tri- and tetrapeptide fragments (**141a**) and (**144a**) was best achieved using the DCCI/HONSu method,[12] the resulting heptapeptide (**149**) being isolated by gel filtration on Sephadex LH20 on eluting with DMF. The *N*-α-protecting group was removed by treatment with 0.05 M HCl in 90% trifluoroethanol at pH 0.5[11] with dimethylsulphide present as the scavenger. The resulting heptapeptide hydrochloride (**149a**) was obtained in excellent yield. Cleavage of the phenyl ester group from the protected pentapeptide (**148**) appeared to be particularly sensitive to solvent. When the cleavage was carried out under the standard conditions[6] using DMF/water as the solvent the reaction did not proceed to completion. However, this problem was readily overcome by using dioxan/water as the solvent for the phenyl ester cleavage.

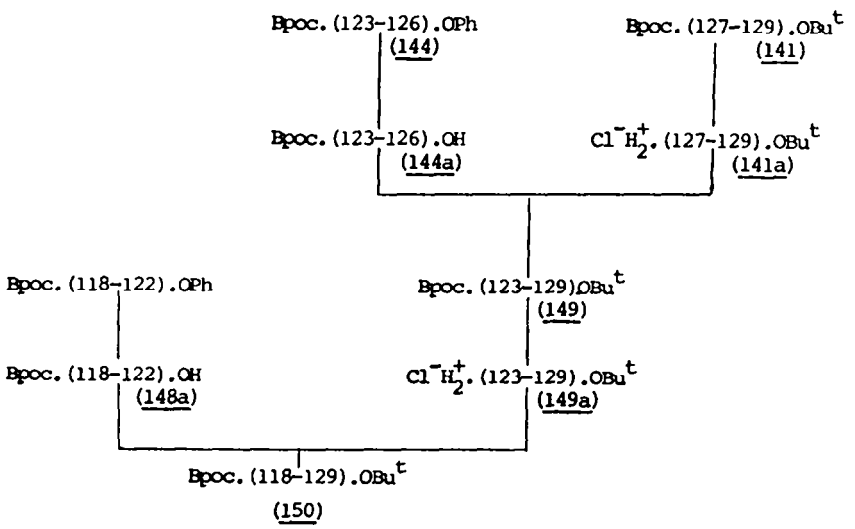
The peptide acid (**148a**) and the hydrochloride (**149a**) were then combined using the DCCI/HONSu method.[12] It was found that a mixture of HMPA and DMF were required to maintain solubility throughout the course of the reaction. Work up of the reaction mixture by direct application to Sephadex LH20 eluting with DMF gave the required dodecapeptide (**150**) in 43% yield. This product was homogeneous by all the normal physical criteria.

EXPERIMENTAL

The abbreviations, TLC systems and general experimental methods are detailed in earlier papers in this series.

Z-Gly-Leu-OBu^t (**140**). Z.Leu.OBu^t (61.0 g, 190 mM) and Tos.OH.H_2O (35.0 g, 190 mM) were dissolved in DMF (300 ml) and hydrogenolysed in the presence of 10% Pd/C (9.6 g) for 20 hr. Filtration and evaporation gave the salt as a white solid after trituration with dry Et_2O (43.0 g, 64%), m.p. 133–134°, $R_f(2) - 0.7$. Z.Gly.OH (23.0 g, 110 mM) and NMM (12.1 ml, 110 mM) were dissolved in DCM (150 ml) and cooled to −20°. Pivaloyl chloride (13.3 g, 110 mM) was added and 25 min allowed for activation. A cooled solution of the above salt (25.8 g, 110 mM) in DMF (100 ml) was added followed by NMM (11.0 ml, 100 mM) and the reaction mixture stirred at room temperature overnight. Evaporation of the solvent gave a residue which was dissolved in EtOAc, this solution was washed with acid and base in the usual way then dried and evaporated. The resulting yellow oil was purified by chromatography on silica gel (650 g) eluting with $CHCl_3$/EtOAc 2:1 the product (**140**) being crystallised from EtOAc/petroleum ether giving (27.6 g, 66%), m.p. 54–55.5°, $[\alpha]_D^{26} - 22.9$ (c = 1, DMF), $R_f(23) - 0.7$, $R_f(22) - 0.6$, $Gly_{1.00}Leu_{0.99}$, (Found: C, 63.25; H.8.05; N, 7.42; $C_{20}H_{30}N_2O_5$ requires: C, 63.47; H, 7.99; N, 7.40%).

Bpoc-Cys(Acm)-Gly-Leu-OBu^t (**141**). The dipeptide ester (**140**) (5.6 g, 14.8 mM) and Tos.OH.H_2O (2.8 g, 14.8 mM) were dissolved in DMF (100 ml) and hydrogenolysed for 6 hr in the presence of 5% Pd/C (1 g). Work up in the usual way gave the salt of the dipeptide as an oil. Bpoc.Cys(Acm).OH (**41**) (7.0 g, 16.3 mM) and NMM (1.8 ml, 16.3 mM) were dissolved in DCM (80 ml) and cooled to −20°. After cooling to −20°, pivaloyl chloride (1.9 g, 15.8 mM) was added and 20 min allowed for activation. The salt from the hydrogenolysis above was dissolved in DMF (50 ml) and cooled to 0°. This solution was then added to the mixed anhydride solution followed by NMM (1.7 ml, 15.4 mM). The reaction mixture was stirred at room temperature overnight and the solvent evaporated. The residue was dissolved in EtOAc and this solution washed with acid base in the normal way. After drying the solution was evaporated and the crude product dissolved in DMF prior to chromatography on Sephadex LH20. The required tripeptide (**141**) eluted with (Ve/Vt) = 0.49 and work up of the appropriate fractions gave



Scheme 4. Fragment condensation giving the (118–129) dodacepeptide.

(4.3 g, 44%), m.p. 72–74°, $[\alpha]_D^{25}$ −37.8 (c = 1, DMF), $R_f(2)$ −0.8, $R_f(9)$ −0.5, $Gly_{1.00}Leu_{1.00}$, (Found: C, 60.62; H, 7.56; N, 8.04; $C_{34}H_{48}N_4O_7S.H_2O$ requires: C, 60.51; H, 7.47; N, 8.30%).

Z-Orn(Adoc)-Gly-OPh (**142**). Z.Orn(Adoc).OH (28.5 g, 64 mM) [prepared from the DCHA salt (**28**) in the usual way] was dissolved in DCM and the solution cooled to −20°. NMM (7.0 ml, 64 mM) and diphenylphosphinic chloride (14.7 g, 62 mM) were added and the solution stirred at −20° for 15 min. A cooled solution containing $Br^-H_2^+$.Gly.OPh (16.3 g, 70 mM) and NMM (7.7 ml, 70 mM) in DMF (250 ml) was added and the reaction mixture warmed to room temperature overnight. The solvent was evaporated and the residue dissolved in EtOAc, this solution being washed with acid and base in the usual way. Drying and evaporation gave a yellow oil which was crystallised from EtOAc and petroleum ether yielding (**142**) (20.7 g, 58%), m.p. 109–110°, $[\alpha]_D^{26}$ −8.4° (c = 1, DMF), $R_f(10)$ −0.2, $R_f(22)$ −0.8, $Orn_{0.96}Gly_{1.04}$, (Found: C, 66.42; H, 6.81; N, 7.33; $C_{32}H_{39}N_3O_7$ requires: C, 66.53, H, 6.81; N, 7.27%).

Z-Val-Orn(Adoc)-Gly-OPh (**143**). The protected dipeptide (**142**) (15.2 g, 26.4 mM) and Tos.OH.H_2O (5.0 g, 26 mM) were dissolved in DMF (110 ml) and 5% Pd/C (3.0 g) added. After 4 hr hydrogenolysis the reaction mixture was worked up in the usual way giving the corresponding salt as an oil $R_f(17)$ −0.6. Z.Val.OH (8.5 g, 33.8 mM) was dissolved in DCM (100 ml) and cooled to −20°. NMM (3.7 ml, 33.8 mM) and diphenylphosphinic chloride (7.7 g, 32.5 mM) were added and 10 min allowed for activation. A cooled solution of the dipeptide salt (16.0 g, 26 mM) in DMF (80 ml) was added followed by NMM (2.9 ml, 26 mM). After overnight reaction at room temperature the solution was evaporated and the residue dissolved in EtOAc. This solution was washed with acid and base then dried and evaporated to yield a white solid. This solid was purified by chromatography on silica gel (600 g) eluting with $CHCl_3$/IPA 20:1, the homogenous product (**143**) being crystallised from a mixture of EtOAc, $CHCl_3$ and petroleum ether giving (11.3 g, 64%), m.p. 138–139°, $[\alpha]_D^{30}$ −9.1° (c = 1, DMF), $R_f(8)$ −0.5, $R_f(22)$ −0.8, $Orn_{0.97}Gly_{1.05}Val_{0.98}$, (Found: C, 64.55; H, 7.13; N, 8.10; $C_{37}H_{48}N_4O_8.0.5\ H_2O$ requires: C, 64.80; H, 7.20; N, 8.17%).

Bpoc-Trp-Val-Orn(Adoc)-Gly-OPh (**144**). Compound (**143**) (15.5 g, 22.8 mM) and Tos.OH.H_2O (4.4 h, 22.8 mM) were dissolved in DMF (110 ml) and hydrogenolysed for 5.5 hr in the presence of 10% Pd/C (1.5 g). Filtration and evaporation gave the corresponding salt as an oil $R_f(17)$ −0.6.Bpoc.Trp.OH (11.7 g, 26.5 mM) in DCM (120 ml) was cooled to −20° and treated with NMM (3.0 ml, 17.3 mM) and pivaloyl chloride (3.2 g, 26 mM) allowing 20 min for activation. The above salt (15.7 g, 22 mM) in DMF (90 ml) was added along with NMM (1.5 ml, 22.7 mM) and the reaction mixture permitted to warm to room temperature overnight. Filtration and evaporation gave a residue which was chromatographed on Sephadex LH20 eluting with DMF. The product (**144**) eluted with (Ve/Vt) = 0.47, evaporation and trituration of the residue with Et_2O gave homogeneous (**144**) (12.0 g, 57%), m.p. 134–135°, $[\alpha]_D^{25}$ −13.5° (c = 1, DMF), $R_f(2)$ −0.6, $R_f(3)$ −0.8, $Val_{0.94}Orn_{0.98}Gly_{1.09}$, (Found: C, 69.36; H, 7.01; N, 8.51; $C_{56}H_{66}N_6O_9$ requires: C, 69.54; H, 6.88; N, 8.69%).

Z-Ser(Bu^t)-Ala-OPh (**145**). Z.Ser(Bu^t).OH (19.6 g, 64 mM) was dissolved in DCM (150 ml) and cooled to −20°. NMM (7.1 ml, 64 mM) and pivaloyl chloride (7.8 g, 64 mM) were added and 25 min allowed for activation. A solution containing $TosO^-H_2^+$.Ala.OPh (20.6 g, 58.3 mM) and NMM (6.4 ml, 58 mM) was added and the reaction mixture stirred at room temperature overnight. The solvent was removed and the resulting residue dissolved in EtOAc. Work up in the usual way and crystallisation from EtOAc and petroleum ether gave the required dipeptide (**145**) (13.5 g, 52%), m.p. 116–117°, $[\alpha]_D^{25}$ −28.9° (c = 1, DMF), $R_f(10)$ −0.7, $R_f(2)$ −0.8, $Ser_{0.79}Ala_{1.00}$, (Found: C, 65.36; H, 6.81; N, 6.56; $C_{24}H_{30}N_2O_6$ requires: C, 65.14; H, 6.83; N, 6.33%).

Z-Val-Ser(Bu^t)-Ala-OPh (**146**). The protected dipeptide (**145**) (4.5 g, 10.2 mM) and Tos.OH.H_2O (1.9 g, 10.2 mM) were dissolved in DMF (130 ml), 10% Pd/C (0.75 g) was added and the reaction mixture hydrogenolysed overnight. Work up in the usual way gave an oil which was used directly in the coupling reaction. Z.Val.OH (2.8 g, 11.3 mM) was dissolved in DCM (40 ml), after cooling to −20°, NMM (1.3 ml, 11.8 mM) and diphenylphosphinic chloride (2.6 g, 10.9 mM) were added and 20 min allowed for activation. A solution of the salt in DMF (35 ml) was added followed by NMM (1.2 ml, 10.5 mM). Work up in the usual way after overnight reaction and crystallisation from EtOAc/petroleum ether afforded the required tripeptide derivative (**146**) as a white solid (3.9 g, 72%), m.p. 164–164°, $[\alpha]_D^{30}$ −23.1° (c = 1, DMF), $R_f(19)$ −0.6, $R_f(2)$ −0.7, $Ser_{0.81}Ala_{0.98}Val_{1.02}$, (Found: C, 64.31; H, 7.19; N, 7.54; $C_{29}H_{39}N_3O_7$ requires: C, 64.31; H, 7.26; N, 7.76%).

Z-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-OPh (**147**). The protected tripeptide (**146**) (7.4 g, 13.6 mM) and Tos.OH.H_2O (2.6 g, 13.6 mM) were dissolved in DMF (90 ml) and 10% Pd/C (1 g) added. Following hydrogenolysis for 5.5 hr the reaction mixture was worked up to yield the salt as a colourless oil (6.5 g, 11.2 mM). Z.Asp(OBu^t).OH(4.0 g, 12.2 mM) was dissolved in DCM and cooled to −20°. NMM (1.4 ml, 12.7 mM) was added followed by pivaloyl chloride (1.5 g, 12.1 mM) and 20 min allowed for activation. A solution of the above salt in DMF (40 ml) was added followed by NMM (1.3 ml, 11.8 mM). After overnight reaction the reaction mixture was processed in the usual way giving the protected tetrapeptide (**147**) (7.6 g, 96%), m.p. 158–160°, $[\alpha]_D^{26}$ −23.5° (c = 1, DMF), $R_f(19)$ −0.7, $R_f(13)$ −0.9, $Asp_{1.02}Ser_{0.81}Ala_{0.99}Val_{0.99}$, (Found: C, 62.63; H, 7.65; N, 8.15; $C_{37}H_{52}N_4O_{10}$ requires: C, 62.34; H, 7.35; N, 7.86%).

Bpoc-Ser(Bu^t)-Asp(OBu^t)-Val-Ser(Bu^t)-Ala-OPh (**148**). Compound (**147**) (3.8 g, 5.4 mM) and Tos.OH.H_2O (1.0 g, 5.4 mM) were dissolved in DMF (50 ml) and hydrogenolysed in the presence of 10% Pd/C (0.4 g) for 5 hr. Work up in the usual way gave the salt as an oil. Bpoc.Ser(Bu^t).OH (1.5 g, 6.2 mM), Bates reagent[9] (3.7 g, 7.2 mM), HONSu (1.7 g, 14.4 mM) and NMM (1.6 ml, 14.4 mM) were dissolved in DMF (60 ml) and cooled to −5°. A cooled solution of the salt in DMF (30 ml) was added and after the addition of NMM (0.5 ml, 4.7 mM) the reaction was stirred at room temperature overnight. The solvent was removed giving a residue which was dissolved in EtOAc. This solution being washed in the usual way to yield a colourless oil. This oil was crystallised from Et_2O/petroleum ether giving the fully protected pentapeptide (**148**) (3.6 g, 78%), m.p. 147–150°, $[\alpha]_D^{26}$ −26.6° (c = 1, DMF), $R_f(2)$ −0.7, $R_f(13)$ −0.8, $Asp_{1.03}Ser_{1.78}Al_{1.02}Val_{0.96}$, (Found: C, 64.39; H, 7.56; N, 7.50; $C_{52}H_{73}N_5O_{12}.0.5H_2O$ requires: C, 64.44; H, 7.70; N, 7.23%).

Bpoc(123–129)*OBu^t* (**149**). (a)*Cl$^-H_2^+$*(127–129)*OBu^t* (**141a**). Bpoc(127–129).OBu^t (**141**) (1.6 g, 4 mM) was dissolved in DCM (10 ml) and DMS (14.6 ml, 200 mM) added.HCl in dioxan (5.45 M, 1.5 ml, 8.18 mM) was diluted with DCM (139 ml) and added to the above solution and the resulting reaction mixture maintained at room temperature for 45 min. The solution was concentrated and added to Et_2O to produce an off white solid, filtration of this solid gave (**141a**) (1.4 g, 79%), m.p. 95–97°, $R_f(2)$ −0.5.

(b) *Bpoc*(123–126).*OH* (**144a**). The protected tripeptide phenyl ester (**144**)

(3.9 g, 4.0 mM) was dissolved in DMF (120 ml), H_2O (20 ml) and DMS (14.1 ml, 193 mM) added and the pH adjusted to 10.6 with 1 M NaOH 100 vol.H_2O_2 (0.4 ml) was added and the pH maintained at 10.6 for 1 hr. The pH was then adjusted to 7 by the addition of citrate buffer (pH 3.5) and the solvent evaporated to give a residue which was partitioned between EtOAc and citrate buffer. The organic phase was washed (×4) with H_2O, dried and evaporated to give a white solid, which was further purified by gel filtration on Sephadex LH20 eluting with DMF. The required acid (**144a**) was isolated from the peak eluting with (Ve/Vt) = 0.46 giving (2.5 g, 68%), m.p. 120–121°, $[\alpha]_D^{25}$ −17.6° (c = 1, DMF), $R_f(3)$ −0.5, $R_f(31)$ −0.7, $Orn_{0.85}Gly_{1.05}Val_{0.95}$, (Found: C, 65.03; H, 7.18; N, 9.31; $C_{50}H_{62}N_6O_9.2H_2O$ requires: C, 64.78; H, 7.18; N, 9.07%).

(c) *Coupling*. The peptide acid (**144a**) (1.02 g, 1.1 mM), the protected tripeptide hydrochloride (**141a**) (0.46 g, 1.0 mM) and HONSu (0.26 g, 2.26 mM) were dissolved in DMF (10 ml) and the solution cooled to −20°. DCCI (0.25 g, 1.2 mM) and NMM (0.12 ml, 1.07 mM) were added and the solution stirred at room temperature for 45 hours. After cooling to 0° further portions of HONSu (0.13 g, 1.1 mM) and DCCI (0.13 g, 0.6 mM) were added and the mixture stirred for a further 48 hours. The reaction mixture was applied directly to Sephadex LH20 eluting with DMF. The required

heptapeptide (**149**) eluted with (Ve/Vt) = 0.41, work up giving (1.06 g, 73%), m.p. 170–172°, $[\alpha]_D^{26}$ − 16.9° (c = 1, DMF) $R_f(2)$ − 0.8, $R_f(13)$ − 0.5, Trp-$Orn_{1.02}Gly_{1.95}$Cys-$Val_{0.99}Leu_{1.03}$, (Found: C, 61.35; H, 7.50; N, 10.84. $C_{68}H_{94}N_{10}O_{13}S.2H_2O$ requires: C, 61.52; H, 7.44; N, 10.56%).

Bpoc(118–129)*OBu'* (**150**). (a) $Cl^-H_2^+$.(123–129)*OBu'* (**149a**). The protected heptapeptide (**149**) (0.92 g, 0.7 mM) was dissolved in 90% aqueous trifluoroethanol (2 ml) and DMS (3.65 ml, 50 mM) added. The pH was adjusted to 0.5 with 0.05 MHCl in 90% aqueous trifluoroethanol and maintained at this value for 45 min. The solvent was evaporated and Et_2O added to yield the salt (**149a**) as a white solid (0.72 g, 82%), $R_f(2)$ − 0.7.

(b) *Bpoc*(118–122)*OH* (**148a**). The protected subfragment (**148**) (3.1 g, 3.3 mM) was dissolved in DMF (35 ml) and H_2O (5 ml) added. The pH was brought to 10.6 with 1 M NaOH and maintained at this value for 1 hr after the addition of 100 vol.H_2O_2 (0.33 ml). The pH was adjusted to 7 with citrate (pH. 3.5) buffer and the solvent evaporated, the residue being partitioned between EtOAc and water. After washing with H_2O (×4) and drying the solution was evaporated and the residue dissolved in DMF and chromatographed on Sephadex LH20. The required protected peptide acid (**148a**) was obtained from the fractions with (Ve/Vt) = 0.46 giving (1.8 g, 62%), m.p. 117–121°, $R_f(2)$ − 0.6.

(c) *Coupling*. The peptide acid (**148a**) (0.82 g, 0.93 mM), protected heptapeptide hydrochloride (**149a**) (0.72 g, 0.57 mM) and HONSu were dissolved in DMF (6 ml) and cooled to − 20°. A solution of DCCI (0.21 g, 1.02 mM) in HMPA (4 ml) and NMM (0.08 ml, 0.69 mM) were added and the solution stirred for 54 hr. After recooling to − 20° further portions of DCCI (1.0 g, 0.48 mM) and HONSu (0.1 g, 0.87 mM) were added and the reaction mixture stirred for a further 24 hr. The solution was applied directly to Sephadex LH20 and eluted with DMF giving the required protected dodecapeptide (**150**) after work up of the peak eluting with (Ve/Vt) = 0.38 (0.54 g, 49%), m.p. 190° dec, $[\alpha]_D^{26}$ − 22.0° (c = 0.5, DMF), $R_f(7)$ − 0.8, $R_f(2)$ − 0.3, $R_f(29)$ − 0.5, Trp-$Orn_{0.96}Asp_{1.00}Ser_{1.71}Gly_{2.13}$Cys-$Ala_{0.93}Val_{1.92}Leu_{1.06}$, (Found: C, 60.52; H, 7.66; N, 10.77; $C_{98}H_{147}N_{15}O_{22}S.H_2O$ requires: C, 60.76; H, 7.75; N, 10.84%).

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This compound numbering sequence follows that established in earlier papers in this series.

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