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PEPTIDES—XXXXIII

SYNTHESIS OF THE 94–104 FRAGMENT OF A LYSOZYME ANALOGUE

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Abstract—The synthesis of the (94–104) fragment of a lysozyme analogue is reported by union of the protected (94–98) and (99–104) subfragments using the *N*-hydroxysuccinimide active ester of the (94–98) fragment. This choice of coupling method was made on the basis of a study employing a range of established methods using the criteria of yield and minimum racemisation.

This paper describes the synthesis of the (94–104) fragment of our lysozyme analogue,[1] in accord with the general methodology described previously.[2] From the sequence of this fragment shown below it may be seen that there are no obvious points at which a fragment coupling may be carried out. Preliminary work clearly indicated that stepwise addition from the *C*-terminal glycine was feasible up to residue 99 but that extension in a stepwise manner past this point was unsatisfactory.

$$\underset{94}{\text{Cys.Ala.Lys.Lys.Ile.Val.Ser.Asp.Gly.Asn.Gly.}}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\underset{98}{}\qquad\qquad\qquad\qquad\underset{94}{}$$

Various coupling points for fragment condensation were considered but as the synthesis of the (99–104) portion could be achieved in a stepwise manner in reasonable yield, it was decided that this portion should constitute one of the fragments in a fragment condensation approach. Thus, the final coupling would be a 5+6 fragment coupling and, therefore, the method of assembly of the pentapeptide portion and the fragment coupling would be the main points of concern.

The (102–104) protected tripeptide fragment (**114**) (see Scheme 1) was assembled using the pivalic mixed anhydride method for both couplings. The dipeptide (**88**) had been synthesised on a previous occasion as part of the (68–75) portion of the lysozyme analogue.[3] After hydrogenolysis, the tripeptide fragment (**114**) was coupled with Z.Asp(OBu^t) *N*-hydroxysuccinimide active ester which had in turn been prepared by the standard procedure.[4] The tetrapeptide was then further extended after hydrogenolysis by the addition of the seryl residue as its protected nitrophenyl ester.[6] This nitrophenyl ester proved to be rather difficult to crystallise but after purification gave the active ester in good yield which enabled us to prepare the (100–104) pentapeptide. The final valine residue of this portion was added as its *N*-hydroxysuccinimide active ester and in this case it was found that crystallisation of the product (**117**) provided an adequate means of purification rendering gel filtration unnecessary.

The (94–98) pentapeptide was assembled from two subfragments (see Scheme 2). Z. Lys(Adoc).OH (**17**)[6] was activated by a reaction with DCCI and HONSu in demethoxyethane to give the corresponding active ester as a red oil which defied all attempts at crystallisation. This active ester was then used to prepare the dipeptide acid (**118**) by a salt coupling procedure using the triethylamonium salt of isoleucine; the required product being obtained in 75% yield as a dry foam. Hydrogenolysis of this compound (**118**) gave incomplete cleavage of the benzyloxycarbonyl function after 24 hr when DMF was used as the solvent, however, a clean deprotection was achieved in 16 hr when the solvent was changed to a mixture of acetic acid and DMF. A further coupling with the lysine hydroxysuccinimide ester described above gave the protected tripeptide acid (**119**) in 85% yield after gel filtration on Sephadex LH20.

The synthesis of the dipeptide (**120**) was approached in a similar manner using the hydroxysuccinimide ester (**98**).[7] This active ester was coupled with the sodium salt of alanine giving the dipeptide as an impure oil which was chromatographed on Sephadex LH20 in order to isolate the dipeptide acid in 81% yield. Treatment of this acid with DCCI/HONSu gave the dipeptide active ester (**120**) as a dry foam. The benzyloxycarbonyl group was then removed from the tripeptide (**119**) by a 3 hr hydrogenolysis in 90% acetic acid. If the hydrogenolysis was carried out with DMF as solvent a low yield of an impure product was obtained. The free tripeptide was then coupled with the dipeptide active ester (**120**), using diisopropylethylamine as the base in order to reduce the possibility of racemisation to give the product (**121**) in 64% yield after purification by gel filtration on Sephadex LH20.

Although the amino acid analysis of this protected pentapeptide (**121**) showed the constituent amino-acids to be present in the correct ratios, 1.7% allo-isoleucine† was observed during amino acid analysis. Further investigations confirmed the optical purity of the isoleucine used in the synthesis and that the allo-isoleucine was not present in the dipeptide (**118**) or tripeptide (**119**). The racemisation must therefore have taken place during the hydrolysis associated with the amino acid analysis, which was supported by the fact that when isoleucine itself was exposed to acid hydrolysis 0.5% allo-isoleucine was detected.

The assembly of the complete (94–104) portion was then investigated using the pentapeptide acid (**121**) and the *p*-toluenesulphonate salt (**117a**) (see Scheme 3) which

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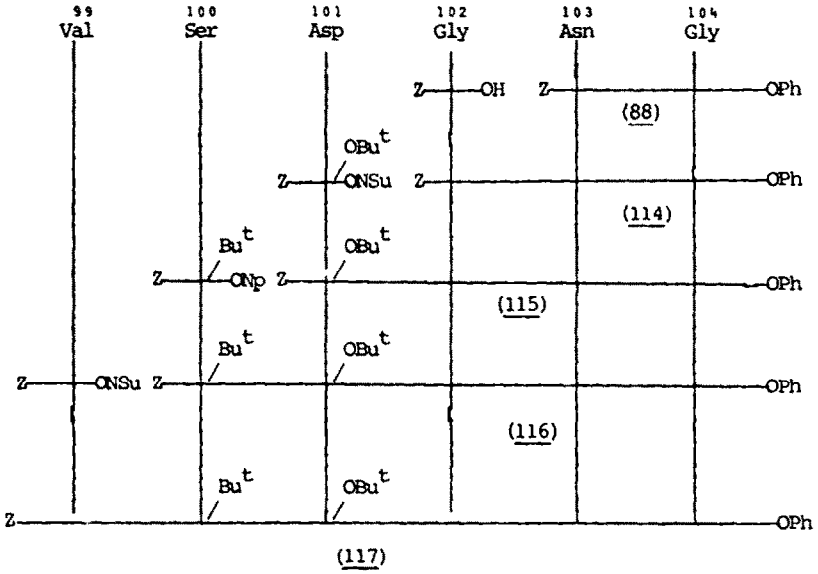
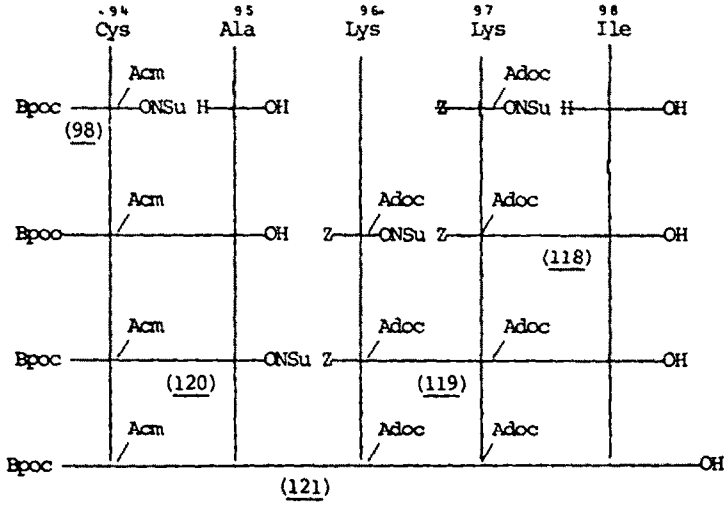
†%allo - isoleucine = allo - isoleucine/(allo - isoleucine + leucine) × 100.



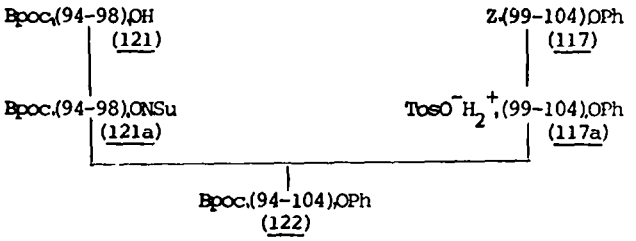
Scheme 1. Synthesis of the protected (99–104) hexapeptide (117).



Scheme 2. Synthesis of the protected (94–98) pentapeptide acid (121).

was obtained by hydrogenolysis of the corresponding benzyloxycarbonyl peptide (117) for 16 hr in the presence of palladium charcoal catalyst and *p*-toluenesulphonic acid in the normal way. In line with the standard strategy employed throughout our synthetic work[2] we first examined coupling using the DCCI/HONSu method[8] employing DMF as solvent and NMM to liberate the amino function. A 4 day reaction followed by purification on Sephadex LH20 gave only a 16% yield of the required fragment (122) which contained 10% allo-isoleucine. The low yield and detected diasteroisomer of isoleucine from conditions which should not give racemisation caused us to examine the coupling in much greater detail. Clearly this isoleucine to valine coupling is highly hindered and approach of nucleophiles is restricted. One must assume that the *O*-acylurea can form reasonably readily but gives rise to a peptide oxazolone rather than being attacked by *N*-hydroxysuccinimide to give the active ester. In order to optimise the coupling a wide variety of conditions were employed giving rise to the results shown in Table 1.

Retaining the coupling method (F) and reducing the activation temperature to $-10°$ whilst increasing the concentration threefold (H) produced as expected an increased yield and a slight decrease in racemisation. The conditions of this experiment would tend to shorten the life of the reactive intermediates but accessibility to the carboxyl group appear still to be sufficiently restricted so as to prevent rapid attack by *N*-hydroxysuccinimide thus giving rise to relatively high racemisation. Change of solvent to HMPA (D) produced dramatic increase in the detected allo-isoleucine (18%) indicating a considerable solvent effect. A further change to a "non-racemising solvent" such as dichloromethane in which the com-



Scheme 3. The preparation of the protected (94–104) fragment (122).

ponents were readily soluble was made in experiment (P) as well as a change to the hindered polymeric Hünig base.[9] A drop in racemisation was observed to 2.2%, however since the yield was still low at 23% the base was then replaced with di-isopropylethylamine (O) giving only a slight increase in the measured allo-isoleucine. Activation of the carboxyl component by the DPP mixed anhydride method[10] (B and C) gave low yields and extensive racemisation as did the addition of hydroxybenzotriazole to the DCCI reaction when the solvent used was HMPA (A). The level of racemisation dropped considerably on using DMF as solvent (E) but again was sufficiently high (11%) to be unacceptable. The use of polyHünig base in DCCI/HOBt[11] reaction (G) reduced both the yield and the detected racemate (7.9%). In order to minimise the problems encountered with dichloromethane in combination with polyHünig base, DMA (K) and DMF (L) were substituted employing an activation temperature of −20°. After an 18 hr reaction similar yields and considerably reduced racemisation were again found. The importance of the low temperature in the initial stages of this reaction is demonstrated by comparing experiment (L) with (I) in which the activation is carried out at 0° giving a greater degree of racemisation (5.9%).

The results show that it is certainly necessary to activate at a low temperature and that non-racemising solvents should be used. Also the use of the minimum amount of base and *N*-hydroxysuccinimide as additive give the best results as far as racemisation is concerned.

It was found that the highest yield and lowest racemisation were brought about using a 2-stage fragment condensation, i.e. pre-activation to give the peptide active ester followed by addition of the amino component. From the Table it can be seen that experiments (M to S) all give reasonably low levels of allo-isoleucine, the yield being optimal in experiment (R) in which the highest concentration of reagents is used. The conditions of experiment (R) giving 60% yield and 1.9% racemisation were those used in the final preparative experiments which resulted in preparation of (122) in 44% yield after gel filtration chromatography on Sephadex LH20. Amino acid analysis indicated the presence of 2.2% allo-isoleucine. It is interesting to note that the active ester shows 7% of allo-isoleucine but that when used in a fragment coupling only 2.2% was detected. This again indicates a variable degree of racemisation during acid hydrolysis. The purity of the final product (122) was determined by TLC in 3 solvent systems, by paper electrophoresis, by combustion analysis and amino acid analysis. Enzymic digestion of the fully deprotected peptide gave a satisfactory analysis although the experimental variation was somewhat greater than the 5% normally associated with acid hydrolysis.

EXPERIMENTAL

The general experimental methods, abbreviations, and TLC are those described in earlier papers in this series.

Z–Gly–Asn–Gly–OPh (**114**). Z–Asn–Gly–OPh (**88**)[2] (40.0 g, 100 mM) and Tos.OH.H_2O (19.1 g, 100 mM) were dissolved in DMF (250 ml) and hydrogenolysed overnight in the presence of 5% Pd/C (5 g). The suspension was filtered and concentrated to 100 ml and set on one side for use in the coupling. Z–Gly–OH(2.20 g, 105 mM) was dissolved in THF (220 ml) containing TEA (1.47 ml, 105 mM). After cooling to −10° pivaloyl chloride (12.6 g, 105 mM) was added and 10 min allowed for activation. The cooled amino-component from above was then added followed by TEA (14.0 ml, 100 mM) and the reaction stirred overnight slowly warming to room temperature. The solvent was evaporated and water added to precipitate the product washing with 5% $NaHCO_3$ solution, 1 M AcOH and reprecipitation from DMF/H_2O gave the required tripeptide (**114**) (34.7 g, 76%); m.p. 200–203°, $[\alpha]_D^{22}$ −15.7° (c = 0.98, DMF), R_f(25) −0.4, R_f(17) −0.7, $Asp_{1.04}$ $Gly_{2.00}$, (Found: C, 58.05; H, 5.42; N, 12.09; $C_{22}H_{24}N_4O_7$ requires: C, 57.89; H, 5.30; N, 12.27%).

Z–Asp(OBu^t)–Gly–Asn–Gly–OPh (**115**). The protected tripeptide (**114**) (34.7 g, 76 mM) and Tos.OH.H_2O (14.5 g, 76 mM) were dissolved in DMF (250 ml) and hydrogenolysed in the presence of 5% Pd/C (5 g). After 5 h the cleavage was complete and the solution filtered and concentrated to 100 ml. Z–Asp(OBu^t)–ONSu (36.9 g, 88 mM) was added along with DIPEA (9.9 g, 76 mM). The reaction mixture set solid after 1 h but was allowed to stand overnight before work up. Trituration with EtOAc followed by recrystallisation from DMF/H_2O gave the required product (36.0 g, 66%) m.p. 184–188°, $[\alpha]_D^{22}$ −22.2° (c = 1.04, DMF), R_f(7) −0.6, R_f(17) −0.5, $Asp_{2.02}$ $Gly_{1.98}$, (Found: C, 55.84; H, 5.82; N, 10.90; $C_{30}H_{37}N_5O_{10}\cdot H_2O$ requires: C, 55.81; H, 6.09; N.10.85%).

Z–Ser(Bu^t)–Asp(OBu^t)–Gly–Asn–Gly–OPh (**116**). The preceding protected tetrapeptide (**115**) (29.6 g, 46 mM) was dissolved in DMF (300 ml) and hydrogenolysed in the presence of Tos·OH·H_2O (9.0 g, 46 mM) and 5% Pd/C (6 g) for 8 h. The reaction mixture was filtered and concentrated to *ca* 120ml and Z-Ser(Bu^t)–ONp (23.5 g, 57.5 mM) and DIPEA (5.93 g, 46 mM) added. After 24 h ninhydrin showed the reaction to be complete and Et_2O was added to precipitate the crude product. This material was triturated with Et_2O then crystallised from DMF/H_2O giving (**116**) (26.5 g, 73%), m.p. 169–175°, $[\alpha]_D^{22}$ −17.1° (c = 1, DMF), R_f(28) −0.85, R_f(7) −0.8, $Asp_{2.03}Ser_{0.89}Gly_{2.00}$, (Found: C, 56.41; H, 6.30; N, 10.73. $C_{37}H_{50}N_6O_{12}\cdot H_2O$ requires: C, 56.33; H, 6.64; N, 10.65%).

Z–Val–Ser(Bu^t)–Asp(OBu^t)–Gly–Asn–Gly–OPh (**117**). The protected pentapeptide (**116**) (26.4 g, 33.5 mM) and Tos·OH·H_2O (6.4 g, 33.5 mM) were dissolved in DMF 250 ml and 5% Pd/C (20 g) added. After overnight hydrogenolysis the reaction mixture was filtered and the filtrate concentrated to *ca.* 80 ml. Z–Val–ONSu (17.5 g, 50 mM) and DIPEA (4.4 g, 33.5 mM) were added to the stirred solution which set solid overnight (ninhydrin negative). The solid was triturated with Et_2O then recrystallised from DMF/H_2O yielding (**117**) (23.3 g, 78%), m.p. 198–202°, $[\alpha]_D^{22}$ −13.2°, (c = 1, DMF), R_f(28) −0.85, $Asp_{2.01}$ $Ser_{0.79}$ $Gly_{1.98}$ $Val_{0.92}$ (14 h hydrolysis), $Asp_{2.00}$ $Ser_{0.60}$ $Gly_{2.01}$ $Val_{0.98}$ (48 h hydrolysis), (Found: C, 56.79; H, 6.88; N, 11.22; $C_{42}H_{59}N_7O_{13}\cdot H_2O$ requires: C, 56.81; H, 6.93; N, 11.04%).

Z–Lys(Adoc)–Ile–OH (**118**). Z–Lys–(Adoc)–OH (**17**) (68.0 g, 148 mM) was dissolved in DMF (300 ml) and cooled to −5°.

Table 1. Coupling conditions used to prepare Bpoc.(94–104)·OPh (122)

	Coupling Method	Base	Solvent	Temperature °C	Reaction Time h	Concentration mM/ml	Yield	Allo-Ile %
A	DCCI/HOBt	DIPEA	HMPA	- 5	18	0.1	53	34
B	DPPCI mixed anhydride	NMM	DMF	0	18	0.033	13	33
C	DPPCI mixed anhydride	NMM	DMF	-20	18	0.036	6	25
D	DCCI/HONSu	NMM	HMPA	- 5	96	0.1	10	18
E	DCCI/HOBt	NMM	DMF	-10	18	0.1	60	11
F	DCCI/HONSu	NMM	DMF	0	96	0.03	16	10
G	DCCI/HOBt	PolyHünig	CH_2Cl_2	0	72	0.1	40	7.9
H	DCCI/HONSu	NMM	DMF	-10	96	0.1	35	7
I	DCCI/HOBt	PolyHünig	DMF	0	18	0.1	60	5.9
J	DCCI/HOBt	NMM	DMF	0	18	0.33	72	5.3
K	DCCI/HOBt	PolyHünig	DMA	-20	18	0.1	48	4.6
L	DCCI/HOBt	PolyHünig	DMF	-20	18	0.15	46	4.2
M	HONSU active ester	DIPEA	CH_2/Cl_2DMA	- 5	24	0.14	47	3.6
N	HONSU active ester	DIPEA	CH_2/Cl_2DMF	- 5	48	0.14	52	3.5
O	DCCI/HONSu	DIPEA	CH_2Cl_2	-10	96	0.1	16	3.3
P	DCCI/HONSu	PolyHünig	CH_2Cl_2	-10	96	0.1	23	2.2
Q	HONSu active ester	DIPEA	CH_2Cl_2/DMF	- 5	48	0.1	31	2.2
R	HONSu active ester	DIPEA	CH_2Cl_2/DMF	- 5	24	0.66	60	1.9
S	HONSu active ester	DIPEA	CH_2Cl_2/DMF	- 5	18	0.33	49	1.7

HONSu (21.0 g, 177 mM) and DCCI (37.0 g, 170 mM) were added and the solution stirred for 18 h at room temperature. A few drops of glacial AcOH were added and the precipitated DCU filtered after 2 h. The filtrate was evaporated and the residue dissolved in Et_2O, this solution being washed with ice cold 5% citric acid, 5% $NaHCO_3$, water and brine. Drying and evaporation gave an oil (67.0 g, 81%). This active ester (24.0 g, 43 mM), H·Ile·OH (9.0 g, 69 mM) and TEA (9 ml, 60 mM) were dissolved in DMF (80 ml) then left overnight. The solvent was evaporated and water added, the pH being adjusted to pH8 with 5% $NaHCO_3$. The aqueous layer was cooled to 0°, then acidified to pH3 with 5% citric acid. The resulting oil was extracted into Et_2O, washed with water and brine then dried and evaporated to give the required dipeptide acid (**118**) as a dry foam (18.3 g, 46%), m.p. 72–76°, $[\alpha]_D^{20}$ +3.5° (c = 1, DMF), $R_f(2)$ −0.2, $R_f(17)$ −0.6, $Lys_{0.97}Ile_{1.04}$, (Found: C, 64.85; H, 7.98; N, 7.47. $C_{31}H_{45}N_3O_7$ requires: C, 65.13; H, 7.93; N, 7.35%).

Z–Lys(Adoc)–Lys(Adoc)–Ile–OH (**119**). A solution of the dipeptide (**118**) (18.0 g, 32 mM) in DMF (250 ml)/MeOH (20 ml)/HOAc (1 ml) was hydrogenolysed for 16 h in the presence of 10% Pd/C (1.6 g). Filtration and evaporation gave the product as a gum (12.1 g, 87%). A portion of this gum (9.5 g, 21.8 mM) was dissolved in DMF (50 ml) along with Z-Lys(Adoc)-ONSu (13.9 g, 25 mm) and TEA (3.1 ml, 21.8 mM). After 2 days at room temperature the solvent was evaporated and the residue partitioned between EtOAc and ice cold 5% citric acid. The organic layer was washed with water and brine then dried and evaporated to give the crude product (17.0 g, 94%). Purification was achieved by gel filtration on Sephadex LH20 eluting with DMF (Ve/Vt) = 0.47 giving the required product (**119**) (15.3 g, 85%), m.p. 103°, $[\alpha]_D^{20}$ −3.9° (c = 1, DMF), $R_f(2)$ −0.4, $R_f(17)$ −0.7, $Lys_{2.00}$ $Ile_{1.00}$, (Found: C, 65.66; H, 7.97; N, 8.05. $C_{48}H_{71}N_5O_{10}$ requires: C, 65.65; H, 8.15; N, 7.98%).

Bpoc–Cys(Acm)–Ala–ONSu (**120**). Bpoc–Cys(Acm)–ONSu (**98**) (25.6 g, 48 mM) was dissolved in dioxan (90 ml) and cooled to 5°. H·Ala·OH (6.4 g, 72 mM) in 2 M NaOH (36.2 ml) was added and the solution stirred for 24 h prior to acidification to pH 3.5 with 10% citric acid. The reaction mixture was evaporated and the residue taken up in DMF, gel filtration of this solution on Sephadex LH20 gave the product (Ve/Vt) = 0.53 as an oil (20.7 g, 81%). This oil (20.7 g, 41 mM), HONSu (9.6 g, 81.9 mM) and DCCI (10.2 g, 49.8 mM) were dissolved in DCM (180 ml) at −5° and the resulting solution stirred for 2 days. The reaction mixture was diluted with EtOAc and the DCU removed by filtration. Washing the combined organic phase with water and brine followed by evaporation gave the active ester (**120**) as a dry foam (20.0 g, 65%). m.p. 40–45°, $[\alpha]_D^{25}$ −27.1° (c = 1, DMF), $R_f(2)$ −0.2, $R_f(8)$ −0.2, (Found: C, 55.16; H, 5.85; N, 8.67, $C_{29}H_{34}N_4O_8S·2H_2O$ requires: C, 54.87; H, 6.03; N, 8.83%).

Bpoc–Cys(Acm)–Ala–Lys(Adoc)–Lys(Adoc)–Ile–OH (**121**). The protected tripeptide acid (**119**) (14.7 g, 16.7 mM) was dissolved in aq.90% HOAc (200 ml) and hydrogenolysed in the presence of 10% Pd/C (0.85 g) for 3 h. Filtration and evaporation gave a residue which was triturated with water, filtered and dried giving (11.2 g, 91%) of the protected (96–98) fragment. This compound (10.4 g, 14 mM) and the active ester (**120**) (9.6 g, 16 mM) were dissolved in DMF (25 ml) and DIPEA (2.1 g, 16 mM) in DMF (40 ml) added. After 2 days the reaction mixture was evaporated and the residue partitioned between EtOAc and ice cold 5% citric acid. The organic phase was washed and dried; evaporation then gave (18.0 g, 100%). Purification was then achieved by gel filtration on Sephadex LH20 eluting with DMF, the product (**121**) was isolated after evaporation and precipitation with ether giving (11.0 g, 64%), m.p. 133°, $[\alpha]_D^{20}$ −14.7° (c = 1, DMF), $R_f(7)$ −0.8, $R_f(28)$ −0.8, $Lys_{1.98}$ $Ala_{0.98}$ $Ile_{1.03}$, (Found: C, 61.76; H, 7.91; N, 9.16. $C_{65}H_{94}N_8O_{13}S·2H_2O$ requires: C, 61.79; H, 7.82; N, 8.87%).

Bpoc(94–104)OPh. (a) Bpoc(94–98)ONSu (**121a**). A solution of DCM (6 ml) containing (**121**) (1.84 g, 1.5 mM) and HONSu (0.34 g, 3 mM) was cooled to −5° and DCCI (0.46 g, 1.25 mM) added. After 18 h stirring at room temperature the mixture was filtered and the solvent evaporated. The residue was redissolved in EtOAc and cooled to −10°, then after standing refiltered and the solution evaporated giving the active ester (**121a**) (1.75 g, 88%), m.p. 112–116°, $R_f(11)$ −0.6, $R_f(2)$ −0.5, $R_f(20)$ −0.7. This compound being used without further purification.

(b) $TosO^-$ H_2^+·(99–104)·OPh (**117a**), Z·(99–104)·OPh (**117**) (0.95 g, 1.08 mM) and $TosOH·H_2O$ (0.21 g, 1.08 mM) were dissolved in DMF (20 ml) and hydrogenolysed in the presence of 10% Pd/C (0.05 g) for 16 h. After filtration, evaporation gave a residue which was triturated with Et_2O to give the salt (**117a**) (0.96 g, 92%), m.p. 118–122°, $[\alpha]_D^{20}$ −5.8° (c = 1, DMF), $R_f(7)$ −0.2, $R_f(17)$ −0.4, (Found: C, 50.82; H, 6.97; N, 10.71. $C_{41}H_{61}N_7O_{14}S·3H_2O$ requires: C, 51.18; H, 7.02; N, 10.20%).

(c) *Coupling.* The active ester (**121a**) (1.75 g, 1.33 mM) and the salt (**117a**) (0.9 g, 1.00 mM) were dissolved in DMF (4 ml) and DIPEA (0.14 g, 1.08 mM) added. Within 15 min the reaction mixture set to a hard gel which could not be agitated. After 18 h at room temperature the reaction mixture was diluted with DMF and purified by gel filtration on Sephadex LH20 (Ve/Vt) = 0.36 eluting with DMF giving (0.92 g, 46%), m.p. 230°, $[\alpha]_D^{20}$ −17.5° (c = 1, DMF), $R_f(7)$ −0.6, $R_f(15)$ −0.2, $R_f(18)$ −0.3, $Lys_{1.88}$ $Asp_{2.08}$ $Ser_{0.80}$ $Gly_{2.02}$ $Ala_{1.08}$ $Val_{0.96}$ $Ile_{0.87}$ (allo-Ile 2.2%), pronase/APM, $Lys_{2.06}$ $Cys(Acm)_{0.95}$ $Asp_{1.01}$ $Asn/Ser_{1.84}$ $Gly_{1.81}$ $Ala_{0.91}$ $Val_{1.07}$ $Ile_{1.12}$ another preparation gave $Lys_{1.97}$ $Asp_{0.96}$ $Cys(Acm)_{0.77}$ $Asn/Ser_{1.82}$ $Gly_{2.02}$ $Ala_{0.77}$ $Val_{1.04}$ $Ile_{1.01}$, (Found: C, 59.54; H, 7.50; N, 11.00; $C_{99}H_{145}N_{15}O_{23}S·3H_2O$ requires C, 59.47; H, 7.61; N, 10.51%).

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REFERENCES

[1]G. W. Kenner, *Proc. R. Soc. Lond. A.* **353**, 441 (1977).
[2]G. W. Kenner, R. Ramage and R. C. Sheppard, *Tetrahedron* **35**, 2767 (1979).
[3]I. J. Galpin, D. Hudson, A. G. Jackson, G. W. Kenner and R. Ramage, *Ibid.* **36**, 2255 (1980).
[4]K. Hofmann, W. Haas, M. J. Smithers and G. D. Zaneth, *J. Am. Chem. Soc.* **87**, 631 (1965).
[5]E. Wünsch and A. Zwick, *Chem. Ber.* **99**, 105 (1966).
[6]I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage and B. Singh, *Tetrahedron* **35**, 2771 (1979).
[7]I. J. Galpin, A. Hallett, G. W. Kenner, P. Noble, R. Ramage and J. H. Seely, *Tetrahedron Ibid.* **37**, 3017 (1981).
[8]E. Wünsch and F. Drees, *Chem. Ber.* **99**, 110 (1966).
[9]Material purchased from Fluka A.G.
[10]A. G. Jackson, G. W. Kenner, G. A. Moore, R. Ramage and W. D. Thorpe, *Tetrahedron Letters* 3627 (1976).
[11]W. Konig and R. Geiger, *Chem. Ber.* **106**, 3626 (1973).