Synthesis of Sequential Polydepsipeptides Involving Depsipeptide Formation by the 2-Nitrophenylsulphenyl *N*-Carboxy α-Amino Acid Anhydride (Nps–NCA) Method

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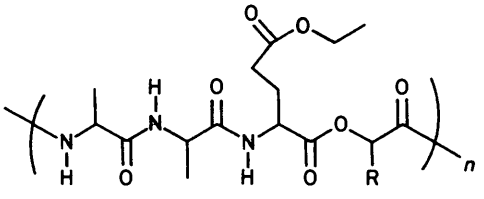
Sequential polydepsipeptides containing a tripeptide sequence Ala-Ala-Glu(OEt) and various α-hydroxy acids have been successfully prepared by polycondensation of tetradepsipeptide pentachlorophenyl esters. The latter were synthesized by preparation of didepsipeptide free acids by the Nps-NCA method followed by active esterification and peptide chain elongation by the so-called 'back-up' procedure.

Polydepsipeptides, copolymers of α-amino acids and α-hydroxy acids, are appropriate model compounds for structural studies of polypeptides and proteins.[1] One of the structural characteristics of polydepsipeptides is that they contain both amide and ester bonds in the main chain, the latter lacking the ability to act as hydrogen-bond donors. Because of this characteristic, the secondary structures in polydepsipeptides are substantially destabilized compared with those in the polypeptides.[2] Although the role of the hydrogen bonds in the stability of protein structures may be evaluated by examining the conformations of polydepsipeptides the greater difficulty in preparing the latter compared with polypeptides has limited the number of such studies published.[3]

In a new method for preparing depsipeptides which we developed, the reaction of 2-nitrophenylsulphenyl (Nps) *N*-carboxy anhydrides (NCA's) of α-amino acids with α-hydroxy acids,[4] has the advantage of rapid formation of the ester bond between an Nps-amino acid and a hydroxy acid without protection. To demonstrate the applicability of this method to other depsipeptide systems, we have synthesized compounds consisting of a peptide sequence Ala-Ala-Glu(OEt) and three kinds of hydroxy acid, 2-hydroxyethanoic acid (Hea), 2-hydroxypropanoic acid (lactic acid, Lac), or 2-hydroxy-4-methylpentanoic acid (Hmp). The polydepsipeptides synthesized in this study have a general formula (**I**).



(**I**) R = H(Hea), Me(Lac), and CH_2CHMe_2(Hmp)

The synthesis involves first preparation and then polycondensation of a depsipeptide active ester monomer. Monomer preparation consisted of three steps: the reaction of the Nps-NCA of γ-ethyl glutamate with α-hydroxy acids to give didepsipeptides; active esterification of the didepsipeptides; elongation of the peptide chains by a so-called 'back-up' procedure.[5]

Preparation and Reaction of N^{α}*-Nps-γ-ethyl Glutamate NCA.*—Nps-Glu(OEt) NCA used for the depsipeptide synthesis was prepared by a modified rapid method. Glu(OEt) NCA (**1**), prepared by the phosgenation of the amino acid with trichloromethyl chloroformate (TCF),[6] was allowed to react with 2-nitrobenzenesulphenyl chloride (Nps-Cl), prepared *in situ* by the reaction of bis-2-nitrophenyl disulphide with chlorine,[7] by dropwise addition of triethylamine. The Nps-Glu(OEt) NCA (**2**) so prepared was purified by repeated recrystallization from ethyl acetate. Subsequently it was allowed to react with an α-hydroxy acid (HA) in the presence of pyridine in tetrahydrofuran at room temperature[4] for 3 h to give didepsipeptides Nps-Glu(OEt)-HA-OH (**3**) in high yields.

Preparation of Monomers.—Didepsipeptides Nps-Glu(OEt)-HA-OH were converted into their active ester derivatives (**4**) by treatment with pentachlorophenol (HOPcp) in the presence of dicyclohexylcarbodi-imide (DCC). The Nps-group of (**4**) was removed by hydrochloric acid in dioxane to give didepsipeptide pentachlorophenyl ester hydrochlorides (**5**), which were allowed to react rapidly with Nps-alanine by the mixed anhydride method using isobutyl chlorocarbonate to give tridepsipeptide pentachlorophenyl esters Nps-Ala-Glu(OEt)-HA-OPcp (**6**). A further elongation of the peptide chains by the 'back-up' procedure gave the tetradepsipeptide esters Nps-Ala-Ala-Glu(OEt)-HA-OPcp (**7**) as *N*-protected monomers.

The monomers and all intermediates were highly crystalline, except Nps-Glu(OEt)-Hmp-OPcp, and easily purified by recrystallization to give a single spot on t.l.c. Results of the syntheses are summarized in Table 1.

Polycondensation of the Monomers.—The protected monomers (**7**) were treated with hydrochloric acid to remove the Nps-protecting group. The monomer hydrochlorides thus obtained were dissolved in dimethyl sulphoxide. Polycondensation was started by addition of triethylamine to the polymerization system. The polymerization proceeded in a viscous solution state. Dilution of the polymerization system with methanol gave the polydepsipeptides which were subsequently reprecipitated from chloroform–diethyl ether. The results of the polymerization are summarized in Table 2.

Experimental

M.p.s are uncorrected and were determined using a Yamato MP-21 apparatus. Optical rotation at the sodium D line were measured with a Jasco DIP-SL polarimeter. I.r. spectra were recorded for KBr disks with a Jasco A-702 spectrophotometer.

Nps-Glu(OEt) NCA.—TCF (9 ml, 75 mmol) was added to a stirred mixture of γ-ethyl glutamate (17.5 g, 0.1 mol) and activated charcoal (0.5 g) suspended in tetrahydrofuran (200

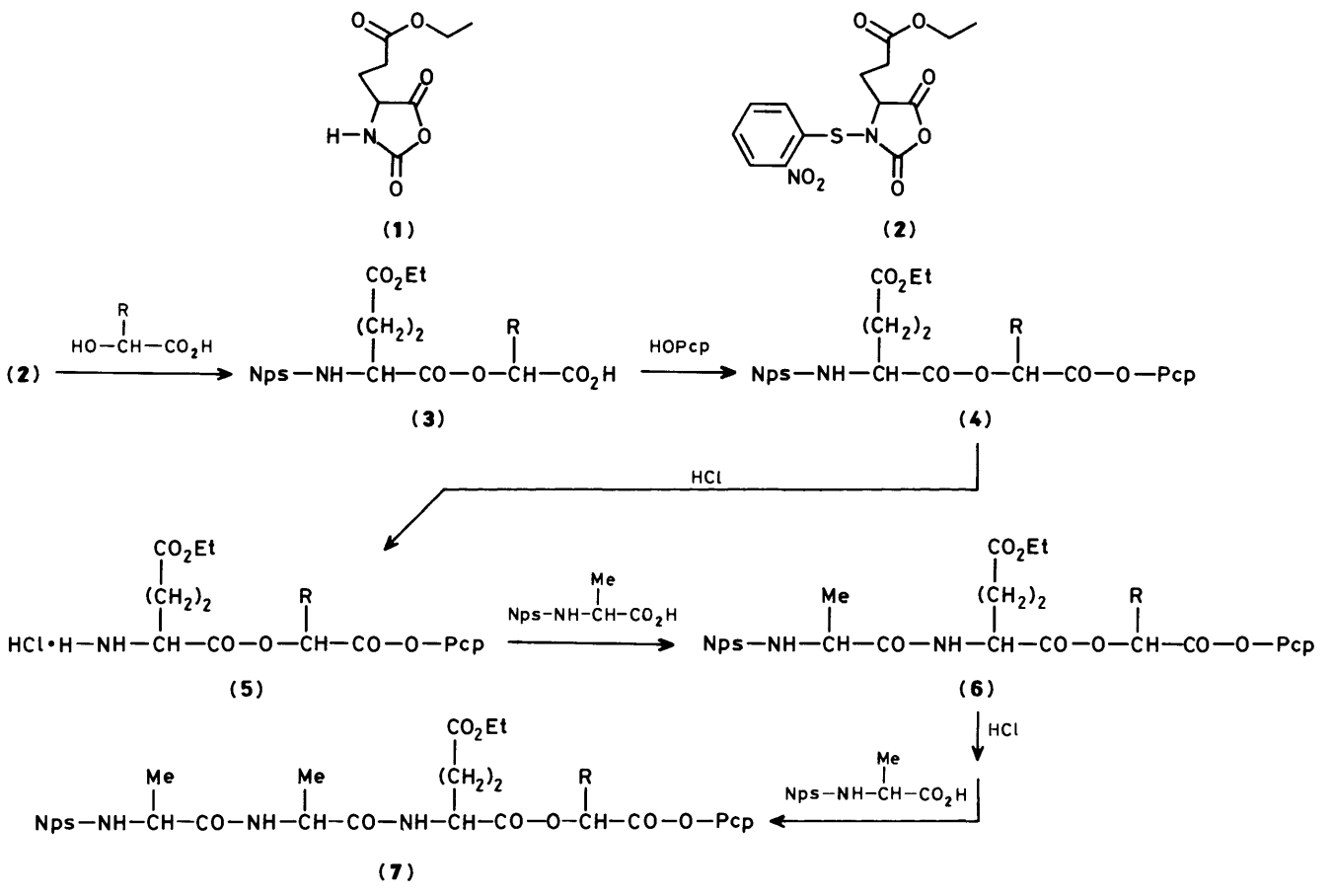
Table 1. Analytical data for oligodepsipeptides*

Depsipeptide	Yield(%)	M.p. (°C)	$[\alpha]_D$ in THF	R_F†
Nps-Glu(OEt)-Hea-OPcp	89	116—117	−56.5 (*c* 1.0)	0.78[a]
Nps-Glu(OEt)-Lac-OPcp	90	93—94	−53.6 (*c* 1.0)	0.82[b]
Nps-Glu(OEt)-Hmp-OPcp	85	Oil		0.74[a]
Nps-Ala-Glu(OEt)-Hea-OPcp	82	161—163	−58.6 (*c* 1.0)	0.38[a]
Nps-Ala-Glu(OEt)-Lac-OPcp	84	155—157	−64.9 (*c* 0.5)	0.63[c]
Nps-Ala-Glu(OEt)-Hmp-OPcp	80	102—104	−65.1 (*c* 0.5)	0.53[a]
Nps-Ala-Ala-Glu(OEt)-Hea-OPcp	84	158—160	−64.8 (*c* 0.5)	0.73[d]
Nps-Ala-Ala-Glu(OEt)-Lac-OPcp	89	142—144	−58.8 (*c* 0.5)	0.72[d]
Nps-Ala-Ala-Glu(OEt)-Hmp-OPcp	90	141—143	−63.8 (*c* 0.5)	0.77[d]

* Satisfactory elemental analyses for C, H, and N were obtained for all compounds: details are available in Supplementary Publication No. 566 98 (2 p.). For details of the Supplementary Publications Scheme, see 'Instructions for Authors' (1987), *J. Chem. Soc., Perkin Trans. 1*, 1987, Issue 1.
† Eluant: [a] EtOAc–benzene (2:5); [b] EtOAc–benzene (1:2); [c] EtOAc–benzene (1:1); [d], THF–benzene (1:1).

Table 2. Analytical data for polydepsipeptides

Polydepsipeptide	Yield (%)	$[\eta]$ in DCA	Found (%)			Required (%)		
			C	H	N	C	H	N
$[Ala\text{-}Ala\text{-}Glu(OEt)\text{-}Hea]_n$	82	0.36	51.0	6.9	11.2	50.4	6.5	11.8
$[Ala\text{-}Ala\text{-}Glu(OEt)\text{-}Lac]_n$	85	0.45	52.0	7.0	10.8	51.7	6.8	11.3
$[Ala\text{-}Ala\text{-}Glu(OEt)\text{-}Hmp]_n$	88	0.69	55.4	7.9	9.9	55.2	7.6	10.2



Scheme.

ml). The amino acid dissolved within a few minutes and stirring was continued for 15 min. The solution was then filtered through Celite to remove the charcoal and the filtrate concentrated under reduced pressure to give an oily residue; this crystallized on addition of hexane. The crude product was recrystallized from ethyl acetate to give a pure Glu(OEt) NCA (18.0 g, 89%) and this, dissolved in tetrahydrofuran (200 ml), was cooled at −10 °C.

A 1M solution of chlorine in carbon tetrachloride (45 ml) was added to a suspension of bis-2-nitrophenyl disulphide (13.9 g, 45 mmol) and iodine (0.5 g) in carbon tetrachloride (100 ml). After dissolution of the disulphide the solution was concentrated under reduced pressure to give crystals of Nps-Cl, which were dissolved in tetrahydrofuran (200 ml). This solution of Nps-Cl was added to the above prepared solution of Glu(OEt) NCA. Triethylamine (6.3 ml, 45 mmol) in tetrahydrofuran (50 ml) was then added dropwise to the mixture at −10 °C with vigorous stirring. After the addition of triethylamine, the solution was stirred for 10 min at −10 °C. The resulting crystalline triethylammonium chloride was filtered off and the filtrate concentrated under reduced pressure to give an oil; this, upon addition of hexane, crystallized. The crude product was recrystallized from warm ethyl acetate to give a pure Nps-Glu(OEt) NCA (29.1 g, 92%), m.p. 135.5—136.5 °C; $[\alpha]_D$ +40.45 (*c* 1.0 in tetrahydrofuran); $\nu_{max.}$ 1 858, 1 780 (the anhydride C=O), 1 717 the ester C=O), 1 593, 1 571 (the substituted benzene C–H), and 1 515 cm^{-1} (the nitro N=O).

Nps-Glu(OEt)-HA-OH.—Pyridine (9.7 ml, 120 mmol) was added to the hydroxy acid (120 mmol) dissolved in tetrahydrofuran (50 ml). A solution of Nps-Glu(OEt) NCA (35.5 g, 0.1 mol) in tetrahydrofuran (200 ml) was then added with stirring; the stirring was continued at room temperature and the reaction was monitored by t.l.c. After completion of the reaction, the solution was diluted with ethyl acetate (200 ml) and washed with 10% citric acid; it was then extracted with 5% aqueous sodium hydrogen carbonate. The extract was acidified with 10% citric acid and the resulting oil was extracted with ethyl acetate: the extract was then washed with water and dried (Na_2SO_4). The solution was concentrated to give an oily residue of Nps-Glu(OEt)-HA-OH, which showed a single spot on t.l.c. The yields were 92, 95, and 93% for Nps-Glu(OEt)-Hea-OH, Nps-Glu(OEt)-Lac-OH, and Nps-Glu(OEt)-Hmp-OH, respectively.

Nps-Glu(OEt)-HA-OPcp.—A solution of a didepsipeptide Nps-Glu(OEt)-HA-OH (0.1 mol) and pentachlorophenol (29.3 g, 0.11 mol) dissolved in tetrahydrofuran (200 ml) was cooled at −10 °C. DCC (22.7 g, 0.11 mol) was then added to the solution and the solution was stirred for 3 h at −10 °C and for 1 h at room temperature. The solution was diluted with ethyl acetate (200 ml), the resulting urea filtered off, and the filtrate washed with 5% aqueous sodium hydrogen carbonate and water and dried (Na_2SO_4). It was then evaporated under reduced pressure and the oily residue crystallized by addition of hexane. The crude product was recrystallized from ethyl acetate–hexane. For Nps-Glu(OEt)-Hmp-OPcp, the crude oily product was purified by silica gel column chromatography using chloroform–hexane (1:1) as an eluant; for Nps-Glu(OEt)-Lac-OPcp $\nu_{max.}$ 1 792, 1 745, and 1 717 cm^{-1} (the ester C=O of OPcp, OLac, and OEt, respectively).

Nps-Ala-Glu(OEt)-Ha-OPcp.—2.5M Hydrochloric acid in dioxane (40 ml) was added to Nps-Glu(OEt)-HA-OPcp (50 mmol) dissolved in tetrahydrofuran (100 ml). Diethyl ether–hexane (1:1; 300 ml) was then added to the solution to give an oily precipitate. This was then extracted with diethyl ether–hexane until it was no longer yellow. The precipitate HCl·H-Glu(OEt)-HA-OPcp was dissolved in tetrahydrofuran (100 ml) and the solution was cooled at −10 °C.

Nps-alanine (18.2 g, 75 mmol) and *N*-methylmorpholine (8.25 ml, 75 mmol) were dissolved in tetrahydrofuran and the solution was cooled at −10 °C. Isobutyl chlorocarbonate (9.9 ml, 75 mmol) was added to the solution with vigorous stirring and the stirring continued for 5 min at −10 °C. To the solution was added the solution of HCl·H-Glu(OEt)-HA-OPcp in tetrahydrofuran. A solution of triethylamine (7 ml, 50 mmol) in tetrahydrofuran (100 ml) was then added dropwise over 1 h at −10 °C to the mixture. The mixture was diluted with ethyl acetate (300 ml), washed with 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated under reduced pressure to give an oil; this crystallized upon addition of hexane. The product was recrystallized from ethyl acetate–hexane: for Nps-Ala-Glu(OET)-Lac-OPcp $\nu_{max.}$ 1 798, 1 764, 1 720, and 1 653 cm^{-1} (the amide C=O).

Nps-Ala-Ala-Glu(OEt)-HA-OPcp.—2.5M Hydrochloric acid in dioxane was added to Nps-Ala-Glu(OEt)-HA-OPcp (50 mmol) dissolved in tetrahydrofuran (150 mmol). Addition of diethyl ether—hexane (2:1; 300 ml) to the solution gave the crystalline hydrochloride, which was collected and washed with diethyl ether until disappearance of the yellow colour. After recrystallization from tetrahydrofuran containing a small amount of methanol, the pure HCl·H-Ala-Glu(OEt)-HA-OPcp was treated with Nps-alanine as above to give an Nps-tetradepsipeptide active ester; this was recrystallized from tetrahydrofuran–hexane: for Nps-Ala-Ala-Glu(OEt)-Lac-OPcp $\nu_{max.}$ 1 799, 1 766, 1 722, and 1 649 cm^{-1}.

[Ala-Ala-Glu(OEt)-HA]$_n$.—2.5M Hydrochloric acid (8 ml) was added to Nps-Ala-Ala-Glu(OEt)-HA-OPcp (10 mmol) dissolved in tetrahydrofuran (100 ml). Addition of diethyl ether (300 ml) to the solution gave the crystalline monomer hydrochloride, which was recrystallized from tetrahydrofuran–methanol. Triethylamine (1.68 ml, 12 mmol) was added with vigorous stirring to the monomer hydrochloride (10 mmol) dissolved in dimethyl sulphoxide (50 ml) and stirring was continued for 2 days at room temperature. The very viscous solution so obtained was diluted with methanol (300 ml) to give a precipitate, which was collected, washed with methanol and diethyl ether, and dried (P_2O_5). The polydepsipeptide so obtained was reprecipitated from chloroform–diethyl ether.

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