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A Base Labile Protecting Group for Peptide Synthesis: 2,2-Bis(4'-nitrophenyl)ethan-1-oxycarbonyl Urethanes

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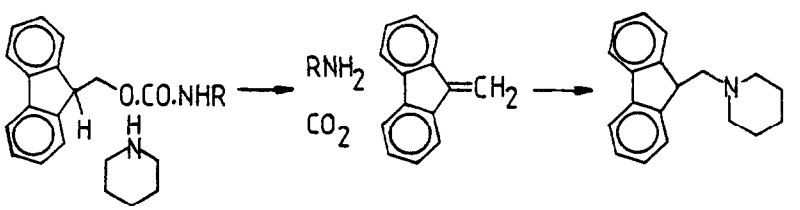
Abstract

A base labile urethane (Bnpeoc) group derived from 2,2-bis(4'-nitrophenyl)ethan-1-ol has been developed as an amine protecting group. This protecting group may be cleaved using the reagents DBN, DBU, DBU/HOAc and piperidine. The preparations of N^{α}-Bnpeoc amino acids and applications to peptide/glycopeptide synthesis are described.

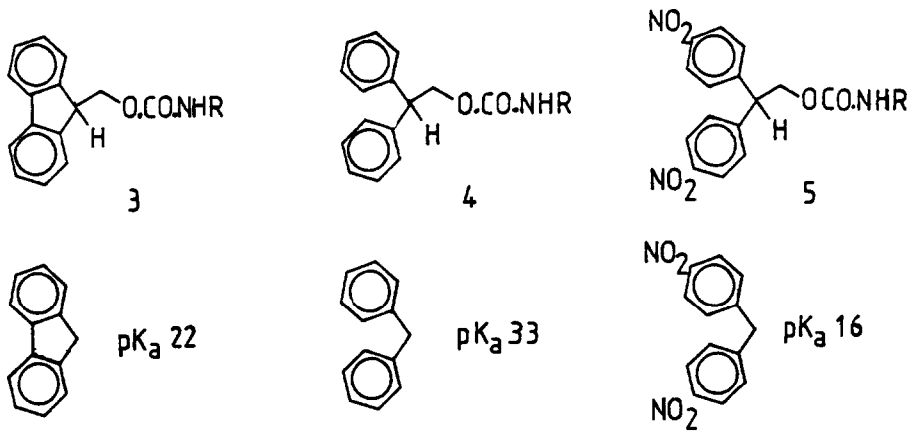
In recent years the Merrifield Solid Phase Peptide Synthesis (SPPS) concept[1] has been modified by introduction of the N^{α}-Fmoc protecting group[2] for α-amino acids. This base labile protecting group allows the side chain functionality to be labile to mild acid such as trifluoroacetic acid (TFA) in order to obtain the required orthogonality between N^{α}- and side chain functionality. In addition the design of a linker unit which can finally release the assembled peptide by TFA means that the crude peptide may be obtained at the end of SPPS using a single treatment by TFA, thus minimising exposure of the synthetic peptide to strong acid. A further degree of orthogonality may be obtained by using extremely acid-labile[3] or fluoride-cleavable linkers[4] which allows the SPPS of protected peptides for subsequent fragment coupling.



1 2

Scheme 1

In addition to the chemical benefits accruing to the N^{α}-Fmoc/Bu^{t} strategy[5] there is another advantage in that the deprotection mechanism (Scheme 1) produces the olefin (1) and the piperidine adduct (2), both of which can be monitored by real time UV spectroscopic measurement. However it must be remembered that for SPPS using automated instruments, all reagents must be soluble in the chosen solvent in a prescribed, short time at room temperature. Some N^{α}-Fmoc α-amino acid derivatives are not sufficiently soluble in dichloromethane, which is used in the Merrifield Boc/Bzl protocol, and hence require the more polar solvent dimethylformamide (DMF). Furthermore the Fmoc/Bu^{t} approach is rendered more expensive because of the cost of N^{α}-Fmoc α-amino acids. Thus we sought to design an alternative base-labile protecting group which would be capable of UV monitoring, give soluble derivatives and be less expensive.



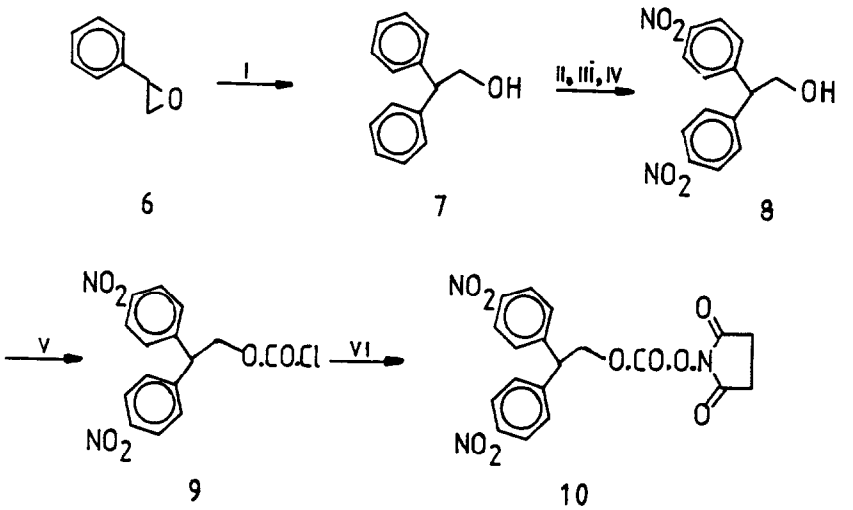
Scheme 2

The design concept, illustrated in Scheme 2, started from the Fmoc group (3) by cleaving the biphenyl bond to give the diphenylethoxycarbonyl system (4) and then introducing NO_2 groups to regain, and increase, the acidity of the β hydrogen according to the pKa values for the parent systems. The target, 2,2-bis(4'-nitrophenyl)ethan-1-oxycarbonyl (Bnpeoc), protecting group (5) was independently investigated by König *et al*,[6] and reported briefly in a conference proceedings. However, only five α-amino acid derivatives were prepared of which only Bnpeoc.Phe.OH was obtained as a crystalline solid. Of various options, the route selected for large scale (1 kg) synthesis of 2,2-bis(4'-nitrophenyl)-ethan-1-ol (8) is shown in Scheme 3.

Synthesis of 2,2-bis(4'-nitrophenyl)ethan-1-ol (8)

The first step in the synthesis of (8) involves the reaction of phenylmagnesium bromide with styrene oxide (6) which could produce two products. However, it had been shown by Kharash and Clapp[7] that the desired product (7) was produced when styrene oxide was added to the Grignard reagent, presumably due to Lewis acid-induced opening of the epoxide favouring the more stable benzylic carbocation intermediate. After acetylation, the nitration of 2,2-diphenylethyl acetate proceeded in 66% yield. The temperature of the reaction mixture proved to be critical since below 0°C the nitration was too slow whereas if the temperature was allowed to rise above 0°C the yield of desired 4',4'- dinitro-product was reduced, probably due to decreased selectivity of the nitration. Subsequent acid-catalysed methanolysis of the acetate then gave the required alcohol (8).

Treatment of the 2,2-bis(4'-nitrophenyl)ethan-1-ol (8) with phosgene (used as a toluene solution) and N-methylmorpholine (NMM) afforded the corresponding chloroformate (9) as a white crystalline solid. In practice, however, (9) was not isolated usually and the NMM hydrochloride



6 7 8

9 10

(i) PhMgBr (60%); (ii) Ac_2O/H^+ (cat.) (>95%);
(iii) H_2SO_4/HNO_3 (66%); (iv) H^+/MeOH (>95%); (v) $COCl_2$/NMM,
(vi) HONSu/NMM (82%); (vii) Et_3N/α-amino acid (65-90%)

Scheme 3

salt formed in the preparation of (9) was removed by filtration, whereupon the crude chloroformate solution was converted directly to the N-succinimidyl carbonate (10) by the addition of a further equivalent each of NMM and N-hydroxysuccinimide. The required product, Bnpeoc-ONSu (10) was isolated as a white solid in 82% yield.

Preparation of N^{α}-Bnpeoc amino acid derivatives

The general methods for the preparation of N^{α}-Bnpeoc amino acids from (10) were adaptations of the methods of Lapatsanis[8] and Rich.[9] A solution of (10) in DMF was added slowly to the α-amino acid in aqueous 10% Na_2CO_3 (2 equiv) and in the case of serine, threonine and tyrosine the yield of product could be improved with the addition of aqueous 5% $NaHCO_3$ (1 equiv). Alternatively the α-amino acid was suspended in dioxan/water (1:1) before addition of triethylamine (1.5 equiv) after which solid Bnpeoc-ONSu (10) was added and the reaction followed by tlc until completion (4-16 h). For the Bnpeoc derivatisation of hindered amino acids such as α-aminoisobutyric acid this was best achieved by using the chloroformate (9). The N^{α}-Bnpeoc amino acid derivatives were isolated by acidifying the reaction mixture followed by solvent extraction. In the case of derivatives which proved difficult to purify, these were converted into the cyclohexylamine (Cha) or dicyclohexylamine (Dcha) salts. These salts were recrystallised and reconverted to the pure N^{α}-Bnpeoc amino acids in near quantitative yields by partitioning the salt between an organic solvent and aqueous 2.0 M $KHSO_4$ solution. The free acids were obtained either as a foam or as a precipitate from chloroform/petroleum ether. The experimental section collates the N^{α}-Bnpeoc amino acids of which Bnpeoc.Ala.OH was subjected to X-ray crystal structure determination and the result is shown in Figure 1.

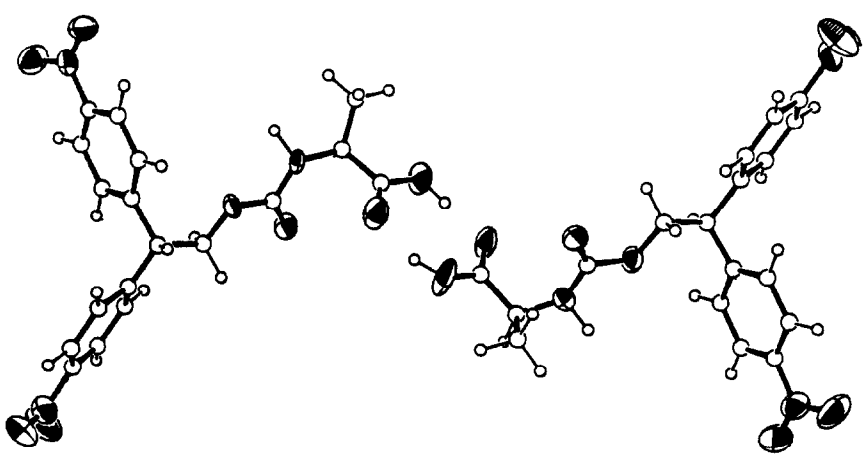


Figure 1 View of the asymmetric unit showing the Bnpeoc.Ala.OH linked carboxylic acid dimeric system

Crystal Data:- $C_{18}H_{17}N_3O_8$, M = 403.31, monoclinic, space group $P2_1$, a = 9.658(7), b = 12.057(6), c = 16.302(8)Å, β = 91.93(5)°, V = 1897Å^3 [from 2θ values of 25 centred reflections with 2θ = 10-25°, λ = 0.71073Å)], Z = 4, D_{calc} = 1.412 g cm^{-3}, T = 295K, colourless column, 0.35 x 0.35 x 0.62 mm, μ = 0.106 mm^{-1}, $F(000)$ = 840.

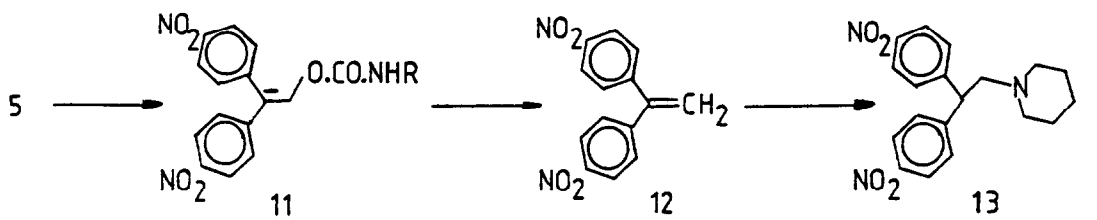
Data Collection and Processing:- Stoë STADI-4 four-circle diffractometer, graphite-monochromated Mo-K_α X-radiation, T = 295K, ω-θ scans with ω scan width (2.10 + 0.347tanθ)°, 2733 data collected ($2\theta_{max}$ 45°, h -10 → 10, k 0 → 12, l 0 → 17), 2422 unique (R_{int} 0.047), giving 1915 reflections with $F \geq 2\sigma(F)$ for use in all calculations. Linear isotropic crystal decay (*ca.* 2%) corrected for during data reduction.

Structure Solution and Refinement:- Automatic direct methods[10] located the positions of all non-H atoms which were then refined (by least-squares on F[11]) with anisotropic thermal parameters, with O-H and N-H distances constrained to be 1.00Å and the remaining H atoms included at fixed, calculated positions. At final convergence R, R_w = 0.0546, 0.0534 respectively, S = 1.227 for 523 refined parameters and the final ΔF synthesis showed no $\Delta\rho$ outside the range ±0.27 eÅ^{-3}. The weighting scheme $w^{-1} = \sigma^2(F) + 0.00133F^2$ gave satisfactory agreement analyses and in the final cycle $(\Delta/\sigma)_{max}$ was 0.29.

Atomic scattering factors were inlaid[11], molecular geometry calculations utilised CALC[12] and the Figure was produced by ORTEPII[13].

Deprotection of Bnpeoc derivatives

The Bnpeoc group was found to be very stable to acids with no modification being detected in TFA over 24 h or in 3 M HCl in methanol after 18 h. Not surprisingly, catalytic hydrogenation (for 2 h using Pd/C) gave two major products, plus starting material, which were shown to be the result of reduction of one or both nitro groups.



Scheme 4

In a study of the stability of N$^\alpha$-Fmoc- and Bnpeoc-Ser.OBzl in several solvents (DMF, DMA and NMP) it was observed that prolonged solution in the solvents could lead to partial deprotection. However the free acids, N$^\alpha$-Fmoc- and Bnpeoc-Thr.OH, were found to be stable over an

extended period (48 h). Bnpeoc derivatives (5) were found to be stable to treatment by pyridine or 2,6-lutidine but the Bnpeoc group may be cleaved by the addition of strong amidine bases such as DBU or DBN with rapid formation of 1,1-bis-(4'-nitrophenyl)ethene (12). A transient blue colour is observed during such a deprotection, characteristic of anions of the type (11), which fades as the reaction approaches completion (Scheme 4). During SPPS this phenomenon can be used as a self-indicator of deprotection since pulsed DBU treatment (5,3,3,1 min) of the N^{α}-Bnpeoc peptide bound to the resin gives an initial deep blue colouration to the resin followed by successive lessening of the colour until the last DBU treatment has no effect on the resin colour. This indicates when the N^{α} Bnpeoc group is fully deprotected. There is a solvent dependency in the deprotection with a slower rate in CH_2Cl_2 compared to DMF. In addition DBU in the presence of acetic acid (1 equiv), using DMF as solvent, also effects a smooth elimination to give (12) although it was found that the pKa of the added acid must be *ca* 4.5 since stronger acids depressed the rate of deprotection.

The Fmoc group is usually cleaved during SPPS by using a solution of 20% piperidine (v/v) in DMF and results in the formation of the piperidine adduct (2), although variable small amounts of the fulvene (1) may be present in the synthesis of longer peptides. Addition of 20% piperidine in DMF to Bnpeoc derivatives (5) resulted in a comparable rate of cleavage to give the corresponding amine and the piperidine adduct (13). This method was, in fact, the method of choice for SPPS. The olefin (12)[14] was prepared separately and treated with 20% piperidine in DMF to give (13). Monitoring of this addition by UV indicated that the olefin (λ_{max} 304 nm) was converted quickly to the piperidine adduct (λ_{max} 270 nm).

The deprotection of the Bnpeoc group was found to be dependent upon solvent polarity, substrate concentration, amount and nature of base, temperature, however a detailed interpretation of the kinetics did not give a clear distinction between El_{cb} and E2. We sought to test the relative stabilities of the N^{α}-Bnpeoc group and the COOMe group towards alkaline hydrolysis using Bnpeoc.Ala.Phe.Gly.OMe (14) as substrate. The best conditions found for the preferential hydrolysis[15] of the ester function were aqueous NaOH (0.1 M, 1 equiv), aqueous H_2O_2 (2 equiv) in 20% aqueous acetone at room temperature which afforded Bnpeoc.Ala.Phe.Gly. OH (15) in 55% yield. A standard sample of (15) was synthesised by SPPS using the Wang resin.[16]

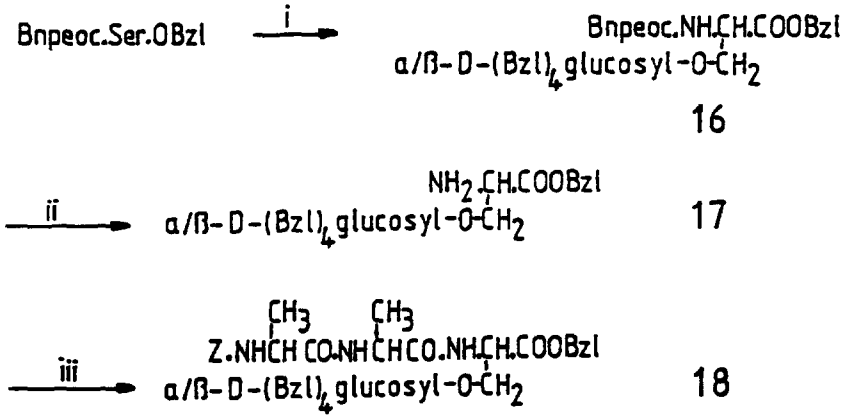
Application of N^{α}-Bnpeoc amino acids to SPPS

An important consideration when selecting α-amino acid derivatives for SPPS using an automated instrument, such as the ABI 430A used in these studies, is the solubility of each of these derivatives and reagents in

the chosen solvents. We sought to improve upon the range of solubilities that N^{α}-Fmoc amino acids exhibit in less polar solvent systems which increase the swelling of polystyrene resins. In general we found the N^{α}-Bnpeoc derivatives to have increased solubility in CH_2Cl_2 and CH_2Cl_2/dioxan mixtures compared with the N^{α}-Fmoc counterparts.

For the synthesis of peptide C-terminal carboxylic acids initial loading of the N^{α}-protected amino acid involves the formation of an ester with the linker unit; in these studies the polystyrene *p*-alkoxybenzyl alcohol system designed by Wang[16] was selected. Due to the depressed nucleophilicity of the OH function, compared with NH_2, it is important to avoid extreme conditions in the reaction of the carboxyl activated, N^{α}-protected amino acid with the OH function, since this can lead to racemisation at the C^{α}-chiral centre. Although this particular consideration does not occur when glycine is loaded under forcing conditions there is the additional problem in the possibility of double incorporation of glycine ie formation of Gly-Gly C-terminus. It has been found that these problems can be overcome for N^{α}-Fmoc amino acids if a catalytic amount of DMAP is included in the loading of N^{α}-Fmoc amino acid symmetrical anhdyrides. We therefore studied the loading process using Bnpeoc.Val.OH and Bnpeoc.Gly.OH by varying the methods of agitation, solvents and types of activation. Using the acid chloride method of Carpino[17] it was found that sonication was greatly superior to conventional shaking (2 x incorporation) and it was also observed that CH_2Cl_2 and DMF gave similar results. It was found that good loading (75%) was achieved with Bnpeoc. Val.Cl (1.1 equiv) using (a) CH_2Cl_2/ pyridine (1:2) as solvent or using (b) DMAP (0.05 mole/mole acid chloride) with NMM (2 equiv) as base with CH_2Cl_2 as solvent and employing mixing by a sonic bath at room temperature for both conditions. The N^{α}-Bnpeoc group was then cleaved by DBU/HOAc in DMA over 10 mins. Al- though racemisation should not occur on activation of the N^{α}-urethane Bnpeoc protected amino acids, by analogy with Fmoc and other N^{α}-urethane protection groups, the question was addressed by coupling (DCC) Boc(L)Ala. OH to the H_2N.Val-resin prepared by method (a) described above. Treatment of the Boc.Ala.Val-resin with TFA afforded the free dipeptide which was analysed by ion-exchange (LKB 4151 alpha plus amino acid analyser, column 24 x 0.6 cm, 75°C, 0.2 M sodium citrate pH 3.49) according to the modified Manning and Moore method. Samples of D,L- and L,L-dipeptide diastereomers were prepared and used as standards, however only the L,L-diastereomer could be identified from the above reaction sequence. The pentapeptides, H.Leu.Ile.Phe.Ala.Gly.OH and H.Thr.Leu.Ser.Ile.Gly.OH were synthesised by conventional N^{α}-Fmoc/ DCC-HOBt and N^{α}-Bnpeoc/DCC-HOBt SPPS methods and it was found that the products were identical in every respect with no allo-Ile observed by amino acid analysis.

Loading of Bnpeoc.Gly.OH (2.5 equiv) or Fmoc.Gly.OH (2.5 equiv) to the *p*-alkoxybenzyl alcohol polystyrene resin using DMAP (0.05 mole/mole amino acid) by the DCC method afforded 6 and 5% Gly.Gly incorporation respectively. It was found that the loading efficiency could be increased to *ca* 80% with <0.2% Gly.Gly content if Bnpeoc.Gly.OH or Fmoc.Gly.OH were incorporated as the acid chlorides using pyridine in CH_2Cl_2 without the necessity of adding DMAP. The acid chloride method of synthesis is limited largely to aliphatic α-amino acids. However, we have found that N^{α}-Bnpeoc and N^{α}-Fmoc amino acid carboxylic-phosphinic mixed anhydrides[18] are very efficient for the initial loading reaction.



(i) $(CF_3SO_2)_2O$, α-D-$(Bzl)_4$ glucose; (ii) DBU/HOAc,DMF; (iii) DCC/HOBt, Z.Ala.Ala.OH

Scheme 5

The synthesis of glycopeptides poses a great challenge for protecting group strategies and we tested the Bnpeoc protocol in the synthesis of Bnpeoc.Ser[α/β-D-$(Bzl)_4$Gluc].OBzl (16) and Z.Ala.Ala.Ser[α/β-D-$(Bzl)_4$-Gluc].OBzl (18) (Scheme 5). The reaction of Bnpeoc.Ser.OBzl (1 equiv) and α-D-$(Bzl)_4$-glucose (1 equiv) mediated by triflic anhydride and performed in a solvent mixture of CH_3CN/CH_2Cl_2 afforded (16) in 34% yield as a 1:1 ratio of α and β anomeric products after a difficult purification procedure. Selective deprotection of the N^{α}-Bnpeoc group of (16) was investigated using DBU/HOAc (1.2 equiv) in either CH_2Cl_2 (3 h) or DMF (20 min). The latter conditions afforded a quantitative yield of (17). Z.Ala.Ala.OH was prepared by the method of Rich[9] from Z.Ala.ONSu and alanine. The coupling of (17) and Z.Ala.Ala.OH was achieved using DCC/HOBt together with Et_3N to adjust the pH of the reaction mixture to

9.0. Purification of (18) was performed by preparative hplc thus it can be concluded that N^{α}-Bnpeoc amino acid derivatives are compatible with glycopeptide synthesis.

EXPERIMENTAL

All L-amino acids used were purchased from the SAS group of companies; protected derivatives were purchased from Applied Biosystems Inc., Bachem or Novabiochem and were used as supplied. Melting points were taken in open capillaries on an electrically heated Buchi 510 melting point apparatus, or on microscope slides on an electrically heated Reichart 7905 melting point apparatus and are uncorrected. Thin layer chromatography (tlc) was carried out on plastic sheets coated with silica gel 60GF-254 (Merck 5735) in the following systems:

A 1/5, ethyl acetate/light petroleum
B 1/4, ethyl acetate/light petroleum
C 30/70, ethyl acetate/light petroleum
D 40/60, ethyl acetate/light petroleum
E 18/2/1, chloroform/methanol/acetic acid
F 16/4/1, chloroform/methanol/acetic acid
G 19/1, ethyl acetate/acetic acid
H 2/1/1/1, ethyl acetate/n-butanol/acetic acid/water
I 3/1/1, n-butanol/acetic acid/water
J 1/1/1, cyclohexane/ethyl acetate/methanol
K 3/1, ethyl acetate/chloroform
L 4/6, methanol/chloroform
M 1/4, methanol/chloroform
N ethyl acetate

Visualisation of the compounds was achieved by using the following methods as appropriate: iodine vapour, ultra-violet absorbtion at 254 nm, Mary's reagent [4,4'-*bis*(dimethylamino)diphenylcarbinol], acidified potassium permanganate and ninhydrin. Hplc was carried out using Applied Biosystems equipment (2 x 1406 A solvent delivery systems, a 1480 A injector mixer and a 1783 A detector controller), or its equivalent. The gradients that were used are summarised in Table 1. The sample was injected, followed by the isocratic and then the gradient phases. The elution of the sample was monitored at 214 or 229 nm.

Gradient	Flow ml/min	Isocratic %A	Time (Min)	Gradient %A (1)	(2)	Time (Min)	Column	Size mm
A	1.0	10	4	10	90	25	ABI RP18	220 x 4.6
B	3.6	30	5	30	50	20	ABI RP18	100 x 10
C	1.0	0	4	0	100	30	ABI RP18	100 x 4.6
D	4.0	5	4	5	15	15	ABI RP18	250 x 10
E	1.0	5	4	5	25	20	ABI RP18	220 x 4.6
F	5.0	5	4	5	35	20	ABI RP18	250 x 10
G	1.0	5	4	5	35	20	ABI RP18	220 x 4.6
H	1.0	5	4	5	40	30	Capital ODS-SB5	250 x 10
I	3.0	5	4	5	15	15	ABI RP8	250 x 10
J	4.0	10	4	10	50	20	ABI RP18	250 x 10
K	3.5	5	5	5	40	30	ABI RP18	250 x 10
L	1.0	5	5	5	50	20	Capital ODS-SB5	250 x 4.5
M	1.0	5	4	5	20	20	ABI RP8	220 x 4.6
N	1.0	-	-	20	100	28	Spherisorb 8	300 x 2.6
O	1.0	-	-	50	100	25	Spherisorb 18	300 x 2.6

Table 1. The conditions used for reverse phase h.p.l.c. buffer A was 0.1% TFA in water. Buffer B was 0.1% TFA in acetonitrile. (1) gives the percentage of A at the start of the gradient and (2) gives the percentage of A at the end of the gradient.

Amino acid analyses were carried out on a LKB 4151A alpha plus amino acid analyser, following hydrolysis in constant boiling hydrochloric acid at 110°C for an appropriate length of time (24-72 hours). Infra-red spectra were recorded on a Perkin Elmer 781 spectrophotometer in the solvent stated or as a nujol mull, using polystyrene (1603 cm^{-1}) as a standard. Ultra-violet spectra were recorded on a Varian Cary 210 spectrophotometer and wavelengths are quoted in nanometers and extinction coefficients are quoted in $dm^3\ mol^{-1}\ cm^{-1}$. Optical rotations were measured using an Optical Activity AA1000 polarimeter. Optical rotation measurements of known compounds were performed under identical conditions (concentration and solvent) to those stated in the literature, unless otherwise noted. Mass spectra (FAB) were measured on a Kratos MS 50TC spectrometer. Proton nuclear magnetic resonance spectra were recorded on Bruker WP80 (80 MHz), WP200 (200 MHz) or WH360 (360 MHz) instruments using tetramethylsilane as the standard (δ = 0.00), or on the Varian VXR600S (600 mHz). Spectra recorded on this instrument were referenced either to H_2O (at 4.8 p.p.m. at 25°C) or to CD_2HOH (at 3.325 p.p.m. in CD_3OH). Elemental analyses were carried out on a Carlo Erba model 1106 elemental analyser. The following solvents were distilled before use and were dried using the reagents given in parentheses: chloroform (phosphorus pentoxide), dichloromethane (calcium hydride), diethyl ether (sodium wire), ethyl acetate, tetrahydrofuran (benzophenone/sodium), ethanol (magenesium ethoxide). Light petroleum refers to the fraction which boils between 40° and 60°C.

2,2-Diphenylethanol (7).

This compound was prepared by the method of Kharash and Clapp[7] using a 10 litre five necked flask. A single crystal of iodine was added to a suspension of magnesium (88.25 g, 3.63 mol) in dry diethyl ether (400 ml) under an atmosphere of argon, then bromobenzene (345.5 g, 3.3 mol) in dry diethyl ether (800 ml) was added cautiously dropwise at a rate sufficient to maintain the reaction at reflux. When the addition was complete the mxiture was heated under reflux for 30 min with stirring to produce the Grignard reagent, phenylmagnesium bromide. The stirred Grignard reagent was then cooled to between 0 and 5°C in an ice bath and a solution of styrene oxide (352.3 g, 3.0 mol) in dry diethyl ether (800 ml) was added dropwise over 30 min. The solution was stirred for a further 30 min after which the solution was poured cautiously onto a mixture of ice (1 l) and water (1 l) and treated with sulphuric acid (4.0 M, *ie* pH1). The organic phase was separated and the aqueous phase extracted with diethyl ether (2 l). The combined organic phase was washed with saturated sodium bicarbonate solution (2 x 500 ml), water (3 x 500 ml) and brine (1 x 500 ml), then dried ($MgSO_4$) and the solvent removed *in vacuo*. The *title compound* was then crystallised from light petroleum as a white solid (286.6 g, 48%): m.p. 60-61°C) (lit.,[7] 56°C); (Found: C, 84.8; H, 7.1. Calc for $C_{14}H_{14}O$: C, 84.8; H, 7.1%); tlc -A,R_f, 0.61.

2,2-Diphenylethyl acetate.

2,2-Diphenylethanol (200.1 g, 1 mol) was added in small portions as a fine solid to acetic anhydride (189.2 g 2 mol) and then sulphuric acid (98%, 15 drops) was added and the mixture was stirred. The solid dissolved after 25 min and the solution became warm. After a further 30 min the products were poured onto crushed ice (800 ml) and then filtered, washed with water (1 l) then dried to constant weight to give the *title compound* as a solid (243.0 g, quantitative) m.p. 54°C, which could be recrystallised from diethyl ether to give 2,2-diphenylethyl acetate as crystalline product (m.p. 54-55°C); tlc -B,R_f 0.31.

2,2-Bis-(4'-nitrophenyl)ethyl acetate.

A carefully prepared mixture of sulphuric (98%; 169 ml) and nitric (63%: 169 ml) acids were cooled, with vigorous stirring, to between -5 and 0°C in an ice/salt bath and 2,2-diphenylethyl acetate (183.0 g, 0.55 mol) was added cautiously in small portions to the mixture, over a period of one and a half hours, whilst the temperature was carefully maintained between -5 and 0°C by periodic addition of salt to the ice/salt bath. The mixture was then stirred for a further two hours and then poured cautiously onto crushed ice (1 l) and extracted with ethyl acetate (3 x 1 l). The combined organic phase was washed with saturated sodium bicarbonate solution until the pH of the aqueous layer was neutral. The organic phase was then washed with water (3 x 500 ml) and brine (1 x 500 ml) and dried ($MgSO_4$). The ethyl acetate was removed *in vacuo*, leaving a yellow oil which was crystallised from diethyl ether to afford the *title compound* (121.1 g, 66%) as a pale yellow solid: m.p. 110-111°C; (Found: C, 58.0; H, 4.2; N, 8.4; $C_{16}H_{14}N_2O_6$ requires C, 58.2; H, 4.4; N, 8.4%); tlc -C, R_f 0.31.

2,2-Bis(4'-nitrophenyl)ethan-1-ol (8).

2,2-*Bis*(4'-nitrophenyl)ethyl acetate (141.3 g, 0.43 mol) was dissolved in methanol (500 ml) and conc. hydrochloric acid (16.9 ml) was added dropwise. The mixture was then heated under reflux for five hours. The mixture was then poured onto crushed ice (500 ml) and the solid product was extracted into ethyl acetate (250 ml). The organic phase was separated and the aqueous phase extracted with ethyl acetate (2 x 250 ml). The combined organic phase was then washed with water (2 x 500 ml) and brine (1 x 500 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was crystallised from chloroform/diethyl ether to afford the *title compound* (122.5 g, 99%) as a white solid: m.p. 109-110°C; (Found: C, 58.4; H, 4.2; N, 9.7; $C_{14}H_{12}O_5N_2$ requires C, 58.5; H, 4.2; N, 9.7%); tlc -D, R_f 0.25.

2,2-Bis(4'-nitrophenyl)ethyl chloroformate (9).

2,2-*Bis*(4'-nitrophenyl)ethan-1-ol (8) (14.4 g, 50 mmol) was dissolved in toluene (200 ml) at 60°C. To this solution, maintained at 40°C, was added phosgene (64.4 mol, 1.5 eq., 12.5% w/w toluene) followed by N-methylmorpholine (6 ml). After 30 mins the precipitated N-methylmorpholine hydrochloride was removed *via* filtration through a grade 3 sintered funnel to leave a clear green solution. The solvent was removed *in vacuo* to produce an oil which was crystallised from chloroform/diethyl-ether to give 2,2-*bis*(4'-nitrophenyl)ethyl chloroformate (7) as a white solid (17.5 g, quantitative) which was recrystallised from chloroform/diethyl ether, m.p. 97-98°C; (Found: C, 51.4; H, 3.11; N, 8.01; $C_{15}H_{11}N_2O_6Cl$ requires C, 51.4; H, 3.14; N, 7.99%); ν_{max} (CH_2Cl_2) 1770 (CO) 1605, 1595, (C-C, aromatic), 1520, 1350 (NO_2), 1160, 1145, 860 and 820 cm^{-1}; λ_{max} 274 (ϵ 20400); δ_H ($CDCl_3$, 200 MHz) 8.19 (4H, d, J_{AB} 8.7 Hz), 7.42 (4H, d, J_{AB} 8.7 Hz), 4.91 (2H, d, J_{AB_2} 7.1 Hz, CH_2), 4.70 (1H, t, J_{AB_2} 7.0 Hz, CH); δ_C ($CDCl_3$, 50 MHz) 150.4 (CO), 147.4 (quat. aromatic C's), 129.0, 124.1 (aromatic C's), 71.6 (CH_2), 48.9 CH); HRMS 351.03839, $C_{15}H_{12}N_2O_6Cl$ requires 351.03838.

2,2-Bis(4'-nitrophenyl)ethyl-N-succinimidyl carbonate (10).

2,2-*Bis*(4'-nitrophenyl)ethan-1-ol (64.77 g, 0.225 mol) was dissolved in dichloromethane (800 ml) and a solution of phosgene in toluene (1.93 M, 176 ml, 1.5 equivs) was added. N-methylmorpholine (25 ml) was added dropwise to this mixture over a period of one hour, with stirring, under an atmosphere of dry nitrogen and then the N-methylmorpholine hydrochloride salt, which had precipitated, was removed by filtration. The solvents were removed *in vacuo* and the residue was redissolved in dry 1,4 dioxan (300 ml). The solution was stirred under an atmosphere of dry nitrogen and N-hydroxysuccinimide (29.35 g, 0.255 mol), was added in small portions. To this mixture was added N-methylmorpholine (25 ml) and more hydrochloride salt was then observed to precipitate. The solution was stirred for 16 hours and then the N-methylmorpholine hydrochloride was

removed by filtration and the dioxan removed *in vacuo* to leave a pale yellow crystalline solid which was recrystallised from acetone/light petroleum to afford the *title compound* (79.15 g, 82%) as a white solid: m.p. 183-186°C; (Found: C, 53.4; H, 3.5; N, 9.85; $C_{19}H_{15}N_3O_9$ requires C, 53.2; H, 3.5; N, 9.8%); tlc -E, R_f 0.65. ν_{max} (nujol) 1829, 1800, 1745, 1610, 1600, 1515, 1350, 835, 820 cm^{-1}; δ_H [$(CD_3)_2CO$, 200 MHz] 8.3 (4h, d J_{AB} 9 Hz), 7.7 (4H, d, J_{AB} 9 Hz), 5.2 (3H, m, CH_2CH_2 Bnpeoc), 2.8 (4H, s); δ_C [$(CD_3)_2CO$, 50 MHz] 168.24(CO), 150.54(CO), 146.54, 145.86 (aromatic C), 128.77(CH), 123.06(CH), 70.37(CH_2), 48.11(CH), 24.14(CH_2); HRMS 430.08866, $C_{19}H_{16}N_3O_9$ requires 430.08864.

*1,1-*Bis*(4'-nitrophenyl)ethene* (12).

2,2-*Bis*(4'-nitrophenyl)ethyl acetate (1.82 g, 5.53 mmol) was dissolved in chloroform (18 ml) and to this was added DBN (1.37 ml. 11.1 mmol). On addition of the base, the initially pale yellow solution turned dark blue and a white precipitate formed. After 15 minutes the reaction mixture was diluted with further chloroform (12 ml) and washed with 2 M HCl (2 x 30 ml), water (1 x 30 ml), 1 M NaOH (2 x 30 ml) and brine (2 x 30 ml). After drying over Na_2SO_4 the solvent was removed *in vacuo* to yield a yellow solid which was recrystallised from chloroform/ light petroleum to afford the *title compound* (1.03 g, 69%) as a yellow solid: tlc -K, R_f 0.75; m.p. 174-175°C (lit.[14] 175-176°C); (Found: C, 61.8; H, 3.73; N, 10.3; Calc. for $C_{14}H_{10}N_2O_4$ C, 62.2; H, 3.70; N, 10.4%); ν_{max} (CH_2Cl_2) 1605, 1520, 1350 (NO_2), 870 cm^{-1}; λ_{max} 304 nm; δ_H (200 mHz, $CDCl_3$) 8.22 (4H, d, J_{AB} 8.0 Hz, Bnpe aromatic CH's), 7.46 (4H, d, J_{AB} 9.0 Hz, Bnpe aromatic CH's), 5.77 (2H, s, CH_2); δ_C (50 mHz, $CDCl_3$) 147.6, 146.4 (3 x quaternary C's), 128.8, 123.7 (aromatic CH's), 120.0 (CH_2); *m/z* (FAB), 271 (MH^+), 255, 240, 176; hplc R_f (B1) 16.8 minutes.

*N-[2,2-*Bis*(4'-nitrophenyl)ethyl]-piperidine* (13).

1,1-*Bis*(4'-nitrophenyl)ethene (12) (0.105 g, 0.39 mmol) was dissolved in DMF (3.0 ml) at room temperature. On addition of piperidine (1.0 ml), the initially pale yellow solution turned dark blue. After 20 minutes the soution had returned to a pale yellow colour. The removal of the solvent *in vacuo* co-evaporating with methylene chloride (twice), afforded an orange oily residue; λ_{max} 275 nm; δ_H (200 mHz, $CDCl_3$) 8.12 (4H, d, J_{AB} 8.9 Hz, Bnpe aromatic CH's), 7.37 (4H, d, J_{AB} 8.9 Hz, Bnpe aromatic CH's), 4.41 (1H, t, J 7.7 Hz, Bnpe CH), 2.89 (2H, d, J 7.7 Hz, Bnpe CH_2), 2.40 (4H, m, 2 x CH_2), 1.41 (6H, m, 3 x CH_2); δ_C (50 MHz, $CDCl_3$) 149.8, 146.5 (quaternary aromatic C's), 129.0, 123.5 (aromatic CH's), 63.3 (Bnpe CH_2), 54.5 (CH_2), 48.3 (Bnpe CH), 25.7, 24.0 (CH_2).

*Preparation of N^{α}-[2,2,-*Bis*(4'-nitrophenyl)ethoxycarbonyl]amino acid derivatives.*

Two methods were used for the preparation of the Bnpeoc amino acid derivatives:

Method 1: is an adaption of the general method of Shute and Rich[9] and was used to prepare compounds which could be readily crystallised. Bnpeoc.Tyr(Bu^t).OH is given in full as an example.

Method 2: was used for the Bnpeoc derivatives which could not be readily crystallised, in which case they were isolated as their crystalline Cha or Dcha salts. The free acid was then liberated to give a foam or an amorphous powder. Bnpeoc.Lys(Boc).OH and its Dcha salt are given in full as examples.

*N^{α}-[2,2-*Bis*(4'-nitrophenyl)ethoxycarbonyl]-(O-*tertbutyl*)-tyrosine.*

Bnpeoc.Tyr(Bu^t).OH

The *title compound* was obtained by method 1 as follows: a solution of triethylamine (0.42 ml, 3 mmol, 1.5 equivs) in dioxan (10 ml) was added to a stirred suspension of H.Tyr(Bu^t).OH. 0.75 H_2O (502 mg, 2 mmol) in water (10 ml). Bnpeoc.ONSu (860 mg, 2 mmol) was then added and the mixture stirred for 16 hours at room temperature. The resultant solution was then diluted with water (20 ml) and the pH adjusted to 3.0 by the addition of $KHSO_4$ (2.0 M). The solution was extracted with ethyl acetate (3 x 100

ml) and the combined organic phase washed with water (3 x 300 ml) and brine (1 x 200 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a clear oil which was crystallised from diethyl ether/light petroleum to give the *title compound* (823 mg, 75%) as a white solid: m.p. 73-76°C; (Found: C, 60.8; H, 5.3; N, 7.6; $C_{28}N_{28}N_3O_9$ requires C, 61.0; H, 5.3; N, 7.6%); tlc -E,R_f 0.65; hplc -A,R_f 24.2 min; $[\alpha]_D^{22}$ -20.0° (c 0.8 in DMF); ν_{max} (CH_2Cl_2) 3420 (NH), 2985, 1760 (sh, carboxylic acid), 1720 (s, urethane), 1610, 1525, 1505, 1390, 1370, 1350 (NO_2), 1160, 1110, 1040, 860, 830; λ_{max} (MeOH) 276 (ϵ 25000); δ_H (80 MHz, $CDCl_3$) 1.30 (9H, s, 3 x CH_3, Bu^t), 3.02 (2H, m, βCH_2, Tyr), 4.4-4.8 (4H, m, $CHCH_2$, Bnpeoc and αCH, Tyr), 5.07 (1H, d, αNH, Tyr), 6.75-7.05 (4H, m, Ar-H, tyr), 7.35 (4H, d, Ar-H, Bnpeoc), 8.15 (4H, d, Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_3$) 28.65 (3 x CH_3, Bu^t), 36.71 (αCH_2, Tyr), 49.65 (αCH, tyr), 54.52 (CH, Bnpeoc), 65.86 (CH_2, Bnpeoc), 78.65 ($C(CH_3)_3$), 123.93, 124.13, 129.09, 129.53 (ArCH's, Bnpeoc and tyr), 130.09, 146.70, 147.07, 154.36 (quaternary C's, Bnpeoc and Tyr), 155.22 (urethane), 175.52 (carboxylic acid), *m/z* 551 (MH^+, 0.6%), 536(1.2), 496 (M^+-Bu^t, 12.1), 450(4.80), 271(13), 225(20), 180(25), 136(38), 107(100), 89(23), 77(24), 48(200); HRMS 550.18257 $C_{28}H_{28}O_9N_3$ requires 550.18254.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-N^{ϵ}-(tert.butyloxycarbonyl)-lysine dicyclohexylammonium salt. Bnpeoc.Lys(Boc).OH,Dcha

The *title compound* was prepared by method 2 as follows: Triethylamine (0.76 ml, 5.5 mmol, 1.5 equivs) in dioxane (10 ml) was added to a stirred suspension of H.Lys(Boc).OH (0.9 g, 3.65 mmol) in water (10 ml). To this mixture was added solid Bnpeoc.ONSu (1.56 g, 3.65 mmol) and the mixture was stirred for 16 hours at room temperature. The solution was then diluted with water (30 ml) and the pH adusted to 3.0 with $KHSO_4$ (2.0 M). This suspension was then extracted with ethyl acetate (3 x 150 ml) and the combined organic phase washed with water (3 x 250 ml) and brine (1 x 250 ml), dried ($MgSO_4$) and the solvent remobved *in vacuo* to give a pale green gum. This was redissolved in dichloromethane (20 ml) and cooled to 0°C. Dicyclohexylamine (1.19 ml, 1 equiv) was then added and the solution stirred for 15 min, after which diethyl ether (100 ml) was added to afford the *title compound* (4.12 g, 93%) as a white solid; m.p. 146-149°C (dec); (Found: C, 61.2; H, 7.4; N, 9.4 $C_{38}H_{55}N_5O_{10}$ requires C, 61.2; H, 7.4; N, 9.4%); $[\alpha]_D^{22}$ +2.9° (1.0 in DMF); tlc -E, R_f 0.55; hplc -A,R_f 24.8 min; ν_{max} (CH_2Cl_2) 3440 (NH), 2970, 2840, 1720, 1635, 1610, 1600, 1525, 1395, 1365, 1350; λ_{max} (MeOH) 275 (ϵ 21300); δ_H (80 MHz; $CDCl_3$) 0.8-2.2 (37H, br m, CH_2 x 3, Lys, 10 x CH_2, Dcha, 2 x CH, Dcha, 3 x CH_3, Boc), 3.0 (2H, m, ϵCH_2, Lys), 3.85 (1H, m, αCH, Lys),4.55 (3H, m, $CHCH_2$, Bnpeoc), 4.65 (1H, αNH, Lys), 7.40 (4H, d, Ar-H, Bnpeoc), 8.10 (4H, d, Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_3$) 22.3(CH_2, Lys), 24.53 (2 x CH_2, Dcha), 25.00 (CH_2, Lys), 28.26 (CH_3 x 3, Boc), 29.23 (CH_2, Dcha), 31.99 (CH_2, Lys), 40.42 (CH_2, Lys), 49.79 (αCH, Lys), 52.44 (CH, Dcha), 55.52 (CH, Bnpeoc), 65.19 (CH_2, Bnpeoc), 78.84 ($C(CH_3)_3$), Boc), 123.85, 129.40 (Bnpeoc aromatic CH's), 146.92, 147.12 (Bnpeoc quaternary C's), 154.96, 155.78 (urethanes), 178.65 (carboxylate); *m/z* (MH^+, 0.3%), 741 (0.76), 560 (0.21), 504 (0.4), 460 (2.1), 306 (3.1), 182 (100), 154 (23); HRMS 742.40266 $C_{38}H_{56}N_5O_{10}$ requires 742.40269.

N^{α}-[Bis(4'-nitrophenyl)ethoxycarbonyl]-N^{ϵ}-(tert.butyloxycarbonyl)-lysine. Bnpeoc.Lys(Boc).OH

Bnpeoc.Lys(Boc-OH.Dcha (4.30 g, 6.75 mmol) was suspended in $KHSO_4$ (2.0 M, 500 ml) and extracted with ethyl acetate (3 x 300 ml). The combined organic phase was washed with water (3 x 500 ml) and brine (1 x 500 ml) and then dried ($MgSO_4$). The solvent was removed *in vacuo* and the residual oil redissolved in dichloromethane and evaporated *in vacuo* to give the *title compound* (3.36 g, 89%) as a yellow foam. A small quantity of the material was precipitated from chloroform/light petroleum as a white amorphous powder; m.p. 76-69°C (Found: C, 55.8; H, 5.8; N, 9.9 $C_{26}H_{32}N_4O_{11}$ requires C, 55.6; H, 6.1; N, 10.0%); tlc -E,R_f 0.55; hplc -A,R_f 24.8 min; $[\alpha]_D^{22}$ + 7.6° (c 1.0 in C HCl_3); ν_{max} (CH_2Cl_2) 3440 (NH), 2960, 1720, 1605, 1600, 1520, 1350, 1240, 1170, 1080, 870; λ_{max} ($CHCl_3$)

275 (ε 19500); δ_H (80 MHz, $CDCl_3$) 1.5 (15H, m, 3 x CH_3, Boc, β, γ and δ CH_2, Lys), 3.05 (2H, m, εCH_2, Lys), 4.25 (1H, m, αCH, Lys), 4.63 (3H, m, $CHCH_2$, Bnpeoc), 5.53 (1H, d, αNH, Lys), 7.37 (4H, d, Ar-H, Bnpeoc), 8.15 (5H, m, εNH, Lys and Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_3$) 22.06 (CH_2 x 2, Lys), 28.04 (CH_3 x 3, Boc), 31.24 (CH_2, Lys), 39.71 (CH_2, Lys), 49.48 (αCH, Lys), 53.47 (CH, Bnpeoc), 65.66 (CH_2, Bnpeoc), 79.30 ($C(CH_3)_3$, Boc), 123.77, 129.04 (Bnpeoc aromatic CH's, 146.81 (Bnpeoc quaternary C's), 156.19 (urethanes), 175.52 (carboxylic acid); *m/z* 561 (MH^+, 0.3%), 506 (MH^+ -Bu^t, 2), 254(10), 178(8), 128(17), 92(17), 85(25), 58(100); HRMS 561.21966 $C_{26}H_{33}N_4O_{10}$ requires 561.21965.

N^{α}-[2,2-Bis(4'nitrophenyl)ethoxycarbonyl]-α-aminoisobutyric acid. Bnpeoc.Aib.OH

The *title compound* was prepared using Bnpeoc.Cl (9) in an aqueous Na_2CO_3 solution diluted with DMF (1:1). The required derivative was isolated in 50% yield as a crystalline compound; m.p. 166-9°C, after crystallisation from diethyl ether/chloroform followed by ethyl acetate/light petroleum: (Found: C, 54.7; H, 4.59; N, 10.1. $C_{19}H_{19}N_3O_8$ requires C, 55.0; H, 4.77; N, 9.90%); ν_{max} (CH_2Cl_2) 3420(NH), 2980, 1720, 1605, 1595, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 275 (ε 19850); δ_H [$(CD_3)_2CO$, 200 MHz] 8.22 (4H, d, J_{AB} 8.8 Hz), 7.69 (4H, d, J_{AB} 8.8 Hz), 4.77 (3H, m, $CHCH_2$ Bnpeoc), 1.45 (6H, s, 2 x CH_3); δ_C [$(CD_3)_2CO$, 50 MHz] 174.2, 153.9, 147.3, 146.5, 128.9, 122.8, 64.5 (CH_2Bnpeoc), 54.9 (αCH), 49.1 (CH Bnpeoc), 23.9 (2 x CH_3); HRMS 418.12499. $C_{19}H_{20}N_3O_8$ requires 418.125.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-alanine. Bnpeoc.Ala.OH

The *title compound* was prepared by method 1 and crystallised from chloroform/diethylether to afford a white solid (8.97 g, 89%); m.p. 148-9°C; (Found: C, 53.5; H, 4.2; N, 10.4. $C_{18}H_{17}N_3O_8$ requires C, 53.5; H, 4.2; N, 10.4%); $[\alpha]_D^{22}$ + 0.8° (C 1.0 in DMF); tlc-E, R_f 0.60; ν_{max} (CH_2Cl_2) 3425, 3040, 2980, 1730, 1605, 1595, 1520, 1350, 890, 860 cm^{-1}; λ_{max} (MeOH) 274 (ε 32400); δ_H [$(CD_3)_2O$; 200 MHz] 1.36 (3H, d, βCH$_2$), 4.20 (1H, q, αCH), 4.70-4.82 (3H, m, Bnpeoc $CHCH_2$), 7.70, 8.22 (2 x 4H, 2 x d, Bnpeoc Ar-H); *m/z* 404 (MH^+, 10%), 257(4), 62(100); HRMS 404.1094. $C_{18}H_{18}N_3O_8$ (MH^+) requires 404.10940.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]N^G-(2,2,5,7,8-pentamethyl-chroman-6-sulphonyl)arginine cyclohexylamine salt. Bnpeoc.Arg(Pmc).OH,Cha

The *title compound* was prepared by method 2 and crystallised from dichloromethane/diethylether as a pale yellow solid (79%); m.p. 136-137°C; (Found: C, 57.8; H, 6.73; N, 11.6. $C_{41}H_{55}N_7O_{11}S$ requires C, 57.7; N, 6.5; N, 11.6%); tlc -E, R_f 0.4; ν_{max} (CH_2Cl_2) 3440 (NH), 1720 (urethane), 1610 (phenyl), 1525 (NO_2); λ_{max} (MeOH) 435 (ε1200), 320 (ε13600), 282 (ε53000), 256 (ε48000); δ_H (80 mHz, $CDCl_3$) 1.0-1.9 (22H, br m, Pmc x x CH_3, Argβ and γ CH_2's, Cha 5 x CH_2), 2.03 (3H, s, Pmc CH_3), 2.55 (2H, m, Pmc CH_2), 3.05 (3H, m, δCH_2, Arg and CH, Cha), 3.89 (1H, m Arg αCH, 4.5-4.7 (3H, m, $CHCH_2$, Bnpeoc), 6.0-7.0 (7H, br m, Arg αNH, guanadino NH's, Cha NH_3^+), 7.32 (4H, d, Ar-H, Bnpeoc), 8.10 (4H, d, ArH, Bnpeoc); *m/z* 754 [Bnpeoc.Arg(Pmc).OH, 7.6%], 366 (29), 326 (2.7), 219 (18), 203 (36), 147 (67), 100 (ChaH$^+$. 100).

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]N^G-(2,2,5,7,8-pentamethyl-chroman-6-sulphonyl)arginine. Bnpeoc.Arg(Pmc).OH

The *title compound* was prepared by method 2 and precipitated from chloroform with light petroleum as a pale yellow solid: [75% from H.Arg(Pmc).OH]; m.p. *ca* 140°C: (Found: C, 55.6; H, 5.8; N, 10.8. $C_{35}H_{42}N_6O_{11}S$ requires C, 55.7; H, 5.6; N, 11.1%); $[\alpha]_D^{22}$ + 3.6° (c 1.0 in $CHCl_3$); m/z 755 (MH^+, 9%), 499 (5), 219 (2), 203 (50), 147 (100); HRMS 755.27104. $C_{35}H_{43}N_6O_{11}S$ (MH^+) requires 755.27103.

N^{α}[2,2-Bis(4'-nitrophenyl)ethoxcarbonyl]-asparagine. Bnpeoc.Asn.OH

The *title compound* was prepared in 75% yield by method 1 and recrystallised from acetone/light petroleum ether to give a solid, m.p.

177-8°C; (Found: C, 50.9; H, 4.05; N, 12.6. $C_{19}H_{18}N_4O_9$ requires C, 51.1; H, 4.04; N, 12.5%); $[\alpha]_D^{22}$ + 0.5° (C 1.0 in DMF); tlc -E, R_f 0.2; ν_{max} ($CHBr_3$ mull), 3430, 3140, 1750 (COOH), 1705, 1675, 1520, 1350, 860 and 840 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 23,200); δ_H [$(CD_3)_2CO$, 200 MHz] 215 (2H, m, Asn βCH_2), 4.26 (1H, q, Asn αCH), 4.7 (3H, m, Bnpeoc CH and CH_2), 6.90 (1H, d, NH), 7.30 (2H, m, NH_2), 7.7- and 8.14 (8H, 2 x d, Bnpeoc aromatic CH); HRMS 447.11522. $C_{19}H_{19}N_4O_9$ (MH^+) requires 447.1152.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-N^{γ}-[bis(4'-methoxyphenyl)-methylasparagine. Bnpeoc.Asn(Mbh).OH

The *title compound* was prepared by method 1 and was crystallised from dichloromethane/diethyl ether as a white solid (87%); m.p. 114-115°C; (Found: C, 60.7; H, 4.8; N, 8.3. $C_{34}H_{34}N_4O_{11}$ requires C, 60.7; H, 5.05; N, 8.3%); tlc -E,R_f 0.68; hplc -A, R_f 24.8 min; $[\alpha]_D^{22}$ -2.1° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 2840, 1750 (sh), 1725, 1685, 1610, 1525, 1515, 1350, 1160, 1035, 860, 835; λ_{max} (CH_2Cl_2) 277 (ϵ 20500); δ_H (200 MHz, $CDCl_3$) 2.83 (2H, q, βCH_2, Asn), 3.72 (3H, s, CH_3O, Mbg), 3.74 (3H, s, CH_3O, Mbh), 4.44 (1H, m, αCH, Asn), 4.5-4.8 (3H, br m, $CHCH_2$, Bnpeoc), 6.00 (1H, d CH, Mbh), 6.08 (1H, d, ex, αNH, Asn), 6.64 (1H, d, βCH_2, Asn), 6.7806.82 (4H, m, Ar-H, Mbh), 7.02-7.08 (4H, m, Ar-H, Mbh), 7.34 (4H, d, *J* 8.78 Hz, Ar-H, Bnpeoc), 8.12 (4H, d, *J* 8.71 Hz, Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_2$) 32.1 (βCH_2, Asn), 49.4 (αCH, Asn), 53.4 (CH, Bnpeoc), 55.1 (CH_3O x 2, Mbh), 56.0 (CH, Mbh), 65.9 (CH_2, Bnpeoc), 113.8, 123.9, 128.3, 129.1 (aromatic CH's, Bnpeoc and Mbh), 133.2, 146.8, 158.6 (quat. C's, Bnpeoc and Mbh), 155.8 (urethane), 172.1 (amide), 173.8 (CO_2H); *m/z* 673 (MH^+, 0.7%), 671(1.0), 656(1.4), 268(7.0), 255(10), 242(50), 227(100), 213(19); HRMS 673.21453. $C_{34}H_{35}N_4O_{11}$ requires 673.21456.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-β-(tert.butyl)aspartic acid dicyclohexylammonium salt. Bnpeoc.Asp(OBu^t).OH, Dcha

The *title compound* was prepared by method 2. The crude product formed a gel from dichloromethane/diethyl ether (69%); m.p. 160-164°C; (Found: C, 61.2; H, 7.01 N, 8.2. $C_{35}H_{48}N_4O_{10}$ requires C, 61.4; H, 7.1; N, 8.2%); tlc -E, R_f 0.66; hplc -A,R_f 23.6 min; $[\alpha]_D^{22}$ +17.8° (*c* 1.0 in $CHCl_3$); ν_{max} 3440, 2830, 2730, 1725, 1640, 1605, 1600, 1525, 1395, 1365, 1350, 1150, 1080, 870, 830; λ_{max} (MeOH), 274 (ϵ 14600); δ_H (80 MHz, $CDCl_3$) 0.9-1.2 (20H, br m, 10 xCH_2, Dcha), 1.38 (9H, x 3 x CH_3, *tert.* butyl), 2.73 (2H, m, 2 x CH, Dcha), 2.85 (2H, m, βCH, Asp), 4.05 (1H, m, αCH, Asp), 4.85 (3H, m, $CHCH_2$, Bnpeoc), 5.83 (1H, d αNH, Asp), 7.38 (4H, d, Ar-H, Bnpeoc), 8.15 (4H, d, Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_3$) 24.39, 29.92 (CH_2's, Dcha), 27.64 (3 x CH_3, *tert.* butyl), 37.63 (βCH_2, Asp), 49.26 (αCH, Asp), 51.24 (2 x CH, Dcha), 53.03 (CH, Bnpeoc), 65.60 (CH_2, Bnpeoc), 80.95 [$C(CH_3)_3$], 123.77, 129.50 (aromatic CH's, Bnpeoc), 146.81, 146.90 (quat. C's, Bnpeoc), 155.16 (urethane), 170.24 (CO_2Bu^t), 174.43 (CO_2H); *m/z* 685 (MH^+, 1.2%), 670 (1.3), 491 (21), 426 (100), 399 (56), 288 (37), 271 (39), 255 (40); HRMS 685.34482. $C_{35}H_{49}N_4O_{10}$ (MH^+) requires 685.34484.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-β-(tert. butyl)aspartic acid. Bnpeoc.Asp(OBu^t).OH.

The *title compound* was prepared by method 2 and precipitated as white solid from chloroform/light petroleum (95%); m.p. 75-79°C; (Found: C, 57.9; H, 6.50; N, 7.55. $C_{23}H_{25}N_3O_{10}$ requires C, 54.9; H, 5.01; N, 8.35%); tlc -E, R_f 0.66; hplc -A, R_f 23.6 min; ν_{max} (CH_2Cl_2) 3440, 2960, 2860, 1725, 1610, 1600, 1525, 1390, 1375, 1350, 1230, 1150, 1079, 870, 830; λ_{max} (MeOH) 277 (ϵ 9700); δ_H (80 MHz, $CDCl_3$) 1.36 (9H, *tert.* butyl), 2.75 (2H, m, βCH_2, Asp), 4.32 (1H, m, αCH, Asp), 4.63 (3H, $CHCH_2$, Bnpeoc), 5.73 (1H, d αNH, Asp), 7.37, 8.15 (4H x 2, d x 2, Ar-H, Bnpeoc); δ_C (50 MHz, $CDCl_3$) 27.66 (CH_3 x 3, Bu^t), 37.09 (βCH_2, Asp), 49.47 (αCH, Asp), 52.53 (CH, Bnpeoc), 65.92 (CH_2, Bnpeoc), 82.05 ($C(CH_3)_3$), 123.82, 129.04 (ArCH's, Bnpeoc), 146.72, 146.87 (quat. C's, Bnpeoc), 155.45 (urethane), 169.96 (CO_2Bu^t, Asp), 174.86 (CO_2H, Asp); *m/z* 502 (0.17%), 448(1.67), 426(1.34), 271(8), 186(16), 182(100), 138(5), 134(7); HRMS 504.16177. $C_{23}H_{26}N_3O_{10}$ (MH^+) requires 504.16180.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-glutamine. Bnpeoc.Gln.OH.

The *title compound* was prepared in 94% yield by method 1 and crystallised from acetone/light petroleum ether to give a white solid, m.p. 189-190°C; (Found: C, 52.4; H, 4.5; N, 11.9. $C_{20}H_{20}N_4O_9$ requires C, 52.2; H, 4.4; N, 12.2%); tlc -F, R_f 0.6; $[\alpha]_D^{22}$ -3.9° (*c* 1.0 in DMF); ν_{max} ($CHBr_3$ mull) 3420, 3340, 1750 (COOH), 1705 (urethane), 1675 (amide), 1520, 1350, 830 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 20000), 215 (22000); δ_H [$(CD_3)_2SO$, 200 MHz] 1.80 (2H, m, glu βCH_2), 2.08 (2H, m, Gln γCH_2), 3.91 (1H, m, αCH), 4.77 (3H, m, Bnpeoc CH and CH_2), 7.68 and 8.19 (8H, 2 x d, Bnpeoc aromatic H's); δ_C (50 MHz, [$(CD_3)SO$] 26.5 (βCH_2, Gln), 31.3 (γCH_2, gln), 48.9 (αCH, Gln), 53.46 (CH, Bnpeoc), 65.05 (CH_2, Bnpeoc), 123.6, 129.5 (Bnpeoc, Ar CH's), 146.4, 148.1 (Bnpeoc, quaternary C's), 255.77 (urethane), 182.4 (carboxylic acid); HRMS 461.13083. $C_{20}H_{20}N_4O_9$ requires 461.1308.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-N^{γ}-[bis(4'-methoxyphenyl)-methylglutamine. Bnpeoc.Gln(Mbh).OH.

The *title compound* was prepared by method 1 and was crystallised from THF light petroleum (b.p. 40-60°C) as a white solid (62%); m.p. 97-107°C; (Found: C, 60.5; H, 5.1; N, 7.9. $C_{35}H_{34}N_4O_{11}$ requires C, 61.2; H, 5.0; N, 8.2%); tlc -E, R_f 0.66; hplc -A R_f 24.9 min; $[\alpha]_D^{22}$ +5.8° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 2740, 1740, 1670, 1610, 1525, 1350; λ_{max} (MeOH) 277 (ϵ 23000); δ_H (80 MHz, $CDCl_3$) 2.00 (2H, m, βCH_2, Gln), 2.25 (2H, m, γCH_2, Gln), 3.71 (6H, s, 2 x OCH_3, Mbh), 4.20 (1H, m, αCH, Gln), 5.5 (3H, br m, $CHCH_2$, Bnpeoc), 5.99 (2H, m, αNH, Gln and CH, Mbh), 6.74 (4H, d, J_{AB} 8.7 Hz, Mbh Ar-H), 7.05 (4H, d, J_{AB} 8.7 Hz, Mbh Ar-H), 7.30 (4H, d, J_{AB} 7.7 Hz, Bnpeoc Ar-H), 8.10 (4H, d, J_{AB} 7.7 Hz); δ_C (50 MHz, $CDCl_3$) 27.72 (Gln βCH_2), 32.08 (Gln γCH_2), 49.45 (Gln αCH), 53.40 (Bnpeoc CH), 55.04 (2 x OCH_3, Mbh), 55.96 (Mbh CH), 65.93 (Bnpeoc CH_2), 118.00, 127.88, 128.29, 129.05 (aromatic CH's, Bnpeoc and Mbh), 133.25 (Mbh Ar v-C), 146.87 (Bnpeoc Ar 1-C and 4-C), 155.74 (urethane), 158.64 (Mbh Ar 4-C), 172.64 (amide), 173.8 (carboxylic acid); *m/z* 686 (MH^+, 73%), 669(100), 578 (MH^+ -PhOMe), 506(42); HRMS 687.23023. $C_{35}H_{34}N_4O_{11}$ requires 687.23021.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-γ-(tert butyl)glutamic acid dicyclohexylammonium salt. Bnpeoc.Glu(OBu^t).OH, Dcha

The *title compound* was prepared by method 2. The crude product formed a gel from chloroform/diethyl ether which was dried *in vacuo* to give a white solid which was then recrystallised from dichloromethane/diethyl ether (88%); m.p. 129-132°C; (Found: C, 62.20; H, 7.30; N, 8.10. $C_{36}H_{50}N_4O_{10}$ requires C, 61.9; H, 7.2; N, 8.05%) $[\alpha]_D^{22}$ + 5.3° (*c* 1.0 in DMF); tlc -E, R_f 0.61, -G, R_f 0.41; ν_{max} (CH_2Cl_2) 3420, 2920, 2870 (CH), 1625, 1640, 1620, 1595, 1525, 1490, 1350 (NO_2), 1160, 860, 830; *m/z* 699 (MH^+, 1.7%), 670(1.4), 491(25), 462(30), 426(100), 254(58); HRMS 699.36050. $C_{36}H_{51}N_4O_{10}$ (MH^+) requires 699.36049.

N^{α}-2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-(γ-tert.butyl)glutamic acid. Bnpeoc.Glu(OBu^t).OH.

The *title compound* was prepared in 82% yield by method 2 and precipitated from chloroform/light petroleum ether as a white amorphous solid; (Found: C, 55.4; H, 5.25; N, 8.1. $C_{24}H_{27}N_3O_{10}$ requires C, 55.7; H, 5.2; N, 8.1%); tlc -E, R_f 0.61; $[\alpha]_D^{22}$ +6.2 (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3920, 1720, 1605, 1595, 1520, 880 and 860 cm^{-1}; λ_{max} (MeOH) 275 (ϵ18900); δ_H (100 MHz, $CDCl_3$) 1.40 (9H, s, tert.butyl), 2.10 (2H, m, Glu βCH_2), 2.25 (2H, m, Glu γCH_2), 4.29 (1H, m, Glu αCH), 4.5-5.0 (3H, m, $CHCH_2$, Bnpeoc), 5.5 (1H, d, αNH, Glu), 7.35 (4H, d, Bnpeoc Ar-H), 8.16 (4H, d, Bnpeoc Ar-H); *m/z* 462 (40%), 416(20), 271(60), 254(100), 225(60), 179(67); HRMS 518.17745. $C_{24}H_{28}N_3O_{10}$ (MH^+) requires 518.17745.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-glycine. Bnpeoc.Gly.OH.

The *title compound* was obtained in 90% yield by method 1 and crystallised from ethyl acetate/petroleum ether to give a white solid, m.p. 156-8°C; (Found: C, 52.4; H, 3.9; N, 10.8. $C_{17}H_{15}N_3O_8$ requires

C, 52.4; H, 3.9; N, 10.8%); tlc -D, R_f 0.23; ν_{max} (CH_2Cl_2) 3470, 1730, 1605, 1520, 1350 cm^{-1}; λ_{max} (MeOH) 275 nm (ϵ 19500); δ_H (200 MHz, $(CD_3)_2CO$) 3.86 (2H, m, Gly CH_2), 4.77 (3H, m, Bnpeoc $CHCH_3$), 6.63 (1H, d, NH), 7.70 and 8.22 (8H, 2d, Bnpeoc aromatic H's); HRMS 390.09372. $C_{17}H_{16}N_3O_8$ requires 390.09370.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-N^{τ}-trityl-histidine. Bnpeoc.His(Trt).OH.

The *title compound* was prepared by method 1 and was then precipitated from chloroform/diethyl ether as a white powder (85%); m.p. 134-138°C; (Found: C, 65.5; H, 4.5; N, 9.6. $C_{40}H_{33}N_5O_8.H_2O$ requires C, 65.8; H, 4.8; N, 9.6%); tlc -E, R_f 0.65; $[\alpha]_D^{22}$ +4.4° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 1760, 1720, 1610, 1525, 1495, 1350, 1130, 865, 850, 830; λ_{max} (MeOH) 274 (ϵ 23900); δ_H (80 MHz, $CDCl_3$) 3.1 (2H, m, βCH_2, His), 4.1-4.7 (4H, m, $CHCH_2$, Bnpeoc and αcH, His), 6.05 (1H, d αNH, His), 6.66 (1H, s), 6.9-7.5 (19H, m, Ar-H, Trt and Bnpeoc), 7.88 (1H, s), 8.10 (4H, d, Ar-H, Bnpeoc), 9.75 (1H, br s, carboxylic acid); δ_C (50 MHz, $CDCl_3$) 28.71 (βCH_2, His), 49.58 (αCH, His), 53.73 (CH, Bnpeoc), 65.66 (CH_2, Bnpeoc), 120.7-137.3 (9 peaks, trityl, His imidazole and Bnpeoc CH's), 146.75, 146.92 (Bnpeoc quaternary C's), 154.99 (urethane), 173.12 (carboxylic acid); *m/z* 712 (MH^+, 2.5%), 470 (MH^+-Trt, 2.3), 243 (Trt^+, 100), 165(52), 136(47), 77(30); HRMS 712.24071. $C_{40}H_{34}N_5O_8$ requires 712.24072.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-isoleucine dicyclohexylammonium salt. Bnpeoc.Ile.OH, Dcha.

The *title compound* was prepared in 91% yield by method 2 and crystallised from CH_2Cl_2/diethylether as a white solid, m.p. 160-2°C; (Found: C, 63.3; H, 7.4; N, 8.9. $C_{33}H_{46}N_4O_8$ requires C, 63.3; H, 7.4; N, 8.9%); tlc -E, R_f 0.6; $[\alpha]_D^{22}$ +7.7 (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 3040, 1730, 1720, 1605, 1595, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 276 nm (ϵ 20300); HRMS 627.33937. $C_{33}H_{47}N_4O_8$ requires 627.33936.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-isoleucine. Bnpeoc.Ile.OH.

The *title compound* was prepared by method 2 and precipitated from chloroform/petroleum ether as a white amorphous solid, m.p. 59-62°C; (Found: C, 56.7; H, 5.3; N, 9.4. $C_{21}H_{23}N_3O_8$ requires C, 56.6; H, 5.2; N, 9.4%); tlc -E, R_f 0.6; $[\alpha]_D^{22}$ +7.9° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 3040, 2980, 1730, 1725, 1605, 1595, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 23200); δ_H (200 MHz, $CDCl_3$) 0.88 (6H, m, Ile 2 x CH_3), 1.23 (2H, m, Ile γCH_2), 1.9 (1H, m, Ile βCH), 4.27 (1H, m, Ile αCH), 4.67 (3H, m, Bnpeoc CH and CH_2), 7.39 and 8.16 (8H, 2 x d, Bnpeoc aromatic H); HRMS 446.15634. $C_{21}H_{24}N_3O_8$ requires 446.15632.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-leucine. Bnpeoc.Leu.OH.

The *title compound* was prepared in 91% yield by method 1 and crystallised from chloroform/diethylether to give a solid, m.p. 68-70°C; (Found: C, 56.9; H, 5.5; N, 9.1. $C_{21}H_{23}N_3O_8$ requires C, 56.9; H, 5.2; N, 9.4%); tlc -E, R_f 0.5; $[\alpha]_D^{22}$ -4.1° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 3030, 2880, 1720, 1605, 1595, 1520, 1350, 870 cm^{-1}; λ_{max} (MeOH) 275 nm (ϵ 17400); δ_H (80 MHz, $CDCl_3$) 0.87 (6H, d, 2 x δCH_3,Leu), 1.58 (3H, m, βCH_2 and γCH,Leu), 4.30 (1H, m, αCH,Leu), 7.38 (4H, d, Ar-H, Bnpeoc), 8.13 (4H, d, Ar-H, Bnpeoc); *m/z* 446 (MH^+, 2%), 271(2), 225(27), 178(30), 86(37), 42(90), 28(100); HRMS 446.15629. $C_{21}H_{24}N_3O_8$ requires 446.15632.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxcarbonyl]-methionine dicyclohexylammonium salt. Bnpeoc.Met.OH, Dcha.

The *title compound* was prepared by method 2 under an atmosphere of N_2 and crystallised from methanol/diethylether as a pale yellow solid (81%); m.p. 186-189°C; (Found: C, 59.3; H, 6.8; N, 8.7. $C_{32}H_{44}N_4O_8S$ requires C, 59.6; H, 6.8; N, 8.7%); tlc -F, R_f 0.60; ν_{max} (CH_2Cl_2) 3440, 2840, 2860, 1720, 1640, 1610, 1600, 1525, 1350, 860, 830; λ_{max} (MeOH) 278 (ϵ 17700); δ_H (360 MHz, $CDCl_3$) 1.0-1.9 (20H, 10 x cH_2, Dcha),

2.0 (5H, βCH_2 and CH_3, Met), 2.43 (2H, m, γCH_2, Met), 2.73 (2H, m, CH x 2, Dcha), 4.07 (1H, m, αCH, Met), 4.5-4.7 (3H, m, $CHCH_2$, Bnpeoc), 5.67 (1H, d, αNH, Met), 7.38 (4H, d, Ar-H, Bnpeoc), 8.15 (4H, d, Ar-H, Bnpeoc); *m/z* 399(10), 182(100), 98(30), 83(43), 56(70), 42(62); HRMS 645.29577. $C_{32}H_{45}N_4O_8S$ requires 645.29579.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-methionine. Bnpeoc-Met-OH

The *title compound* was prepared by route 2 and was obtained in 79% yield as a foam from dichloromethane - all operations under N_2; tlc -F, R_f 0.6; $[\alpha]_D^{22}$ +8.1 (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 3020, 2970, 2860, 1720, 1520, 1350, 860 cm^{-1}; λ_{max} 274 nm (ϵ 15500); (δ_H (100 MHz, $(CD_3)_2CO$) 2.0 (5H, m, ϵCH_3 and γCH_2, Met), 2.5 (2H, m, βCh_2, Met), 4.30 (1H, m, αCH, Met), 4.77 (3H, m, $CHCH_2$, Bnpeoc), 7.70 (4H, d, Ar-H, Bnpeoc), 8.22 (4H, d, Ar-H, Bnpeoc); *m/z* 464 (11%), 257(30), 217(100), 199(40), 181(90).

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-L-norvaline. Bnpeoc.Nva.OH.

The required compound was prepared by method 1 in 75% yield as an amorphous powder; (Found: C, 55.3; H, 5.12; N, 9.75. $C_{20}H_{21}N_3O_8$ requires C, 55.7; H, 4.87; N, 9.70%); $[\alpha]_D^{27}$ -1.3° (*c* 1.0 in DMF); tlc -D, R_f 0.5; ν_{max} (CH_2Cl_2) 3425, 2970, 1720, 1605, 1595, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 274 (ϵ 19840); δ_H ($CDCl_3$, 200 MHz) 0.87 (3H, m, δCH_3), 1.30 (2H, m, γCH_2), 1.70 (2H, m, βCH_2), 4.25 (1H, m, αCH), 4.70 (3H, m_{AB_2}, $CHCH_2$-Bnpeoc), 7.39 (4H, d, J_{AB} 8.6 Hz), 8.15 (4H, d, J_{AB} 8.6 Hz); δ_C ($CDCl_3$, 50 MHz) 13.3 (δCH_3), 18.4 (γCH_2), 34.0 (βCH_2), 49.8 (CH, Bnpeoc), 53.6 (αCh), 65.9 (CH_2, Bnpeoc), 123.9, 129.1 (aromatic CH's), 146.8, 147.1 (quat. aromatic C's), 155.5 (CO, urethane), 176.6 (CO, acid); HRMS 432.14069, $C_{20}H_{22}N_3O_8$ requires 432.14068.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-phenylalanine. Bnpeoc.Phe.OH

The *title compound* was prepared by route 1 and was recrystallised from diethyl ether to afford a white crystalline solid (93%); m.p. 77-80°C; (Found: C, 57.8; H, 4.5; N, 8.3. $C_{24}H_{21}N_3O_8.H_2O$ requires C, 57.9; H, 4.6; N, 8.4%); tlc -F, R_f 0.8; $[\alpha]_D^{22}$ -16.5° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3420, 3020, 2980, 1730, 1605, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 22000); δ_H (200 MHz, CD_2Cl_2) 3.12 (2H, m, Phe βCH_2), 4.61 (4H, m, Phe αCH, Bnpeoc CH and CH_2), 7.15-7.26 (5H, m, Phe, aromatic H), 7.41 and 8.17 (8H, 2 x d, Bnpeoc aromatic H); δ_C (50 MHz, $CDCl_3$) 37.24 (βCH_2, Phe), 49.52 (αCH, Phe), 54.41 (CH, Bnpeoc), 65.73 (CH_2, Bnpeoc), 123.56, 127.11, 128.47, 129.01 (Ar CH's, Bnpeoc and Phe), 135.23 (Ar quat. C, Phe), 146.64, 146.90 (Ar quat. C's, Bnpeoc), 155.18 (urethane), 175.59 (carboxylic acid); HRMS 480.14071. $C_{24}H_{22}O_8N_3$ requires 480.14068.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-proline. Bnpeoc.Pro.OH

The *title compound* was prepared by method 1 and precipitated from chloroform/petroleum ether, m.p. 78-9°C; (Found: C, 55.9; H, 4.81; N, 9.1. $C_{20}H_{19}N_3O_8$ requires C, 55.9; H, 4.4; N, 9.8%); tlc -E, R_f 0.4; $[\alpha]_D^{22}$ -18.7° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3040, 2960, 1760, 1705, 1605, 1595, 1520, 1350, 860 cm^{-1}; λ_{max} (EtOH) 272 nm (ϵ 22700); δ_H (200 MHz, $CDCl_3$) 2.0 (4H, m, Pro β and γCH), 3.4 (2H, m, δCH_2), 4.12 (0.5 H, m, αCH), 4.37 (0.5 H, m, αCh), 4.60 (3H, m, Bnpeoc CH and CH_2), 7.40 and 8.20 (8H, 2 x d, Bnpeoc aromatic H); HRMS 430.12500. $C_{20}H_{20}N_3O_8$ requires 430.12500.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-serine. Bnpeoc.Ser.OH

The *title compound* was prepared in 71% yield by method 1 and recrystallised from acetone/light petroleum to give a white solid: tlc -L, R_f 0.27 (A2) 0.27; m.p. 171-173°C; $[\alpha]_D^{27}$ +2.9° (*c* = 1 DMF); (Found: C, 51.2; H, 4.03; N, 9.87. $C_{18}H_{17}N_3O_9$ requires C, 51.6; H, 4.05; N, 10.0%); ν_{max} (bromoform null) 1730, 1670, 1520, 1350, 1155 cm^{-1}; δ_H (200 MHz, CD_3COCD_3) 8.21 (4H, d, J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.69 (4H, d J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 6.43 (1H, bd, NH), 4.77 (3H, m, Bnpeoc CH,CH_2), 4.28 (1H, m, Ser αCH), 3.88 (2H, m, Ser βCH_2); δ_C (50 MHz,

CD_3COCD_3) 170.3 (acid C=O), 154.9 (urethane C=O), 147.1, 146.3 (Bnpeoc quat. aromatic C's), 128.8, 122.8 (Bnpeoc aromatic CH's), 64.9 (Bnpeoc CH_2), 61.0 (Ser βCH_2), 55.3 (Ser αCH), 48.8 (Bnpeoc CH); *m/z* (FAB), 420 (MH^+), 325, 307; HRMS found 420.10427. $C_{18}H_{18}N_3O_9$ requires 420.10429.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-(O-tertbutyl)serine. Bnpeoc.Ser(^{t}Bu).OH

The *title compound* was prepared in 93% yield by method 2 and isolated as a yellow foam; tlc R_f -I 0.77; $[\alpha]_D^{27}$ +13.7° (*c* = 1, methanol); ν_{max} (CH_2Cl_2) 3430, 1770, 1730, 1605, 1595, 1525, 1350, 860 cm^{-1}; λ_{max} 274 nm (ϵ = 19811); δ_H (200 MHz, CD_3COCD_3) 8.25 (4H, d, J_{AB} 8.9 Hz, Bnpeoc aromatic CH's), 7.73 (4H, d, J_{AB} 8.9 Hz, Bnpeoc aromatic CH's), 4.80 (3H, m, Bnpeoc CH,CH_2), 4.32 (1H, m, Ser αCH), 3.71 (2H, m, Ser βCH_2), 1.10 (9H, s, 3 x CH_3); δ_C (50 MHz, CD_3COCD_3) 170.1 (acid C=O), 154.8 (urethane C=O), 147.2, 146.3 (Bnpeoc quat. aromatic C's), 128.8, 122.8 (Bnpeoc aromatic CH's), 72.0 (^{t}Bu quat. C), 64.8 (Bnpeoc CH_2), 60.8 (Ser βCH_2), 53.6 (Ser αCH), 48.8 (Bnpeoc CH), 25.8 (CH_3); *m/z* (FAB), 476 (MH^+), 420, 254, 225; hplc R_f (B2) 17.8 minutes. Bnpeoc. Ser(^{t}Bu).OH, Cha salt: m.p. 182-5°C; (Found: C, 57.8; H, 6.7; N, 9.4. $C_{28}H_{38}N_4O_9$ requires C, 57.9; H, 6.5; N, 9.6%); tlc -E, R_f 0.6; $[\alpha]_D^{22}$ +11° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3440, 1720, 1595, 1525, 1350, 860 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 17900); HRMS 575.271687. $C_{28}H_{39}N_4O_9$ requires 575.271680.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-threonine. Bnpeoc.Thr.OH

The required compound was prepared in 85% yield by method 1 and crystallised from acetone/petroleum ether; (Found: C, 53.0; N, 4.41; N, 9.78. $C_{19}H_{19}N_3O_9$ requires C, 52.7; H, 4.39; N, 9.70%); $[\alpha]_D^{27}$ +3.8°; tlc -D, R_f 0.1; ν_{max} ($CHBr_3$ mull) 3620, 3405, 1765, 1750, 1670, 1660, 1605, 1595, 1520, 1350, 1550, 1325, 870, 860 and 835 cm^{-1}; λ_{max} 275 (ϵ 20000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.21 (4H, d, J_{AB} 8.8 Hz), 7.70 (4H, d, J_{AB} 8.8 Hz), 6.15 (1H, m, NH), 4.78 (3H, m, $CHCH_2$ Bnpeoc), 4.30 (1H, m, αCH), 4.19 (1H, m, Thr βCH), 1.15 (3H, d, J 6.4 Hz, Thr γCH_3); δ_C [$(CD_3)_2CO$, 50 MHz] 170.61 (CO, acid), 155.4 (CO, urethane), 147.2, 146.5 (quat. aromatic C's), 128.9, 122.9 (aromatic CH's), 66.2 (Thr βCH), 65.0 (CH_2, Bnpeoc), 58.7 (αCH), 49.0 (Thr, γCH_3); HRMS 434.11991. $C_{19}H_{20}N_3O_9$ requires 434.1199.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-(O-tertbutyl)threonine. Bnpeoc.Thr(^{t}Bu).OH

The *title compound* was prepared in 78% yield by method 2 which gave crude product which was purified by dry flash chromatography on silica (eluting with chloroform) to yield the product as a yellow foam; tlc -A4, R_f 0.48; $[\alpha]_D^{27}$ +9.7° (*c* = 1 MeOH); ν_{max} (CH_2Cl_2) 3420, 1770, 1730, 1525, 1350, 860 cm-^{1}; δ_H (100 MHz, CD_3COCD_3) 8.22 (4H, d J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.71 (4H, d, J_{AB} 8.8 Hz Bnpeoc aromatic CH's), 4.80 (3H, m, Bnpeoc CH,CH_2), 4.22, 4.12 (2H, m, Thr α/β), 1.12 (12H, m, Thr CH_3, 3 x ^{t}Bu CH_3); δ_C (50 MHz, CD_3COCD_3) 170.5 (acid C=O), 155.2 (urethane C=O), 147.3, 147.2, 146.3 (Bnpeoc quat. aromatic C's), 128.8, 122.8 (Bnpeoc aromatic CH's), 72.8 (^{t}Bu quat. C), 64.8 (Bnpeoc CH_2), 66.3, 58.6 (Thr α/βCH), 48.9 (Bnpeoc CH), 26.9 (^{t}Bu CH_3), 19.1 (Thr CH_3); hplc R_f -N 12.0 minutes. Bnpeoc.Thr (^{t}Bu).OH, CHA salt: m.p. 153-6°C; (Found: C, 59.5; H, 7.0; N, 9.4. $C_{29}H_{40}N_4O_9$ requires C, 59.2; H, 6.9; N, 9.5%); tlc -E, R_f 0.5; $[\alpha]_D^{22}$ +11.0° (*c* 1.0 in DMF); ν_{max} (nujol) 3410, 1720, 1525, 1530, 860 cm^{-1}; λ_{max} (MeOH) 276 nm (ϵ 19800); *m/z* (RAB) 599 (MH^+, 0.2%), 434(1.0), 254(1.9), 100(ChaH$^+$, 90), 83 (20), 58(100).

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-valine. Bnpeoc.Val.OH

The required compound was prepared in 73% yield by method 1 and crystallised from ethyl acetate/petroleum ether; m.p. 142-5°C; (Found: C, 55.8; H, 4.7; N, 9.7. $C_{20}H_{21}N_3O_8$ requires C, 55.8; H, 4.9; N, 9.6%); $[\alpha]_D^{27}$ +7.5° (*c* 1.0 in DMF); ν_{max} (CH_2Cl_2) 3430, 2970, 1725, 1610, 1520, 1350, 860 cm^{-1}; λ_{max} (MeOH) 274 nm (ϵ 23000); δ_H [200 MHz, $(CD_3)_2CO$] 0.9 (6H, m, Val CH_3), 2.1 (1H, m, Val βCH), 4.1 (1H, m, Val αCH), 4.8 (3H, m, Bnpeoc CH and CH_2), 7.69 (4H, d, J_{AB} 8.7 Hz), 8.21 (4H,

d, J_{AB} 8.7 Hz); δ_C [50 MHz, $(CD_3)_2CO$] 16.4 and 17.7 (CH_3 x 2), 29.6 (Val βCH), 49.0 (Bnpeoc *C*H), 58.5 (Val αCH), 64.9 (*C*H_2 Bnpeoc), 122.8 and 128.9 (aromatic *C*H), 147.3 and 146.4 (quat. C, aromatics), 155.2 (*C*O, methane), 171.5 (*C*O, acid); HRMS 432.14069. $C_{20}H_{22}N_3O_8$ requires 432.14068.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-tryptophan. Bnpeoc.Trp.OH.
The required compound was prepared by route 1 as for the Met derivative in 60% yield and the product crystallised from chloroform; m.p. 92-7°C; (Found: C, 60.4; H, 4.4; N, 10.6. $C_{26}H_{22}N_4O_8$ requires C, 60.2; H, 4.3; N, 10.8%); $[\alpha]_D^{20}$ -24.0° (*c* 1.0 in DMF); ν_{max} (nujol) 3450, 1730, 1670, 1605, 1595, 1520, 1350, 860 and 830 cm^{-1}; λ_{max} 274 (ϵ 27000); δ_H [$(CD_3)_2CO$, 200 MHz] 3.26 (2H, m, Trp CH_2), 4.6 (1H, m, Trp αCH), 4.68 (3H, m, CH and CH_2 Bnpeoc), 7.10 (3H, m, Trp), 7.40 (1H, d, J = 8.1 Hz, Trp 2-CH), 7.65 (5H, m, 4H Bnpeoc and 1H Trp), 8.18 (4H, d, J_{BA} 8.6 Hz); HRMS 519.15155. $C_{26}H_{23}N_4O_8$ requires 519.1515.

N^{α}-Bnpeoc amino acid chlorides.
These were prepared by the following general method and, in the cases of Pro, Val and Trp these were isolated as rather unstable solids; m.p. 54-63°, 140-160° and 97-105° respectively. The acid chlorides were used immediately after preparation.

Bnpeoc.Gly.Cl
Thionyl chloride (1.61 ml, 22.0 mmol) was added to Bnpeoc.Gly.OH (0.857 g, 2.20 mmol) in DCM (100 ml) and heated under reflux for 90 minutes under nitrogen. The solvent and excess thionyl chloride were removed *in vacuo*, re-evaporating three times with dichloromethane to ensure complete removal of the thionyl chloride. The resulting foam was used without further manipulation: ν_{max} (CH_2Cl_2) 3425 (NH), 1805 (acid chloride C=O), 1740 (urethane C=O), 1525, 1350 (NO_2) cm^{-1}.

N^{α}-[2,2-Bis(4'nitrophenyl)ethoxycarbonyl]-glycyl-p-alkoxybenzyl alcohol resin. Bnpeoc.Gly($OCH_2C_6H_4$-OR)
To p-alkoxybenzyl resin (Bachem, 1.01 mmol/g) was added sufficient dichloromethane (1 ml) to dry swell the resin, sufficient pyridine (2 ml) to obtain a slurry and Bnpeoc.Gly.Cl (0.14 mmol in 1 ml dichloromethane). This mixture was allowed to stand in a sonic bath for 90 minutes after which the resin was filtered off and washed thoroughly with dichloromethane (5 x 5 ml). The resin and filtrate were analysed by i.r. and CHN; resin, ν_{max} (KBr) 3420 (NH), 1740 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); filtrate, ν_{max} (CH_2Cl_2) 2500-1950 (pyridinium salt), 1720 (C), urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 2.04. $C_{17}H_{13}N_3O_7$-resin requires N, 2.25); esterification = 88%. Both Fmoc.Gly.OH and Bnpeoc.Gly.OH loading in this way showed no Gly.Gly incorporation by amino acid analysis at pH 3.49.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-valyl-p-alkoxybenzyl alcohol resin. Bnpeoc.Val.(O.$CH_2C_6H_4$.OR)
(i) To p-alkoxybenzyl alcohol resin (0.93 g, 0.94 mmol) was added dichloromethane (2 ml), pyridine (4 ml) and Bnpeoc.Val.Cl (0.94 mmol in 1.5 ml dichloromethane). The mixture was allowed to stand in a sonic bath for 2 hours and worked-up as for Bnpeoc.Gly.resin; ν_{max} (KBr) 3570 (OH), 3454 (NH), 1730 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 2.06. $C_{20}H_{19}N_3O_7$-resin requires N, 3.0%); esterification = 60%.

(ii) The above experiment was repeated with p-alkoxybenzyl alcohol resin (1.49 g, 1.51 mmol) in dichloromethane (3 ml) and pyridine (6 ml) to which was added Bnpeoc.Val.Cl (1.65 mmol, 1.1 eq) in 2 ml dichloromethane. Work-up after 2 hours in a sonic bath was as described before to give the loaded resin (1.8 g); ν_{max} (KBr) 3420 (NH), 1730 (CO, urethane, ester), 1520 and 1350 cm^{-1}(NO_2); (Found: N, 2.45. $C_{21}H_{19}N_3O_7$-resin requires N, 3.0%); esterification = 75%.

Racemisation study on Bnpeoc.Val-resin [prepared by method (ii) above]
Bnpeoc.Val-resin (1.8 g, 1.15 mmol) was swollen in dichloromethane (4

ml) and pyridine (10 ml) to which was added acetic anhydride (0.71 ml, 5 mmol). The resin was filtered and washed with dichloromethane after 1 hour in a sonic bath. Treatment with a solution of DBU/acetic acid (15 mmole) in dimethylacetamide afforded the valine benzyl ester resin.

To the above resin (0.129 g, 0.084 mmol) in dichloromethane (1.5 ml) was added Boc.Ala.OH (49 mg, 0.25 mmol) in dichloromethane (2 ml) and dicyclohexylcarbodiimide (54 mg, 0.25 mmol). The mixture was stirred under argon for 2 hours after which the resin was washed thoroughly with dichloromethane. The resin was treated with a trifluoroacetic acid/ dichloromethane mixture (5 ml, 1:1) for 1 hour in a sonic bath. The resin was then filtered off and washed thoroughly. The residue obtained on removal of the solvent from the filtrate was dissolved in 100 ml 0.2M sodium citrate buffer (pH 3.49), and from which was taken 120 μl and placed on an ion-exchange column at 75°C for amino acid analysis. There was only one peak observed due to the L,L-diastereoisomer by comparison with authentic samples of the two diastereoisomers.

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-alanylphenylalanylglycine. Bnpeoc.Ala.Phe.Gly.OH (15)

(i) The synthesis of (15) was accomplished using the functionalised resin, Bnpeoc.Gly.(O-$CH_2C_6H_4$-OR), and employing a repetitive solid-phase cycle on a manual shaker which involved (1) washing the functionalised resin - dichloromethane (2 x 1 minute), (2) capping of any unreacted sites - acetic anhydride (2.08 ml, 22.0 mmol) and pyridine (1.78 m., 22.0 mmol) in dichloromethane (35 ml) (1 hour), (3) washing - dichloromethane (2 x 1 minute), DMF (2 x 1 minute), dichloromethane (2 x 1 minute), (4) removal of N^{α}-protecting group - DBU (600 μl, 4.02 mmol) and acetic acid (230 μl, 4.02 mmol) in DMF (35 ml) (1 hour), (5) washing - DMF (2 x 1 minute), dichloromethane (2 x 1 minute), (6a) coupling of Bnpeoc.Phe.Cl (2.40 mmol) in N-methylmorpholine (6.60 ml, 60.0 mmol) and DCM (35 ml) (2 hours) (double couple), (6b) coupling of Bnpeoc.Ala.Cl (2.40 mmol) in N-methylmorpholine (6.60 ml, 60.0 mmol) and dichloromethane (35 ml) (2 hours) (single couple), (7) washing - dichloromethane (2 x 1 minute), DMF (2 x 1 minute), dichloromethane (2 x 1 minute). Samples of the resin were removed after steps (6a) and (6b) and subjected to the Kaiser test to determine the extent of coupling.

The cleavage of the peptide from the support was performed by reaction with trifluoroacetic acid (25 ml) in dichloromethane (25 ml). The reaction mixture was shaken for 1 hour under nitrogen at room temperature, before the resin was filtered off and washed with DMF (3 x 1 minute) and dichloromethane (3 x 1 minute). The combined filtrates were concentrated *in vacuo* to give a yellow oil, which was triturated with ethyl acetate/ light petroleum to give a yellow solid. This material was purified by gel filtration on Sephadex LH20, eluting with methanol to afford the *title compound* (0.458 g, 38%) as a white solid: tlc -M, R_f 0.10, -I, R_f 0.80; m.p. 113- 115°C; ν_{max} (CH_2Cl_2) 1725, 1670, 1525, 1350 cm^{-1}; δ_H (200 MHz, CD_3COCD_3) 8.20 (4H, d, J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.68 (4H, d, J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.23 (5H, m, Phe aromatic CH's), 4.75 (4H, m, Phe αCH, Bnpeoc CH,CH_2), 4.06 (1H, m, Ala αCH), 3.99 (2H, d, J 3.0 Hz, Gly CH_2), 3.07 (2H, m, Phe βCH_2), 1.21 (3H, d, J 7.2 Hz, Ala CH_3); δ_C (50 MHz, CD_3COCD_3) 171.4, 170.4, 169.7, (2 x amide C=O, acid C=O), 154.8 (urethane C=O), 147.3, 146.6 (Bnpeoc quat. aromatic C's), 136.8 (Phe quat. aromatic C0, 129.0-122.9 (aromatic CH's), 65.2 (Bnpeoc CH_2), 53.2, 50.2, 49.1 (Bnpeoc CH, 2 x αCH), 40.0, 37.0 (Phe αCH_2, Gly CH_2), 16.7 (Ala CH_3); *m/z* (FAB), 608 (M^+), 592, 323, 307, 271; HRMS 608.19921 $C_{29}H_{30}N_5O_{10}$ requires 608.19925; amino acid analysis, Gly_1 0.99, Ala_1 1.02, Phe_1 0.99; hplc -B4, R_f 22.8 minutes.

(ii) The synthesis of (15) was accomplished by hydrolysis of the corresponding methyl ester (14).

Bnpeoc.Ala.OH (1.0 g, 1.5 mmol) was suspended in dry dichloromethane at -10°C. To this was added Dpp.Cl (2.5 mmol, 8.6 ml of 0.7 g Dpp.Cl in 10 ml dichloromethane) and NMM (0.24 ml, 2.5 mmol). After 5 mins, H.Phe.Gly.OMe TFA salt (0.75 g, 1.15 mmol) in DMF (5 ml) was added to the reaction mixture keeping the temperature at -5°C, then NMM (0.16 ml, 2.25

mmol) and 2,6-lutidine (0.26 ml, 2.25 mmol) were added and the reaction left for 1 h at 0°C followed by 1 h at 12°C. After the usual work-up the *titled compound* was isolated (1 g, 75%) as a crystalline product m.p. 159-162°; (Found: C, 57.7; H, 4.92; N, 11.2; $C_{30}H_{31}N_5O_{10}$ requires C, 58.0; H, 5.03; N, 11.3%); ν_{max} (CH_2Cl_2) 3420 (NH), 1750, 1730, 1680, 1525, 1350 cm^{-1}; δ_H (200 mHz, CD_3COCD_3) 8.22 (4H, d, J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.69 (4H, d, J_{AB} 8.8 Hz, Bnpeoc aromatic CH's), 7.25 (5H, m, Phe aromatic CH's), 6.68 (1H, bd, urethane NH), 4.74 (4H, m, Phe αCH, Bnpeoc CH,CH_2), 4.10 (1H, m, Ala αCH), 3.95 (2H, m, Gly CH_2), 3.65 (3H, s, OCH_3), 3.10 (2H, m, Phe βCH_2), 1.22 (3H, d, J 7.7 Hz, Ala CH_3); δ_C (50 MHz, CD_3COCD_3) 171.2, 170.4, 169.1 (2 x amide C=O, ester C=O), 154.8 (urethane C=O), 147.3, 146.5 (Bnpeoc quat. aromatic C's), 136.7 (Phe quat. aromatic C), 129.3-122.9 (aromatic CH's), 65.0 (Bnpeoc CH_2), 53.1, 50.5, 50.2, 48.9 (Bnpeoc CH, OCH_3, 2 x αCH), 39.5, 37.0 (Phe βCH_2, Gly CH_2), 16.6 (Ala CH_3); *m/z* (FAB), 622 (M^+), 254, 237, 167; amino acid analysis, Gly_1 1.03, Ala_1 0.99, Phe_1 0.98; hplc -O, R_f 17.0 minutes.

Bnpeoc.Ala.Phe.Gly.OMe (14) (0.117 g, 0.91 mmol) was dissolved in acetone/water (8.2) (8 ml), and to this was added 0.1M aqeous NaOH (1.92 ml, 0.19 mmol) and H_2O_2 (3% solution in water, 216 μl, 0.19 mmol). The reaction mixture was stirred for 40 minutes at room temperature. The pH of the solution was adjsuted to 1.5 by the addition of conc. HCl, extracted with ethyl acetate (3 x 40 ml), and dried over Na_2SO_4. Concentration of the reaction mixture *in vacuo* afforded a yellow oil, which was purified by preparative hplc, employing an Aquapore C_{18} reverse phase prep. column with water/acetonitrile (+0.05% TFA) as the eluent and monitoring at 254 nm. The removal of the solvents by lyophilisation gave the *title compound* (0.063 g, 55%) as a white solid: tlc R_f -M 0.19, R_f -I 0.80; m.p. 110-113°C; δ_H (200 MHz, d_6-DMSO) 8.32 (1H, bt, amide NH), 8.19 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic CH's), 7.87 (1H, bd, NH), 7.68 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic CH's), 7.33 (1H, d, NH), 7.21 (5H, s, Phe aromatic CH's), 4.64 (4H, m, Phe αCH, Bnpeoc CH,CH_2), 3.95 (1H, m, Ala αCH), 3.77 (2H, d, Gly CH_2), 2.97 (2H, m, Phe βCH_2), 1.07 (3H, d, J 7.0 Hz, Ala CH_3); *m/z* (FAB), 608 (MH^+), 585, 329, 176; HRMS 608.19921 $C_{29}H_{30}N_5O_{10}$ requires 608.19925; amino acid analysis, Gly_1 1.02, Ala_1 0.98, Phe_1 1.00; hplc -B4, R_f 22.8 minutes

N^{α}-[2,2-Bis(4'-nitrophenyl)ethoxycarbonyl]-serine-(O-2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)benzyl ester (16). Bnpeoc.Ser[α/β-D-(Bzl)$_4$Gluc]-OBzl

Trifluoromethanesulphonic anhydride (0.175 ml, 1.05 mmol) was added to a solution of Bnpeoc.Ser.OBzl (1.07 g, 2.10 mmol) in acetonitrile/dichloromethane (1:1) (10 ml) at -15°C. 2,3,4,6-Tetra-O-benzyl-α-D-glucopyranose (0.65 g, 1.20 mmol) in dichloromethane (12 ml) was added, maintaining the temperature at -15°C. The reaction mixture was then allowed to warm to room temperature and stirred for a further 90 minutes. Water (30 ml) was added and the aqueous solution was extracted with dichloromethane (3 x 30 ml). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (2 x 50 ml), water (1 x 50 ml) and dried over Na_2SO_4. The removal of the solvent *in vacuo* gave a yellow oil, an aliquot of which was purified by, firstly, flash chromatography on silica (employing a gradient of light petroleum/diethyl ether), and secondly, preparative hplc, using an Aquapore C_8 reverse phase prep. column with acetonitrile/ water (+0.05% TFA) as the eluent and monitoring at 254 nm. Removal of the solvents by lyophilisation afforded the *title compound* (0.145 g, calculated yield = 34%) as a yellow solid: tlc R_f -K 0.78, 0.80; δ_H (360 MHz, $CDCl_3$) 8.12 (4H, m, Bnpeoc aromatic CH's), 7.21 (29H, m, Bnpeoc aromatic CH's, benzyl CH's), 5.94 + 5.67 (1H, d + d, J 8.6, 8.0 Hz, NH), 5.15 (2H, m, benzyl ester CH_2), 4.91-4.27 (13H, m, anomeric CH, 4 x benzyl ether CH_2, Ser αCH, Bnpeoc CH,CH_2), 4.12-3.29 (8H, m, Ser βCH_2, 4 x glucose CH, glucose CH_2); *m/z* (FAB), 1032, (MH^+), 1002, 558, 423, 325; HRMS 1032.39184 $C_{59}H_{58}N_3O_{14}$ requires 1032.39185; hplc -B5, R_f 21.8, 22.6 minutes.

(O-2,3,5,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-serine benzyl ester (17). H.Ser[α/β-D-(Bzl)$_4$Gluc]-OBzl

Bnpeoc.Ser[α/β-D-(Bzl)$_4$Gluc]-OBzl (18) (0.194 g, 0.19 mmol) was dissolved in DMF (8 ml), and to this was added DBU [1.57 ml of a solution of 0.219 g in 10 ml DMF (0.22 mmol)] and glacial acetic acid [(0.72 ml of a solution of 0.187 g in 10 ml DMF (0.22 mmol)]. The reaction was stirred for 45 minutes, monitoring by tlc. Ethyl acetate (150 ml) and water (150 ml) were added, and the separated organic layer washed with water (1 x 100 ml), an aqueous solution of 5% citric acid (1 x 100 ml), water (1 x 100 ml) and brine (1 x 100 ml), and dried over Na_2SO_4. The removal of the solvent *in vacuo* afforded a yellow oil which was purified by flash chromatography on silica (employing a gradient of light petroleum/ethyl acetate/methanol). The removal of the solvent *in vacuo* gave the *title compound* (0.126 g, 93%) as a yellow oil: tlc R_f -N 0.54; δ_H (360 MHz, $CDCl_3$) 7.28 (25H, m, benzyl CH's), 5.15 (2H, m, benzyl ester CH_2), 4.94-4.27 (10H, m, Ser αCH, anomeric CH, 4 x benzyl ether CH_2), 4.14-3.34 (8H, m, Ser βCH_2, glucose CH_2, 4 x glucose CH); *m/z* (FAB), 718 (MH^+), 628; HRMS 718.33801 $C_{44}H_{48}NO_8$ requires 718.33797.

N^{α}-(Benzyloxycarbonyl)-alanylalanyl(O-2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-serine benzyl ester (18).

Z.Ala.Ala.Ser[α/β-D-(Bzl)$_4$Gluc]-OBzl

Bnpeoc.Ser[α/β-D-(Bzl)$_4$Gluc]-OBzl (16) (0.100 g, 0.097 mmol) was dissolved in DMF (4.0 ml) and to this was added DBU [2.78 ml of a solution of 0.062 g in DMF (10 ml), (0.11 mmol)] and glacial acetic acid [0.60 ml of a solution of 0.096 in DMF (10 ml), (0.11 mmol)]. The reaction mixture was stirred for 40 minutes, monitoring by tlc. Ethyl acetate (50 ml) and water (50 ml) were added, and the separated organic layer was washed with water (2 x 50 ml) and dried over Na_2SO_4. The removal of the solvent *in vacuo* afforded (17) as a yellow oil which was used without further manipulation.

Z.Ala.Ala.OH (0.057 g, 0.19 mmol) was dissolved in DMF (2.0 ml) and to this was added HOBt (0.026 g, 0.19 mmol) in DMF (0.5 ml). After stirring for 2 minutes 1,3-dicyclohexylcarbodiimide (0.040 g, 0.19 mmol) was added and the reaction mixture stirred for a further 2 minutes. The N^{α}-deprotected glyco-amino acid above was dissolved in DMF (1.0 ml), added to the reaction mixture and the pH of the resulting solution adjusted to 9.0 by the addition of triethylamine. The reaction mixture was stirred for 18 hours at room temperature, before filtering and isolating the desired product by preparative hplc, employing an Aquapore C_{18} reverse phase prep. column, with water/acetonitrile (+0.05% TFA) as eluent and monitoring at 229 nm. The removal of the hplc solvents by lyophilisation afforded the *title compound* (0.015 g, 15%) as a white solid: tlc R_f -N 0.71; δ_H (360 MHz, D_2O/CD_3COCD_3) 7.27 (30H, m, Z-, benzyl ether, benzyl ester aromatic CH's), 5.16-4.40 (14H, m, 4 x benzyl ether CH_2, benzyl ester CH_2, anomeric CH, Ser αCH, 2 x Ala αCH), 4.10-3.68 (6H, m, 4 x glucose CH, glucose CH_2), 3.55 (2H, m, Ser βCH_2), 1.25 (6H, m, 2 x Ala CH_3); *m/z* (FAB), 994 (M^+), 610, 562, 454, 367; HRMS 994.44904 $C_{58}H_{63}N_3O_{12}$ requires 994.44896; hplc -B13, R_f 28.2, 28.4 minutes.

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REFERENCES

1. Merrifield, R.B., *J. Amer. Chem. Soc.*, 1963, 85, 2149.
2. Carpino, L.A. and Han, G.Y., *J. Amer. Chem. Soc.*, 1970, 92, 5748.
3. Sheppard, R.C. and Williams, B.J., *Int. J. Pept. Protein Res.*, 1982, 20, 451.
4. Ramage, R., Barron, C.A., Bielecki, S. and Thomas, D.W., *Tetrahedron Lett.*, 1987, 28, 4105; Mullen, D.B. and Barany, G., *J. Org. Chem.*, 1988, 53, 5240.
5. Atherton, E. and Sheppard, R.C., in 'The Peptides, Analysis, Synthesis, Biology', ed. Udenfriend, S. and Meienhofer, J., Academic Press, New York, 1987, Vol 9, p 1; Atherton, E., Logan, C.J. and Sheppard, R.C., *J. Chem. Soc., Perkin Trans. 1*, 1981, 538.
6. König, W., Büchner, D., Knüttel, R., Lindner, K. and Volk, A., *Proceedings of the Akabori Conference*, Grainau-Eibsee, Bavaria, 1985, p 32.
7. Kharasch, M.S. and Clapp, H.G., *J. Org. Chem.*, 1939, 3, 355.
8. Lapatsanis, L., Millias, G., Froussios, K. and Kolovos, M., *Synthesis*, 1983, 671.
9. Shute, R.E. and Rich, D.H., *Synthesis*, 1987, 346.
10. SHELX86, program for crystal structure solution. G.M. Sheldrick, University of Göttingen, Germany, 1986.
11. SHELX76, program for crystal structure refinement. G.M. Sheldrick, University of Cambridge, England, 1976.
12. CALC, program for molecular geometry calculations. R.O. Gould & P. Taylor, University of Edinburgh, Scotland, 1985.
13. ORTEPII, interactive version. P.D. Mallinson & K.W. Muir, *J. Appl. Cryst.* 1985, 18, 51.
14. Casalone, G. and Simonetta, M., *J. Chem. Soc. (B)*, 1971, 1180.
15. Galpin, I.J., Hardy, P.M., Kenner, G.W., McDermott, J.R., Ramage, R., Seely, J.H. and Tyson, R.G., *Tetrahedron*, 1979, 35, 2577.
16. Wang, S.-S., *J. Amer. Chem. Soc.*, 1973, 95, 1328.
17. Carpino, L.A., Cohen, B.J., Stephens, K.E., Sadat-Aalaee, S.Y., Tien, J.-H. and Langridge, D.C., *J. Org. Chem.*, 1986, 51, 3734; Carpino, L.A., Rice, N.W., Mansour, E.M.E. and Triolo, S.A., *J. Org. Chem.*, 1984, 49, 836.
18. Ramage, R., Hopton, D., Parrott, M.J., Richardson, R.S., Kenner, G.W. and Moore, G.A., *J. Chem. Soc., Perkin Trans. 1*, 1985, 461; Ramage, R., Atrash, B., Hopton, D. and Parrott, M.J., *ibid.*, 1985, 1617.