Pergamon

Tetrahedron 55 (1999) 4733–4746

TETRAHEDRON

Internal Resin Capture - A Self Purification Method for the Synthesis of C-Terminally Modified Peptides

Michael Davies and Mark Bradley*

Department of Chemistry, University of Southampton, Highfield, Southampton, UK SO17 1BJ.

Received 8 October 1998; revised 28 January 1999; accepted 11 February 1999

Abstract:

A synthetic strategy which allows for the general modification of peptides at the C-terminus has long been the goal of the synthetic chemist. We report here the full synthetic details of our inversion and modification methodology demonstrating the method with the synthesis of peptide amides, alcohols, nitriles and a range of other modified peptides.

Keywords: combinatorial; peptides; solid-phase; linkers.

Introduction:

Many naturally occurring peptides and proteins are modified at the C-terminus. These modifications can range from simple primary amides found in a huge number of biologically important peptides, for example oxytocin [1], LH-RH [2], substance P [3] and the neurokinins [4], to more exotic examples such as the cysteinyl farnesyl methyl esters found at the termini of the *Ras* membrane-bound lipoproteins [5]. Other modifications include the addition of lipids as in the fungal lipopeptide mating pheromones [6] and ethanolamine linked glycosylphosphatidyl-inositols [7] found in a large number of proteins. C-terminally modified peptides have also been used for many years in a variety of research applications, in particular as substrates and inhibitors for a variety of proteolytic enzymes [8]. Especially important are the chromogenic peptide substrate nitroanilides, 7-amido-4-methyl-coumarins

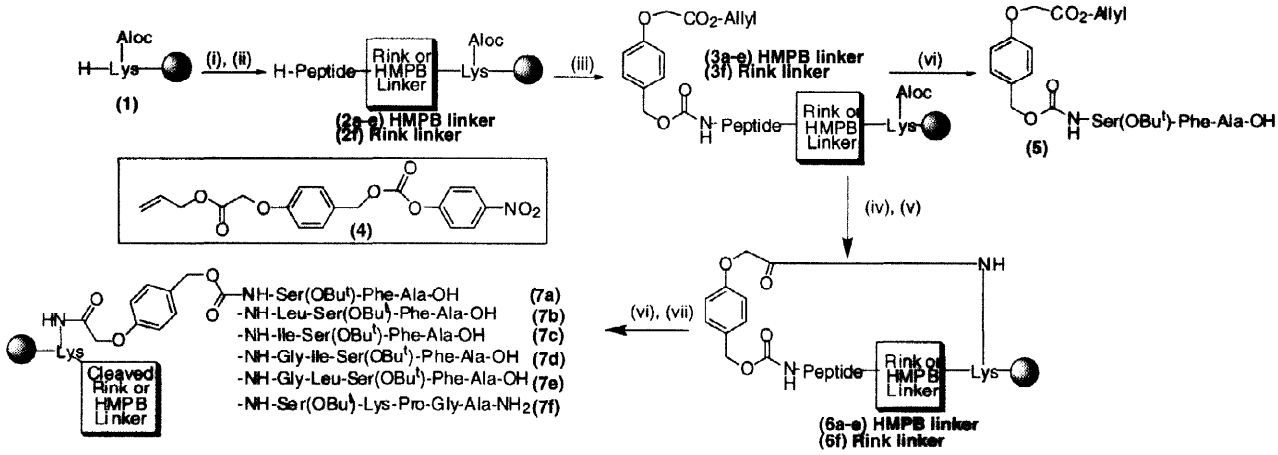
0040-4020/99/$ - see front matter
PII: S0040-4020(99)00146-5

[9] and the β–napthylamides, [10] while peptidyl fluoro- and chloro-ketones, [11] peptidyl (acyloxy)methylketones [12] and peptidyl sulfonium salts [13] have all been examined as irreversible site directed inhibitors in the study of a variety of different proteases, an area which has been extensively reviewed [14]. Peptides modified at the C-terminus are therefore an important class of compounds. Some C-terminally modified peptides are directly accessible using solid phase techniques, for example peptide amides. [15] Others such as *p*-nitroanilides are available using specialised linkers [16] while recent reports on the use of the Weinreib [17], thiazolidine [18] and semicarbazone [19] linkers to give peptide aldehydes have also been reported, as has the use of ozonolysis [20]. For a number of protease based applications in our laboratories we needed a routine method of synthesising a broad range of C-terminally modified peptides and desired a method applicable to split and mix library synthesis. Possible solutions to this problem are: (i) the synthesis the peptide in the non traditional N->C manner [21] (although this necessitates the preparation all the amino acids in a C-terminally protected form, and epimerisation following C-terminal activation is a cause of concern); (ii) the use of back-bone linkers [22] and; (iii) the inversion of the peptide following conventional synthesis and modification of the C-terminus. By its very nature this last process would also be "self purifying" since only those peptides modified with the "inversion linker" and subsequently cyclised would remain on the resin. Although the inversion of resin bound peptides has been effected [23,24] no method has been reported which allows inversion and release of the peptide or modified peptide. We report here the full synthetic details of our inversion methodology [25] for the general solid phase synthesis of C-terminally modified peptides. We demonstrate the method with the synthesis of peptide nitriles and alcohols and a range of other modified peptides.

Results and Discussions:

The general synthetic process, outlined in Scheme 1, was followed by cleavage of materials from the resin at each step of the synthesis followed by analysis by HPLC, MALDI-TOF MS and ESMS and full characterisation of the final compounds. Thus Fmoc-Lys(Aloc)-OH was attached to 1% crosslinked polystyrene aminomethyl resin (1.4mmol/g) or TentaGel resin (0.3mmol/g) to give **(1)**, following Fmoc removal with 20% piperidine in DMF. The α-amine was coupled with either the HMPB [26] (4-(4-hydroxymethyl-3-methoxyphenoxy)-butanoic acid) or Fmoc-Rink linker [27] (*p*-[α-[1-(9*H*-fluoren-9-yl)methoxyformamido]-2,4-

dimethoxybenzyl]-phenoxyacetic acid) to give, following solid phase peptide synthesis using traditional Fmoc chemistry (**2a-f**) [28].



Scheme 1: Inversion Methodology: (i) Fmoc Rink linker or HMPB, DIC, HOBt, DCM; (ii) Fmoc Peptide Synthesis; (iii) (**4**), pyridine, DMF; (iv) $Pd(PPh_3)_4$, dimedone, DCM, THF (1:1); (v) PyBroP, DMAP, DIPEA, DCM; (vi) 1% TFA, DCM (HMPB linker), 5% TFA, DCM (Rink linker); (vii) MeOH or Triisopropylsilane quench.

These peptides were converted to (**3a-f**) by coupling to carbonate (**4**). Carbonate (**4**) was prepared in an overall yield of 44% from 4-formylphenol by alkylation with allylchloroacetate in acetonitrile with potassium carbonate, followed by reduction with sodium cyanoborohydride in ethanol and treatment with *p*-nitrophenylchloroformate and pyridine in dichloromethane. Removal of the Aloc and Allyl groups from (**3a-f**) was accomplished using Pd(PPh3)4 in degassed CH_2Cl_2/THF in the presence of excess 5,5-dimethylcyclohexane-1,3-dione [29,30] and was followed by MS analysis and quantitative ninhydrin assay [30]. Cyclisations were carried out using PyBroP [31] and 2 eq of DIPEA for 2 hours to give the resin bound cyclic peptides (**6a-e**). Treatment of (**6a-e**) with 1% TFA for 12 h gave the protected, inverted and resin bound peptides (**7a-e**), while treatment of (**6f**) with 5% TFA for 12 h gave the protected, inverted and resin bound peptide amide (**7f**). Any peptide not attached to the resin via the second urethane linker at this stage will be removed from the resin by the first acid cleavage step. The cyclisation was followed predominantly by the ninhydrin assay but also in initial studies by amino acid analyses prior to cyclisation (peptides (**2**)) and after the 1% TFA cleavage (peptides (**7**)) using the lysine residue as an internal standard (see experimental data). The initial cyclisations as assessed by amino acid analysis preceded in an average yield of 60%, though subsequent cyclisations,

monitored using the ninhydrin assay, typically gave yields in excess of 70%. The unmodified peptides (**7a-e**) were cleaved from the solid support using 95% TFA/DCM and isolated by Et_2O precipitation for characterisation purposes prior to on resin C-terminal modification. HPLC analysis showed the crude materials to be of excellent purity. Treatment of (**3a**) with 1% TFA in DCM overnight gave the N-protected peptide (**5**) which was fully characterised and clearly demonstrates the success of the carbonate coupling and the orthogonality of the carboxyl and amino linkers. This is further illustrated by Figure 1 which shows the complete synthetic process as followed by ESMS for linear peptide synthesis, carbamate linker attachment, allyl deprotection and C-terminal modification.

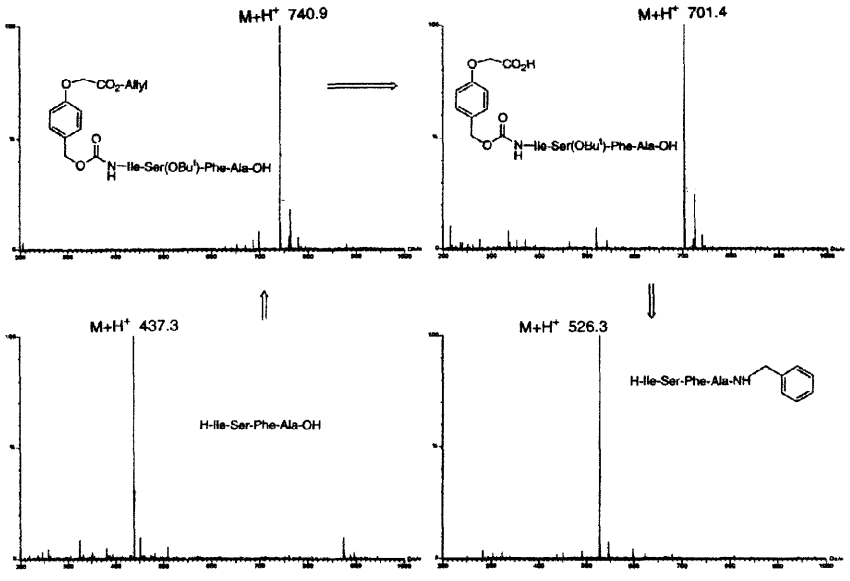
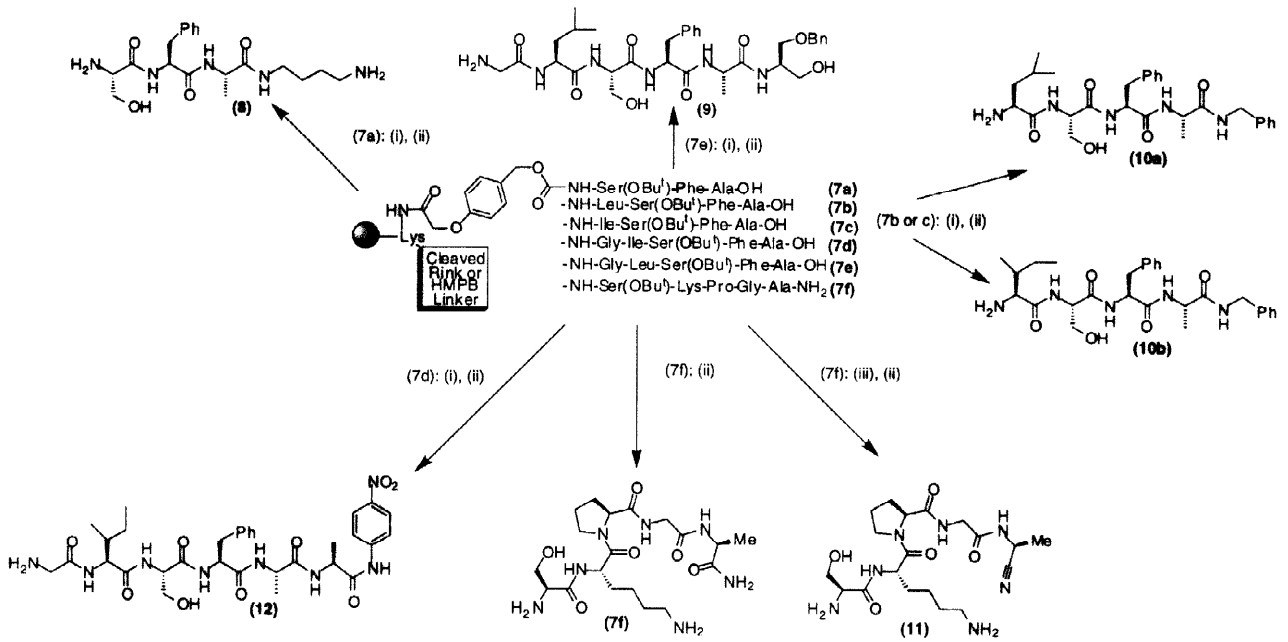


Figure 1: ESMS data for the synthesis of peptide amide H-Ile-Ser-Phe-Ala-$NHCH_2Ph$ (**11**) *via* inversion methodology.

Peptides (**7a-f**), now displaying a free carboxylic acid/amide, were then modified and cleaved as shown in Scheme 2. Thus the resin bound inverted peptides (**7a-e**) were treated with PyBroP and DIPEA and a variety of amines. This included: (i) 4-*tert*-butoxycarbonylaminobutylamine to give (**8**), (ii) serinol(OBn) to give (**9**) and (iii) benzylamine to give (**10**). The resin bound inverted peptide amide (**7f**) was treated with cyanuric chloride [32] to give the peptide nitrile (**11**). All compounds gave a single major peak by RP-HPLC and the expected analytical data.



Scheme 2: (i) RNH_2 (4eq), PyBroP (2eq), DIPEA (2eq), DCM; (ii) 95% TFA/DCM; (iii) cyanuric chloride, DMF.

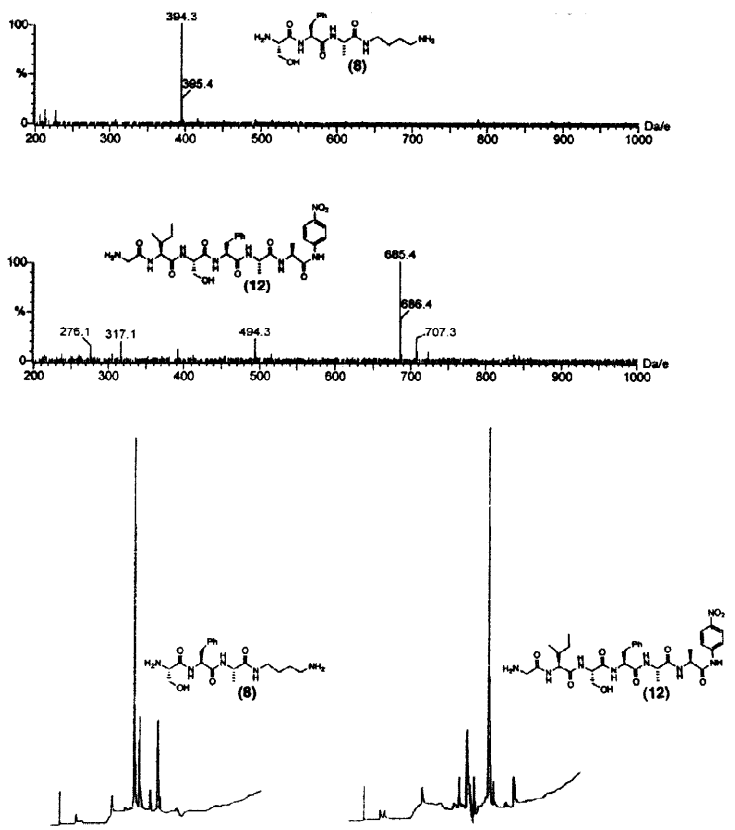


Figure 2: HPLC and ES MS data for (a) Ser-Phe-Ala-NH$(CH_2)_4NH_2$ (**8**); (b) Gly-Ile-Ser-Phe-Ala-Ala-nitroanilide (**12**).

Although activation of a terminal carboxylic acid group in a peptide allows oxazolone formation, the coupling conditions reported here resulted in minimal loss of stereochemical integrity at the carboxyl terminus, typically being <10% (by HPLC and NMR). The methods reported elsewhere [21] for C-terminal couplings were also found be excellent. In one series of experiments over-vigorous treatment with a mixture of PyBroP and DIPEA in the presence of DMAP for extended periods did cause serious epimerisation problems; however reversion to the original methods solved this problem.

Conclusion:

We have developed a novel and practical method of synthesising a broad range of C-terminally modified peptides. Our technology is applicable to library generation either by MPS or by using split and mix methodology. The synthetic route is very efficient, produces materials in excellent overall yield and in a high state of purity and is amenable to standard automated solid phase synthesis. Peptides of varying lengths (3-8 residues) cyclise well and give very pure final products as a natural consequence of the "on resin" clean up process. One issue that might influence this efficiency is the productive nature of both inter and intramolecular cyclisations. This inversion and capture strategy is ideal for single bead screening applications since the products generated will by the nature of the process be of exceptional purity. As part of our continuing interest in proteases we are now preparing substantial libraries of peptide nitriles and aldehydes using this methodology for single bead screening.

Experimental:

NMR spectra were recorded on Bruker AC-300, Bruker AM-360 or Jeol JNM-GX 270 spectrometers in the solvents indicated at 298 K. Chemical shifts are reported on the δ scale in ppm and were referenced to residual protonated solvent. Mass spectra were obtained on a VG platform single quadropole mass spectrometer in electrospray positive ionisation mode. Analytical RP-HPLC was performed on a Hewlett Packard HP 1100 equipped with a Phenomenex Prodigy reverse phase C_{18} column (150 x 3 mm i.d.) with a flow rate of 0.5 mL/min, monitoring at 220 nm and eluting with (A) 0.1% TFA in water and (B) 0.042% TFA in MeCN with a gradient of 0% B to 100% B over 20 min. Amino acids and coupling reagents were purchased from Novabiochem. IR's were recorded in ATR mode on a Golden Gate with neat sample (solid or liquid).

Peptide couplings.

Solid phase amide couplings were carried out using a five fold excess of hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC) in dichloromethane and the appropriate Fmoc amino acid, adding a few drops of DMF where necessary to aid dissolution. Fmoc deprotections were achieved by treating the resin bound peptide with a 20% solution of piperidine in DMF (10mL/g of resin) for 5min followed by washing with DMF (x2) and the process repeated.

Coupling linker (4).

Pre-swollen resin **(3a-d)** (1 mL of DCM per 200 mg resin) was suspended in peptide synthesis grade DMF (2ml) and pyridine (5 eq.) added followed by the inversion linker **(4)** (2 eq.). The reaction vessel was sealed and kept at 50°C until coupling was complete as determined by the ninhydrin test. The solution was removed by filtration, and the resin washed with DMF (10x10mL), DCM (10x10mL) and diethyl ether (10x10mL) and dried under vacuum.

Palladium mediated allyloxycarbonyl and allyl deprotections.

Pre-swollen resin supported peptide (1mL of DCM per 200mg of resin), *tetra*kistriphenylphosphine palladium (1 eq.) and 5,5-dimethylcyclohexane-1,3-dione (10 eq.) were suspended in a mixture of degassed DCM and THF (2mL, 1:1). The reaction vessel was sealed, light excluded and the reaction agitated vigorously for 2 h. The solution was removed by filtration and the resin washed with a solution of 0.5% DIPEA and 0.5% diethyldithiocarbamic acid sodium salt in DMF (10x10mL) to remove the palladium residues. The resin was further washed with DCM (2x10mL) and diethyl ether (2x10mL) and dried under vacuum.

Peptide cyclisations.

The resin bound peptide bearing the inversion linker free acid and the lysine free amine was treated with PyBroP (2 eq.), DMAP (1 eq.) and DIPEA (4 eq.) in DCM (1mL per 100mg of resin) for 12 h. The consumption of amine was monitored using the ninhydrin test. The solvent was removed by filtration and washed with DCM (5x10mL) and diethyl ether (5x10mL) and dried under vacuum.

Cyclisation efficiency.

The peptide resin samples were acid hydrolysed (6M HCl, overnight) and then derivatised with phenylisothiocyanate before HPLC analysis of the PTC-amino acid derivatives. The commercially available Picotag system of Waters was used in this process.

	Relative Amino Acid Ratios						% Cyclisation
Peptide	Ser	Ala	Phe	Ile	Gly	Lys	
3a	0.45	0.80	0.70	-	-	1	
7a	0.32	0.67	0.56	-	-	1	78
3c	0.41	0.77	0.66	0.65	-	1	
7c	0.25	0.45	0.23	0.35	-	1	54
3d	0.43	0.78	0.69	0.65	0.73	1	
7d	0.22	0.40	0.33	0.32	0.35	1	49

In other cases the quantitative ninhydrin assay [31] was used to monitor cyclisation by the difference between the allyloxycarbonyl deprotected lysine residue and the cyclised product. The average cyclisation efficiency was >70%.

Peptide linearisation.

The macrocyclic peptide resin (**6a-c**) was treated with either 1% TFA in DCM (for the HMPB linker) or 5% TFA in DCM for the Rink linker (**6d**) (15 hours, 10mL per 100mg of resin). The resin was washed with DCM (5x10mL), MeOH (5x10mL) and then with DCM (5x10mL) and Et_2O (5x10mL).

C-terminal coupling reactions.

The resin bound inverted peptide was treated with a variety of amines (4 eq.), PyBroP (2 eq.) and DIPEA (2 eq.) in DCM (1mL per 100mg of resin) for 2 h. The resin was collected by filtration and washed with DCM (5x10mL) and Et_2O (5x10mL) and dried under vacuum.

Cleavage of modified peptides.

Peptide resin, preswollen in DCM (1mL per 100mg of resin), was treated with 95% TFA in DCM (10ml/g of resin) for 2 hrs . The supernatant was treated with Et_2O (30mL/g of resin)

and the resultant precipitate collected by filtration. The solids were dried *in vacuo*, dissolved in water (5mL) and freeze-dried to give white fluffy, hydroscopic solids.

4′-Nitrophenyl-(4-hydroxymethylphenoxyallylacetate)carbonate (4).

4-Formylphenol (2.22 g, 18.2 mmol), potassium carbonate (6.04 g, 43.7 mmol) and potassium iodide (0.10 g, 0.6mmols) were suspended in acetonitrile (50mL). To this suspension was added, dropwise, 2-chloro(allyl)acetate (2.53mL, 21.8mmol). The reaction mixture was refluxed for 2 hr, allowed to cool, filtered and concentrated *in vacuo*. The residue was taken up in ethyl acetate (50mL) and again filtered and concentrated *in vacuo* to give 3.94g (99%) of a pale yellow oil. 4-Formylphenoxyallylacetate was used in the following reaction without further purification. A small portion was purified by column chromatography (silica gel, eluting with 50:50 hexane:ethyl acetate) for characterisation and afforded a colourless viscous oil; $\upsilon_{max.}/cm^{-1}$ (Film) 1757, 1692 , 1600, 1580, 1508; δ_H (300MHz, CDCl): 9.80 (s, 1H, C<u>H</u>O); 7.76 (d, J = 8, 2H, $ArH^{3,5}$); 6.91 (d, J = 8, 2H, $ArH^{2,6}$); 5.84 (ddd, J = 18, 12, 6, 1H, $OCH_2C\underline{H}CH_2$); 5.27 (d, J = 18, 1H, $CHCH\underline{H}$); 5.00 (d, J = 12, 1H, $CHC\underline{H}H$); 4.72 (s, 2H, $OC\underline{H}_2C(O)$); 4.63 (d, J = 6, 2H, $OC\underline{H}_2CHCH_2$); δ_C (75MHz, $CDCl_3$): 190.85 (<u>C</u>HO), 167.91 (<u>C</u>O_2), 162.68 (ArC), 132.06 (ArC), 131.38 (CH_2=<u>C</u>H), 130.82 (ArC), 119.38 (CH=<u>C</u>H_2), 115.02 (ArC), 66.13 ($O\underline{C}H_2C(O)$), 65.18 ($O\underline{C}H_2CHCH_2$); HRMS (EI-MS) Found 220.0743, $C_{12}H_{12}O_4$ Requires 220.0736.

4-Formylphenoxyallylacetate (1.0g, 4.55mmol) was dissolved in ethanol (10ml) with a trace of bromophenol blue. A few drops of 2M ethanolic HCl were added to the reaction mixture, followed by sodium cyanoborohydride (0.46g, 4.55mmol). The reaction mixture was kept below pH 4 by the occasional addition of one or two drops of 2M ethanolic HCl for 30 min. The solvent was removed *in vacuo* and the residue taken up in brine (50mL) which was extracted with ethyl acetate (2x50mL). The organic phase was dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give 0.97g (96%) of 4-Hydroxymethylphenoxyallylacetate as a yellow oil which was used in the following reaction without further purification. $\upsilon_{max.}/cm^{-1}$ (Film) 3372, 1740, 1510; δ_H (300MHz, CD_3SOCD_3): 7.15 (d, J = 9, 2H, ArH); 6.82 (d, J = 9, 2H, ArH); 5.85 (ddd, J = 18, 11, 6, 1H, $OCH_2C\underline{H}CH_2$) 5.26 (dd, J = 18, 1, 1H, $CHCH\underline{H}$); 5.17 (dd, J = 11, 1, 1H, $CHC\underline{H}H$); 4.75 (s, 2H, $OC\underline{H}_2C(O)$); 4.56 (d, J = 6, 2H, $OC\underline{H}_2CHCH_2$); 4.34 (d, J = 6, 2H, $CC\underline{H}_2OH$); δ_c (75MHz, CD_3SOCD_3): 168.66 (<u>C</u>O_2H) 156.54 (ArC^1), 135.41 (ArC) 132.32 (CH_2=<u>C</u>H) and

127.96 (ArC), 118.21 (CH=$\underline{C}H_2$), 114.17 (ArC), 64.96 (O$\underline{C}H_2$C(O)), 64.66 (O$\underline{C}H_2$CHCH$_2$), 62.55 ($\underline{C}H_2$OH); HRMS (EI-MS) Found 222.0886, $C_{12}H_{14}O_4$ Requires 222.0892.

4-Hydroxymethylphenoxyallylacetate (0.97g, 4.55mmol) was dissolved in pyridine (0.44mL, 5.46mmol) and dichloromethane (10mL) at 0°C and 4-nitrophenyl chloroformate (1.01 g, 5.01mmol) in dichloromethane (5mL) was added dropwise. The reaction allowed to come to ambient temperature and stirred for 16 h. The yellow precipitate formed during this time was removed by filtration and the solvent removed *in vacuo*. The residue was taken up in diethyl ether (25mL), filtered and concentrated *in vacuo*. The crude product was recrystallised from hexane: ether to give 0.57g (49%) of a white microcrystalline solid. Mpt 54°C; $\upsilon_{max.}/cm^{-1}$ (Solid) 1760, 1613, 1523, 1348; δ_H (300MHz, $CDCl_3$): 8.28 (d, J = 8, 2H, $O_2NArH^{2,6}$), 7.40 (d, J = 8, 2H, $O_2NArH^{3,5}$), 7.38 (d, J =8, 2H, $ArH^{2,6}$); 6.94 (d, J = 8, 2H, $ArH^{3,5}$), 5.95 (ddt, J = 17, 13, 7, 1H, OCH$_2$C$\underline{H}$CH$_2$), 5.35 (dd, J = 17, 4, 1H CHCH$\underline{H}$); 5.28 (dd, J = 13, 4, 1H, CHC$\underline{H}$H), 5.25 (s, 2H, O$\underline{C}H_2$C(O)), 4.72 (d, J = 7, 2H, O$\underline{C}H_2$CHCH$_2$), 4.70 (s, 2H, C$\underline{C}H_2$); δ_c (75MHz, $CDCl_3$): 168.51 ($\underline{C}O_2$), 158.54 (O$\underline{C}O_2$), 155.68, 152.58 and 145.51 (ArC), 131.50 (OCH$_2$$\underline{C}HCH_2$), 130.87, 127.63, 125.69, and 121.94 (ArC), 119.38 (OCH$_2$CH$\underline{C}$H$_2$), 115.01 (OC($\underline{C}$H)$_2$), 70.83 ($\underline{C}H_2$O), 66.10 (O$\underline{C}H_2$C(O)), 65.38 (O$\underline{C}H_2$CHCH$_2$); HRMS (FAB, MNOB matrix) Found 387.0942, $C_{19}H_{17}NO_8$ Requires 387.0954.

p-(CH$_2$=CHCH$_2$OOCCH$_2$O)C$_6$H$_4$CH$_2$OCO-Ser(O^{t}Bu)-Phe-Ala-OH (N-(1-oxyallylacetate-4-benzyloxycarbonyl)-Ser(O^{t}Bu)-Phe-Ala-OH) (5).

The title compound was cleaved from the resin with 1% TFA in DCM (2mL/100mg of resin) for 3 hrs and the resin removed by filtration. Toluene (2mL/100mg of resin) and Pyridine (added dropwise until basic) were added and the solvent and salts were removed *in vacuo* to give a white powder in quantitative yield based on resin loading; δ_H (360MHz, CD$_3$SOCD$_3$): 8.70 (d, J = 7, 1H, N$\underline{H}$), 7.98 (d, J = 8, 1H, N$\underline{H}$), 7.18-7.43 (m, 8H, N$\underline{H}$, C$_6\underline{H}_5$CH$_2$, $ArH^{2,6}$), 7.20 (d, J = 8, 2H, $ArH^{3,5}$), 6.30 (ddt, J = 6, 11, 17, 1H, OCH$_2$C$\underline{H}$CH$_2$), 5.43, (br dd, J = 1, 17, OCH$_2$CHC$\underline{H}$H), 5.33 (br dd, J = 11, 1, 1H, OCH$_2$CHCH$\underline{H}$), 5.06 (m, 2H, OC$_6$H$_4$C$\underline{H}_2$O), 4.93, (s, 2H, OCH$_2$CO), 4.75 (br d, J = 6, 2H, O$\underline{C}H_2$CHCH$_2$), 4.72-4.63 (m, 1H, *Phe*-C$\underline{H}\alpha$), 4.23 (m, 1H, *Ala*-C$\underline{H}\alpha$), 4.12 (dt, J = 8, 6, 1H, *Ser*-C$\underline{H}\alpha$), 3.43 (m, 2H, *Phe*-C$\underline{H}_2$), 1.36 (d, J = 7, 3H, C$\underline{H}_3$CHCO$_2$H), 1.07 (s, 9H, (C$\underline{H}_3$)$_3$C); δ_C (67.5MHz, CD_3SOCD_3): 174.1, 170.06, 169.33 and 168.35 (amides, acid and ester carbonyls), 157.26 (urethane), 156.10 and 137.43 (ArC), 132.16 ($\underline{C}$H=CH$_2$), 129.61, 129.49, 129.29, 127.84 and 126.10

(ArCH), 116.09 (CH=$\underline{C}H_2$), 114.33 (ArC), 72.83 ($\underline{C}(CH_3)_3$), 65.18 ($\underline{C}H_2O$), 64.84 ($\underline{C}H_2O$), 64.59 ($\underline{C}H_2O$), 61.78 ($\underline{C}H_2O$), 55.54, 53.27 and 47.91 (3x$\underline{C}H\alpha$), 37.61 (*Phe*-$\underline{C}H_2$), 27.08 (($\underline{C}H_3)_3C$), 17.49 (*Ala*-$\underline{C}H_3$); HRMS (FAB, MNOB matrix) Found 628.2895, $C_{32}H_{42}N_3O_{10}$ Requires 628.2870.

H-Ser-Lys-Pro-Gly-Ala-NH_2 (7f).

White lyophilised solid, 75% (HPLC); δ_H (360 MHz, CD_3SOCD_3): 8.71 (d, J = 7, 1H, *Lys*-N$\underline{H}$), 8.40 (t, J = 6, 1H, *Gly*-N$\underline{H}$), 8.28 (brs, 3H, *Ser*- N$\underline{H}_3^+$), 7.95 (brs, 3H, *Lys*-N$\underline{H}_3^+$), 7.86 (d, J = 7, 1H, *Ala*-N$\underline{H}$), 7.35 and 7.11 (2 x brs, 2H, CON$\underline{H}_2$), 4.64 (dt, J = 8, 8, 1H, *Lys*-αCH), 4.38 (dd, J = 5, 5, 1H, *Pro*-$\alpha C\underline{H}$), 4.26 (dq, J = 7, 7, 1H, *Ala*-$\alpha C\underline{H}$), 3.88-3.60 (m, 6H, *Gly*-$C\underline{H}_2$, *Pro*-$\delta C\underline{H}_2$ and *Ser*-$C\underline{H}_2$), 2.91-2.82 (m, 2H, *Lys*-$\varepsilon C\underline{H}_2$), 2.21-1.86 (m, 4H, *Pro*-$\gamma C\underline{H}_2$ and *Pro*- $\beta C\underline{H}_2$), 1.70-1.54 and 1.86-1.73 (m, 4H, *Lys*-$\delta C\underline{H}_2$ and *Lys*-$\beta C\underline{H}_2$), 1.51-1.40 (m, 2H, *Lys*-$\gamma C\underline{H}_2$), 1.33 (d, J = 7, 3H, *Ala*-$C\underline{H}_3$); δ_C (67.5MHz, CD_3SOCD_3): 174.29, 172.14, 169,69, 168.41 and 166.70 (5xamide carbonyls), 60.03 (*Ser*-$\beta\underline{C}H_2$), 59.91 (*Pro*-$\underline{C}H\alpha$), 54.01 (*Ser*-$\underline{C}H\alpha$), 50.51 (*Lys*-$\underline{C}H\alpha$), 48.02 (*Ala*-$\underline{C}H\alpha$), 46.89 (*Pro*-$\delta\underline{C}H_2$), 42.09 (*Gly*-$\underline{C}H_2$), 38.59 (*Lys*-$\varepsilon\underline{C}H_2$), 29.05 (*Pro*-$\beta\underline{C}H_2$), 26.95 (*Lys*-$\delta\underline{C}H_2$), 26.65 (*Lys*- $\beta\underline{C}H_2$), 24.51 (*Pro*-$\gamma\underline{C}H_2$), 21.69 (*Lys*-$\gamma\underline{C}H_2$), 18.04 (*Ala*-$\underline{C}H_3$); HRMS (FAB, MNOB matrix) Found 458.2719, $C_{19}H_{36}N_7O_6$ Requires 458.2727.

H-Ser-Lys-Pro-Gly-NH-CH(Me)-CN (11).

Peptide resin (**7d**), preswollen in DMF (100mg/mL) was treated with 2 equiv of cyanuric chloride for 12 hrs. The resin was removed from the solution by filtration and washed with DCM (5x10mL), methanol (5x10mL) and Et_2O (5x10mL) and dried under vacuum. Peptide (**11**) was then cleaved from the resin using the standard cleavage conditions to give following lyophilisation a white solid, 48% (HPLC); $\upsilon_{max.}/cm^{-1}$ (Solid) 2223; δ_H (360MHz, CD_3SOCD_3): 9.56-8.37 (brs, 6H, *Lys*-N$\underline{H}_3^+$ and *Ser*-N$\underline{H}_3^+$), 8.49 (t, J = 6, 1H, *Gly*-N$\underline{H}$), 8.38 (2xd, 2H, J =7, *Lys*-N$\underline{H}$, d, J = 7, *Ala*-N$\underline{H}$), 4.70-4.51 (m, 2H, *Lys*-$C\underline{H}\alpha$ and *Ser*-$C\underline{H}\alpha$), 4.88 (dt, J =7, 1H, *Ala*-$C\underline{H}\alpha$), 4.35 (t, J 6, 1H, *Pro*$C\underline{H}\alpha$), 3.96-3.45 (m, 6H, *Gly*-$C\underline{H}_2$, *Pro*-$\delta C\underline{H}_2$ and *Ala* $\beta C\underline{H}_2$), 3.28 (dt, J = 6, 6, 2H, *Lys*-$\varepsilon C\underline{H}_2$), 2.21-1.87 (m, 4H, *Pro*-$\beta C\underline{H}_2$ and *Pro*-$\gamma C\underline{H}_2$), 1.84-1.71 (m, 2H, *Lys*-$\beta C\underline{H}_2$), 1.70-1.49 (m, 5H, *Ala*-$C\underline{H}_3$ and *Lys*-$\delta C\underline{H}_2$), 1.48-1.28 (m, 2H, *Lys*- $\delta C\underline{H}_2$); δ_C (67.5MHz, CD_3SOCD_3): 171.98, 170.17, 168.97, 167.86 (4xamide carbonyls), 120.09 (*Ala*-$\underline{C}N$), 60.97 (*Ser*-$\beta\underline{C}H_2$), 59.94 (*Pro*-$\underline{C}H\alpha$), 53.89 (*Ser*-$\underline{C}H\alpha$), 50.47 (*Lys*-$\underline{C}H\alpha$), 46.95 (*Pro*-$\delta\underline{C}H_2$), 41.67 (*Gly*-$\underline{C}H_2$), 38.96 (*Lys*-$\varepsilon\underline{C}H_2$), 35.48

(*Ala*-CHα), 30.30 (*Pro*-βCH$_2$), 27.96 (*Lys*- βCH$_2$), 27.14 (*Lys*- δCH$_2$), 28.98 (*Pro*- γCH$_2$), 22.08 (*Lys*- γCH$_2$), 18.09 (*Ala*-CH$_3$).

H-Ser-Phe-Ala-NH(CH$_2$)$_4$NH$_2$ (8).

White lyophilised solid, 75% (HPLC); δ_H (360MHz, CD_3SOCD_3): 8.69 (d, J = 8, 1H, *Phe*-NH), 8.31 (d, J = 8, 1H, *Ala*-NH), 8.16 (br s, 3H, NH$_3^+$), 7.93 (t, J = 6, 1H, CH_2CH_2NHCO), 7.84 (br s, 3H, NH$_3^+$), 7.40-7.27 (m, 5H, ArH), 4.69 (dt, J = 6, 8, 1H, *Phe*-CHα), 4.32 (dq, J =7, 7, 1H, *Ala*-CHα), 4.02-3.94 (br m, 1H, *Ser*-CHα), 3.89 and 3.80 (AB part of ABX system, 2H, J= 11,6,4, *Ser*-CH$_2$), 3.11 and 2.94 (AB part of ABX system, 2H, J= 14, 9, 6, *Phe*-CH$_2$), 3.24-3.06 and 2.94-2.80 (2xm, 4H, CH$_2$NH$_3^+$ and CH_2CH_2NHCO); 1.66-1.46 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.31 (d, J = 7, 3H, *Ala*-CH$_3$); δ_C (67.5MHz, CD_3SOCD_3): 172.01, 170.3 and 167.01 (3xCO), 137.68, 129.34, 128.26 and 126.53 (ArC), 60.59 (CH$_2$O), 54.28, 54.04 and 48.11 (3xCHα), 37.83 and 37.57 (2xNHCH$_2$CH$_2$ and *Phe*-CH$_2$), 26.14 (CH$_2$), 24.57 (CH$_2$),18.55 (*Ala*-CH$_3$); HRMS (FAB, MNOB matrix) Found 394.2458, $C_{19}H_{32}N_5O_4$ Requires 394.2454.

H-Leu-Ser-Phe-Ala-NHCH$_2$Ph (10).

White lyophilised solid, 85% (HPLC); δ_H (360MHz, CD_3SOCD_3): 8.68 (t, J = 8, 1H, *Ser*-NH), 8.37 (t, J = 6, 1H, PhCH$_2$NH), 8.28 (d, J = 8, 1H, *Phe*-NH), 8.22 (d, J = 7, 1H, *Ala*-NH), 8.17 (br s, 3H, NH$_3^+$), 7.23-7.44 (m, 10H, $C_6H_5CH_2NH$ & $C_6H_5CH_2CH$), 4.65 (dt, J = 9, 4, 1H, *Phe*-CHα), 4.57-4.46 (m, 1H, *Ser*-CHα), 4.45-4.32 (m, 3H, PhCH$_2$NH and *Ala*-CHα), 3.97-3.87 (m, 1H, *Leu*-CHα), 3.74-3.61 (br m, 2H, *Ser*-CH$_2$), 3.20 and 3.14 (AB part of ABX system, J = 14, 9, 5, 2H, *Phe*-CH$_2$), 1.63-1.73 (m, 1H, $(CH_3)_2CHCH_2$), 1.61-1.55 (m, 2H, $(CH_3)_2CHCH_2$), 1.35 (d, J= 7, 3H, *Ala*-CH$_3$), 0.97 and 0.94 (2xd, J =7, 6H, *Leu*-CH$_3$); δ_C(67.5MHz, CD_3SOCD_3): 172.15, 170.57, 169.62 and 169.07 (4xCO), 139.45, 137.85, 129.32, 128.42, 128.12, 127.13, 126.35 and 126.90 (ArC); 61.89 (CH_2O), 54.96, 53.98, 50.84, 48.53 (4xCHα), 42.11 and 37.24 (2x$C_6H_5CH_2$), 39.7 (*Leu*-CH$_2$), 23.63 (*Leu*-CH$_3$), 22.83 (*Leu*-CH$_3$), 21.98 (CH), 18.42 (*Ala*-CH$_3$); HRMS (FAB, MNOB matrix) Found 526.3042, $C_{28}H_{40}N_5O_5$ Requires 526.3030.

H-Gly-Leu-Ser-Phe-Ala-Serinol(OBn)-OH (9).

White lyophilised solid,45% (HPLC); δ_H (360MHz, CD_3SOCD_3): 8.56 (d, J = 8 , 1H, *Leu*-NH), 8.26 (d, J = 8, 1H, *Ser*-NH), 8.17 (d, J = 8, 1H, *Ala*-NH), 8.06 (br s, 3H, NH$_3$), 8.05

(d, J = 8, 1H, *Phe*-NH), 7.76 (d, J = 8, 1H, *Serinol*-NH), 7.47-7.22 (m, 10H, 2xAr), 4.66-4.53 (m, 4H, PhCH_2O, *Leu*-CHα and *Phe*-CHα); 4.33-4.44 (m, 2H, *Ser*-CHα and *Ala*-CHα); 4.00 (ddt, J = 6, 3, 3, 1H, *Serinol*-CHα), 3.49-3.73 (m, 6H, 2xCH_2OH and CH_2OCH_2Ph), 3.15 and 2.90 (AB part of ABX J = 14, 9, 4, 2H, *Phe*-CH_2), 1.59-1.74 (m, 1H, $(CH_3)_2$CHCH_2), 1.46-1.55 (m, 2H, $(CH_3)_2$CHCH_2), 1.29 (d, J = 7, 3H, *Ala*-CH_3CH), 0.97 and 0.95 (2xd, J = 7, 6H, 2x *Leu*-CH_3); δ_C (67.5MHz, CD_3SOCD_3): 172.05, 171.76, 170.41, 169.99 and 165.73 (5xCO), 138.60, 137.83, 129.39, 128.39, 128.15, 127.63 and 126.36 (ArC); 72.34 (PhCH_2O), 68.93 (CH_2OBn), 61.83 and 60.42 (2xCH_2O), 55.14, 53.93, 50.87, 50.82, 48.40 (5xCHα), 42.11 (*Gly*-CH_2), 39.7 (*Leu*-CH_2), 37.31 (*Phe*-CH_2), 24.22 (*Leu*-CH_2), 23.30 (*Leu*-CH_3), 21.69 (*Leu*-CH_3), 18.68 (*Ala*-CH_3); HRMS (FAB, MNOB matrix) Found 657.3599, $C_{33}H_{49}N_6O_8$ Requires 657.3612.

Acknowledgements:

We would like to acknowledge the Royal Society for a University Research Fellowship to MB and the BBSRC/EPSRC (BMS) for a project grant (No C02966). We thank John Horton of the Ferring Institute for the amino acid analysis.

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