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Solid Phase Peptide Synthesis: Fluoride Ion Release of Protected Peptide Fragments

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Abstract

A linker unit for solid phase peptide synthesis has been designed on the basis of organosilicon chemistry which allows efficient release, by fluoride ion nucleophilic attack at Si, of t-butyl-derived protected peptide fragments. Such protected fragments would thus be available for subsequent fragment condensation.

The strategy for the synthesis of polypeptides may be conceptually characterised by two protocols; namely total stepwise addition of protected α-amino acids and the convergent approach involving the union of smaller constituent protected peptide fragments. The fully stepwise method of synthesis for large polypeptides suffers from the disadvantage of the cumulative effect of yields of less than 100% in the assembly and in the final deprotection stages, although the latter consideration is common to both the stepwise and fragment condensation approaches. Applying the Merrifield Solid Phase Peptide Synthesis (SPPS) methodology[1] it is now feasible to construct polypeptides of 50-100 α-amino acid residues relatively efficiently and in pure form. There is a considerable need for study and design of methodology for the union of large peptide fragments to allow the convergent strategy to be capable of constructing large molecules of *ca* 150-200 α-amino acid residues, which would allow chemical synthesis to make a contribution to protein structure-biological activity problems.

A prerequisite for the synthesis of protected peptide fragments by SPPS, for subsequent carboxyl activation and union with an amino terminus of another protected peptide, is that the fully protected peptide may be released from the resin by a specific chemical reaction. Consideration

of the strategies for SPPS led us to select the N^{α}-Fmoc/side chain TFA labile protection approach.[2] This, in turn, suggested a linker (L) design based upon organosilicon chemistry illustrated in Figure 1. Our approach to the design of such a linker was based upon the mechanism proposed by Carpino[3] for the rapid β-elimination of the 2-(trimethylsilyl)ethoxycarbonyl group by fluoride ion. The basic design was to incorporate the target system (1) as the linker for SPPS in which circumstance the system shown in Scheme 1 would be susceptible to fluoride-induced fragmentation by tetrabutylammonium fluoride (TBAF) to afford the Bu_4N^+ salt of the peptide leaving the bis-quinone methide attached to the resin.[4] Such a reactive intermediate would spontaneously tautomerise to the resin-bound cinnamic amide. Independently, Barany[5] developed a linker based upon fluoride cleavage of the organosilicon linker (2).

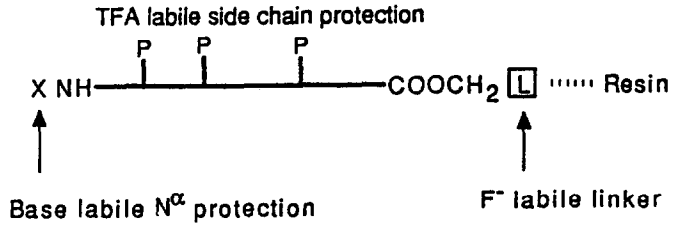
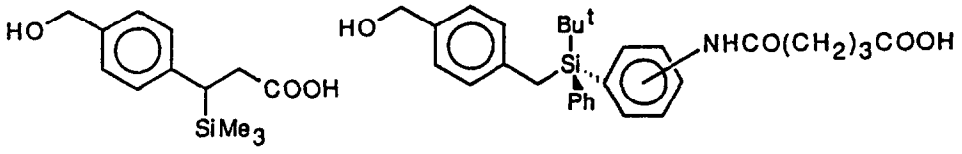


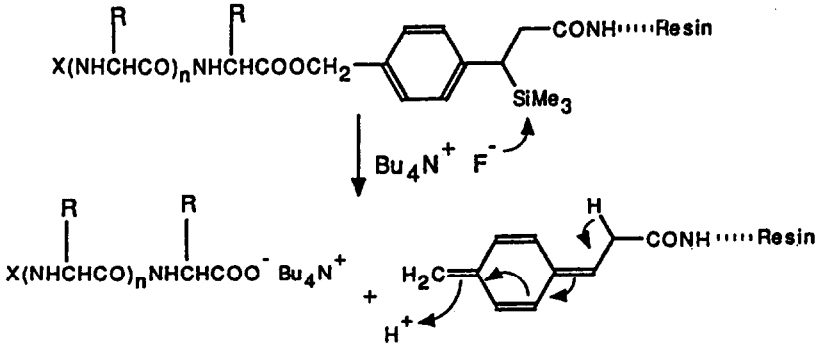
Figure 1. Orthogonal strategy.



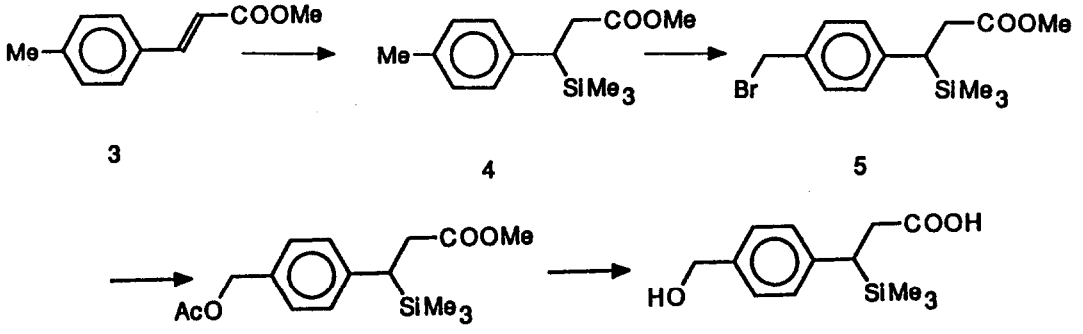
1 2

The linker molecule (1) was synthesised according to Scheme 2, which utilises the readily available methyl 4-methylcinnamate (3) as starting material, however the conversion to (4) by the method of Picard[6] presented problems associated with the use of HMPA as complexing agent for the reagent, $(CH_3)_3Si.MgCl$ and also the control of the exothermic reaction resulting in variable yields. Use of N,N-dimethylethyleneurea (DMEU),

with careful temperature control, afforded the 1,4-addition product which was brominated by NBS mainly at the aryl methyl group followed by bromide displacement of (5) by KOAc, in the presence of 0.04% 18-Crown-6, to give the acetoxy ester (6). This was subsequently hydrolysed to the desired linker reagent (1). The syntheses of analogues (11) and (12) were



Scheme 1

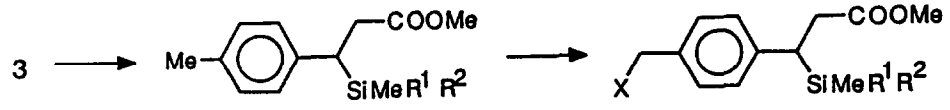


3 4 5

6 Scheme 2. 1

accomplished from (3) using the method of Fleming[7] for the introduction of the silyl groups *via* silyl cuprates derived from $(CH_3)_2PhSiCl$ and $(CH_3)Ph_2SiCl$ respectively (Scheme 3). Elimination of the silyl moiety from (6), (11) and (12) by TBAF in the range of solvents DCM (slowest) CH_3CN and DMF (fastest) showed interesting structure and solvent dependence. In DMF solution the fragmentation of (6) was almost instantaneous whereas (11) and (12) were cleaved to the extents of 72% and

87% respectively after 1 h. Since a resin bound peptide would be expected to add further steric retardation, it was decided to proceed with the linker (1) for SPPS. In order to test the compatibility of such a linker (1) with N^{α}-Boc amino acids it was considered important to determine whether the Si moiety could participate in enhanced acid cleavage of the benzyl ester function of (6). Such a reaction would result in the formation of (3) however, although such a pathway did not intervene, it was observed that transesterification leading to the trifluoroacetate corresponding to (6) did occur. Thus the linker (1) should not be used in conjunction with acid labile N^{α}-protecting groups in SPPS. In the following examples it will be shown that the strategy is compatible with N^{α}-Fmoc or N^{α}-Bnpeoc protected amino acids.



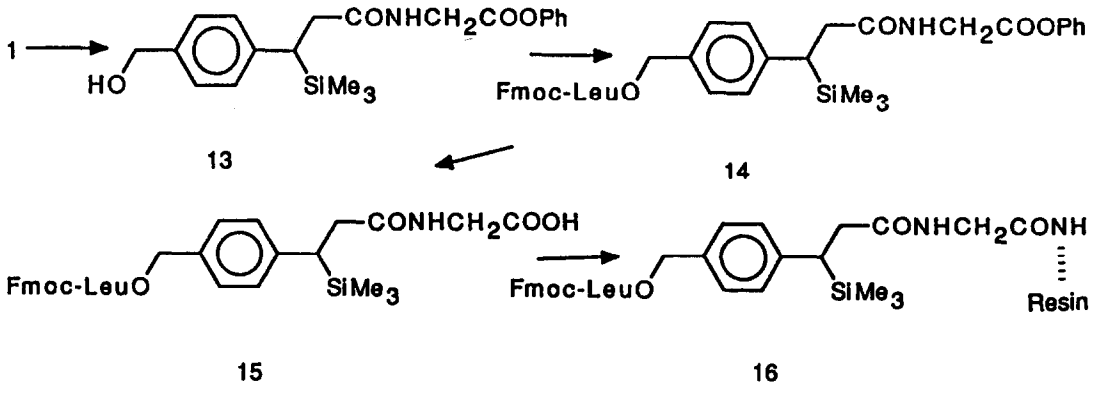
7; R^1=Me, R^2=Ph
8; R^1=R^2=Ph

9; R^1=Me, R^2=Ph, X=Br
10; R^1=R^2=Ph, X=Br
11; R^1=Me, R^2=Ph, X=OAc
12; R^1=R^2=Ph, X=OAc

Scheme 3.

The first application of (1) to SPPS involved attachment to the commercially available crosslinked polystyrene aminomethyl resin. Since it was considered that the carboxylic acid function of (1) would be sterically hindered it was decided to link (1) and H.Gly.OPh to form (13) in order to afford a less encumbered carboxyl group for further attachment to the resin. Subsequent investigation of larger spacer units, such as undecanoic acid, between the linker (1) and the resin gave less satisfactory products derived from SPPS. The details of attachment of (1) to the resin are shown in Scheme 4 where the first N^{α}-Fmoc amino acid was esterified with (13) prior to facile hydrolysis of the intermediate phenyl ester to the corresponding acid[8] followed by subsequent amide formation with the resin to afford (16). In this sequence of reactions it was found that the phenyl ester could be hydrolysed by base faster than the Fmoc urethane and the relatively hindered leucyl ester functions. As will be seen later this control is not always achievable. Base induced deprotection of the N^{α}-Fmoc group in (16) occurred satisfactorily and was

followed by stepwise SPPS using 2 equiv. of N^{α}-Fmoc amino acids (except for the N^{α}-terminal residue which was incorporated as the N^{α}-Dpp derivative[9]) activated by the phosphinic-carboxylic mixed anhydride method.[10] This afforded the protected hexapeptide Dpp.Leu.Val.Gly.Phe.Ala.Leu bound to the resin. Each coupling incorporated 2 additional equiv. of 2,6-lutidine to sequester the diphenylphosphinic acid liberated during the mixed anhydride couplings. Cleavage of the peptide from the resin, by the mechanism shown in Scheme 1, was achieved by TBAF (2 equiv.) in DMF within 5 min and Dpp.Leu.Val.Gly.Phe.Ala.Leu.OH (17) was isolated in 62% yield after purification.



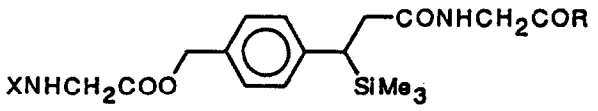
Dpp Leu Val Gly Phe Ala Leu OH

17

Scheme 4

It was then decided to apply the above strategy to the synthesis of the fully protected (1-35) fragment (24) of ubiquitin. In this case the C-terminal residue required is glycine and this presented a problem with the direct application of the above protocol in that the required selectivity of phenyl ester hydrolysis in (18) could not be achieved satisfactorily in the presence of the N^{α}-Fmoc urethane and glycyl ester functions. Instead we applied N^{α}-Bnpeoc protected[11] glycine whereupon the sequence of reactions shown in Scheme 4 could be achieved satisfactorily. Thus the phenylester (19) could be hydrolysed to the corresponding acid (20) which was coupled to the aminomethyl resin to afford (21). Deprotection of the N^{α}-Bnpeoc group was achieved

efficiently to give (22) thus allowing continuation of the SPPS of fully protected (1-35) fragment (23) of ubiquitin attached to the resin. Pulsed TBAF cleavage conditions gave a series of fractions of the protected fragment (1-35).OH which were purified separately by LH20/DMF gel filtration[12] then the appropriate fractions were pooled to give the desired protected (1-35) fragment (24) of ubiquitin having the free C-terminal carboxylic acid function in 48% yield.



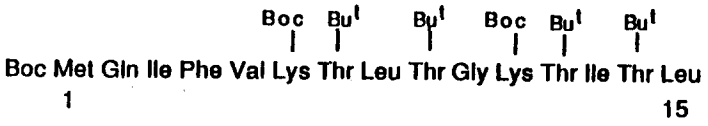
18; X=Fmoc, R=OPh

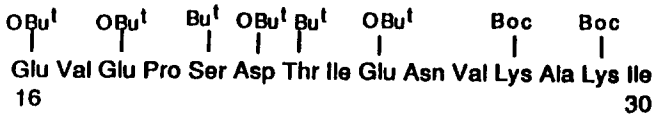
19; X=Bnpeoc, R=OPh

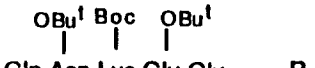
20; X=Bnpeoc, R=OH

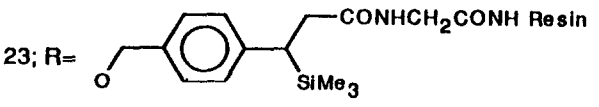
21; X= Bnpeoc, R= NH Resin

22; X= H, R= NH Resin









24; R=OH

EXPERIMENTAL

The general procedures have been described previously.[11] Automated peptide syntheses were performed using an ABI 430 instrument. High-performance liquid chromatography (HPLC) was carried out using either a Water system, *ie* 2 x 600A pumps, a U6K injector, a 680 automatic gradient controller, a model 441 ultraviolet detector, and a 308 computing integrator; or an Applied Biosystems system, *ie* 2 x 1406A solvent delivery systems, a 1480A injector/mixer, and a 2783A detector/controller. Analytical separations were carried out on the following columns: (i) ODS3 5μ Partisil (4.6 x 250 mm) (reverse phase), (ii) ODS2 5μ Spherisorb (4.6 x 150 mm) (reverse phase), (iii) μ Porasil (3.9 x 300 mm) (normal phase) using with column (i) a gradient, as specified in parentheses, between solvent A (0.05% TFA in water) and solvent B (0.05% TFA in acetonitrile). The flow rate was 1 ml/min, and elution of the samples was monitored by ultraviolet absorption at 214, 229 or 254 nm as indicated. Halogen analyses were carried out using the oxygen flask combustion technique followed by mercurimetric titration.

Methyl 3-(4'-methyl)phenyl-3-trimethylsilylpropanoate (4)

(1) Using hexamethylphosphoramide as complexing agent.

Methyl 4-methylcinnamate (3) (17.6 g, 100 mmol) was dissolved in THF (250 ml) and HMPA (54 g, 300 mmol). Magnesium turnings (3.6 g, 150 mmol) were added and the mixture was cooled with an ice bath under a nitrogen atmosphere. The flask was equipped with good mechanical stirring and a dropping funnel through which was cautiously added trimethylsilylchloride (33 g, 300 mmol). This must be done sufficiently quickly to initiate the reaction; however a violent exothermic reaction must be avoided as no product can be isolated subsequently. After addition of the halide was complete the mixture was maintained at reflux for 12 h. cooled and poured onto crushed ice. The aqueous layer and residues were extracted with several 50 ml portions of ethyl acetate, the organic layer was dried ($MgSO_4$) and evaporated to a brown oil. At this stage residual starting material crystallised out, and was removed by filtration. The remaining oil was purified by flash chromatography on silica gel, eluting with petrol and ether (4:1) to give the *title compound* (4) as a pale yellow oil (20 g, 80%); ν_{max} (thin film) 2960, 1740, 1435 and 1250 cm^{-1}, δ (200 MHz $CDCl_3$) -0.05 (9H, s, $SiCH_3$), 2.29 (3H, s, $ArCH_3$), 2.70 (3H, m, CH_2CO_2, t, $SiCH$), 3.55 (3H, s, OCH_3–, 6.85-7.15 (4H, d of d, Ar), *m/z* 250 (M^+) HRMS 250.1389; $C_{14}H_{22}O_2Si$ requires 250.13890.

(2) Using N-alkyl ureas as complexing agents.

Substitution of tetramethylurea for HMPA failed to give any product. Both dimethylpropylene urea (DMPU) and dimethylethylene urea (DMEU) facilitated the reaction; the former gave a less pure product and therefore the procedure using DMEU is given.

Powdered magnesium (8.8 g, 365 mmol) was suspended in dry THF (350 ml) along with 1,3-dimethyl-2-imidazolidinone (DMEU) (41.8 g, 600 mmol) in a 3-necked 3 L flask equipped with good mechanical stirring, a condenser and thermometer. Trimethylsilyl chloride (79.5 g, 730 mol) was added cautiously and the contents were heated to 50°C, over a water bath, in an atmosphere of nitrogen. At this stage no reaction was apparent. Ester (1) (43.0 g, 245 mmol) dissolved in THF (50 ml) was added slowly over a period of 1 h, not allowing the temperature to rise above 53°C. When the addition was complete the temperature was raised to 65°C for 4 h. After cooling the mixture was poured onto ice and extracted with diethyl ether

(2 x 500 ml). The organic layer was dried ($MgSO_4$) and evaporated to a small volume, whereupon a crystalline material was formed which was filtered off, washed with a little ether and dried. This by-product (~4 g, <10%) proved to be dimethyl 3,4-di(4'-methylphenyl)-hexane-1,6-dioate; m.p. 148-9°C; (Found: C, 74.5; H. 7.4, $C_{22}H_{26}O_4$ requires C, 74.6; H, 7.3%); δ_H (80 MHz, $CDCl_3$) 2.30 (6H, s, ArCH_3), 2.42 (4H, m, CH_2CO), 3.22 (2H, m, ArC*H*), 3.48 (6H, s, OCH_3), 7.25 (8H, m, Ar); *m/z* 354 (M^+), 323, 280. The remaining oil (44.3 g, 72%) was either purified by dry flash chromatography prior to further reaction (Silica gel 60 H; Merck 7736, gradient from hexane to ether), or preferably distilled under vacuum. Collection of the fractions b.p. 100-114°C (0.45 mmHg) gave the *title compound* (4) as a very low melting point solid/pale yellow liquid (17.7 g, 29%); m.p. 14-15°C (other data, *vide supra*).

Methyl 3-[(4'-bromomethyl)phenyl]-3-trimethysilylpropanoate (5)

The above silane (4) (5 g, 20 mmol) was dissolved in carbon tetrachloride (50 ml). N-Bromosuccinimide (3.9 g, 22 mmol) and dibenzoylperoxide (~50 mg) were added and the suspension was heated under reflux with concomitant irradiation from a tungsten lamp (500 w) during 1 h, or until no more succinimide floated to the surface. After cooling the solid was removed by filtration and discarded, the filtrate was evaporated to an oil, taken up in ethyl acetate (50 ml) and washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried ($MgSO_4$) and evaporated to an oil which was covered with a layer of petrol and chilled. The crystals which formed were filtered off; they appeared to be methyl 2-bromo-3-[(4'-bromomethyl)phenyl]-3-trimethylsilylpropanoate (1.41 g); m.p. 91-3°C, δ_H ($CDCl_3$, 200 MHz), 0.01 (9H, s, $SiCH_3$), 3.26, 3.65 (2 x 1H, 2 x d, SiC*H*, C*H*BrCO), 3.61 (3H, s, OCH), 4.48 (2H, s, $-CH_2Br$), 7.25-7.45 (4H, m, Ar); *m/z* (M^+) 406. Evaporation of the solvent gave the *title compound* (5) as a yellow oil (3.95 g, 60%); ν_{max} (thin film) 2960, 1740, 1515, 1440, 1250, 840 cm^{-1}, δ_H ($CDCl_3$, 200 MHz) -0.05 (9H, s, $SiCH_3$), 2.73 (3H, m, SiC*H* and CH_2CO_2), 3.55 (3H, s, OCH_3), 4.48 (2H, s, CH_2Br), 6.95-7.35 (4H, d of d, Ar); *m/z* 329 (M^+) HRMS 328.0496; $C_{14}H_{21}BrO_2Si$ requires 328.04946.

Methyl 3-[(4'-acetoxymethyl)phenyl]-3-trimethylsilylpropanoate (6)

The above bromide (5) (1.0 g, 3 mmol) was dissolved in dry DMF (20 ml) in which was suspended anhydrous sodium acetate (0.27 g, 3.3 mmol). The mixture was stirred and heated to 100°C, and maintained at that temperature for 4 h. After cooling the solution was poured into water (50 ml) and the precipitated solid was separated by decantation of the supernatent, which was discarded. The solid residue was extracted into ethyl acetate (50 ml) dried ($MgSO_4$) and evaporated to give the *title compound* (6) as an oil (0.57 g, 61%); after subsequent solidification m.p. 45-7°C. (Found: C, 62.35; H, 7.95, $C_{16}H_{24}O_4Si$ requires C, 62.34; H, 7.79%); ν_{max} (thin film) 2960, 1735, 1235 cm^{-1}, δ_H ($CDCl_3$, 200 MHz) -0.05 (9H, s, $SiCH_3$), 2.08 (3H, s, CH_3CO), 2.67 (3H, m, $CHCO_2$ and SiCH), 3.50 (3H, s, OCH_3), 5.03 (2H, s, OCH_2Ar), 7.00-7.25 (4H, d of d, Ar), *m/z* 308 (M^+), 293, 176, 134. A modest improvement in yield (68%) is possible if the bromide is heated under reflux with potassium acetate (1.02 equiv.) and 18-crown-6 (0.04 equiv.) in acetonitrile (10 ml $mmol^{-1}$) for 0.5 h. Isolation of the compound in this case is by evaporation of the solvent and filtration of the residue, dissolved in carbon tetrachloride, through silica gel.

3-[(4'-Hydroxymethyl)phenyl]-3-trimethylsilylpropanoic acid (1)

The above diester (6) (24.3 g, 79 mmol) was dissolved in dioxan (100 ml) and stirred with sodium hydroxide solution (12.6 g, 100 ml H_2O) during 24 h. The dioxan was removed by evaporation under reduced pressure, along with some of the water. The remaining solution was adjusted to pH3 with sulphuric acid (2 M) and extracted with ethyl acetate (250 ml). The organic solution was washed with water (X3) dried ($MgSO_4$) and evaporated to an oil. Dry flash chromatography (silica gel 60 H, ether), followed by evaporation of the solvent gave a pale yellow solid. This was recrystallised from ethyl acetate and petrol (40-60) to give the *title compound* (1) (10.9 g, 55%); m.p. 123-4.5°C; (Found: C, 61.8; H, 8.0. $C_{13}H_{20}O_3Si$ requires C, 61.9; H, 8.0%); ν_{max} (CH_2Cl_2) 3600 (OH), 3550-2500 (COOH), 1710 cm^{-1}, λ_{max} 278 (ϵ = 423), 269 (554), 227 nm (14,152); δ_H (800 MHz, $(CD_3)_2CO$), -0.05 (9H, s, $SiCH_3$), 2.65-2.80 (3H, m, $SiCHCH_2$), 4.57 (2H, s, $ArCH_2$), 6.0 (2H, bs, CO_2H OH), 6.98 (2H, d, ArH), 7.20 (2H, d, ArH); δ_C (20 MHz, $(CD_3)_2CO$), -3.5 ($Si(CH_3)_3$), 32.3 (SiCH), 34.2 ($SiCHCH_2$), 63.9 (Ar CH_2), 126.8 (Ar(2),(6)), 127.4 (Ar(3),(5)), 139.0 (Ar(4)), 141.8 (Ar(1)), 173.8 (CO_2H), *m/z* (M^+, 7.7%), 162(20), 134(100), 120(29), 117(92).

Methyl 3-[(4'-methyl)phenyl]-3-phenyldimethylsilylpropanoate (7)

Phenyldimethylsilyl chloride (1.85 g, 11 mmol) was added dropwise to a flask containing lithium (980 mg, 140 mmol) in THF (100 ml), the reaction was stirred at 20°C for 18 h under nitrogen and then cooled to -25°C. This solution was transferred slowly to another flask containing copper(I) iodide (1.03 g, 5 mmol) also at -25°C. The suspension was stirred at this temperature for a further 4 h to ensure formation of the silyl-cuprate was complete. Methyl p-methylcinnamate (0.95 g, 5 mmol) dissolved in dry THF (3 ml) was added and the reaction was stirred for 0.75 h at -25°C. After being brought to room temperature the solution was poured onto ice (150 g), acidified with HCl (2 M, 50 ml) and extracted with $CHCl_3$ (3 x 200 ml). The organic layer was washed successively with 200 ml portions of HCl (2 M), water, saturated aqueous $NaHCO_3$ and water, then dried ($MgSO_4$) and evaporated *in vacuo* to a yellow liquid. Dry flash chromatography (silica gel 60 H, hexane to ether gradient) gave the *title compound* (7) as a colourless liquid (1.13 g, 66%); ν_{max} (thin film) 3100-2860, 1740, 1430, 1250, 1165 cm^{-1}; δ_H (800 MHz, $CDCl_3$), -0.05, 0.00 (6H, 2 x s, $Si(CH_3)_2$, 2.30 (3H, s, $ArCH_3$), 2.56-2.88 (3H, m, $SiCHCH_2$), 3.48 (3H, s, CO_2CH_3), 6.75-7.13 (4H, 2 x d, $ArCH_3$), 7.38 (5H, s, Ph); *m/z* 312 (M^+, 60%), 118(100); HRMS 312.1538; $C_{19}H_{24}O_2Si$ requires 312.1545.

Methyl 3-[(4'-bromomethyl)phenyl]-3-phenyldimethylsilylpropanoate (9)

Ester (7) (169 mg, 0.86 mmol) was dissolved in carbon tetrachloride (20 ml), together with N-bromosuccinimide (169 mg, 0.95 mmol) and a little dibenzoylperoxide (~10 mg) as initiator. The mixture was heated under reflux, and illuminated by a tungsten lamp (500 W) during 2 h. After removal of the resulting succinimide the solvent was removed under vacuum and the residue was dissolved in ethyl acetate (15 ml). This solution was washed with successive 15 ml portions of water, $NaHCO_3$ (sat) and brine, dried ($MgSO_4$) and evaporated to give an orange oil. Dry flash chromatography (silica gel 60 H, gradient from hexane to ether) gave the *title compound* (9) as a colourless oil (316 mg, 94%); ν_{max} (thin film) 3100-2840, 1740, 1510, 1430, 1255, 1170 cm^{-1}; δ_H (80 MHz, $CDCl_3$), 0.22, 0.28 (6H, 2 x s, $SiCH_3$), 2.60-2.92 (3H, m, $SiCHCH_2$), 3.48 (3H, s, CO_2CH_3), 4.48 (2H, s, CH_2Br), 6.85-7.30 (4H, m, Ar), 7.35 (5H, s, Ph), *m/z* 392 (M^+, 20%), 311(30), 83(100); HRMS 390.0648; $C_{19}H_{23}O_2SiBr$ requires 390.0651.

Methyl 3-[(4'-acetoxymethyl)phenyl]-3-phenyldimethylsilylpropanoate (11)

Bromide (9) (316 mg, 0.81 mmol) and sodium acetate (73 mg, 0.89 mmol) were dissolved in dry DMF and heated at 100°C during 4 h. The solution was cooled and diluted with sufficient water to form a precipitate. This was extracted into ethyl acetate (2 x 20 ml), and the combined organic layer was washed with water (4 x 40 ml), dried ($MgSO_4$) and evaporated to give the crude material as an orange oil. Dry flash chromatography (silica gel 60 H, gradient from hexane to ether) gave the *title compound* (11) as a pale yellow oil (95 mg, 32%); ν_{max} (thin film) 2960, 1780, 1705 (weak), 1440, 1240 cm^{-1}; λ_{max} 226 nm, δ_H (80 MHz, $CDCl_3$) 0.24, 0.25 (6H, 2 x s, $SiCH_3$), 2.11 (3H, s, CH_3CO), 2.60-2.92 (3H, m, $SiCHCH_2$), 3.48 (3H, s, CO_2CH_3), 5.06 (2H, s, $ArCH_2O$), 6.80-7.26 (4H, d of d, Ar), 7.38 (5H, s, Ph); HRMS 370.16002, $C_{21}H_{26}O_4Si$ requires 370.16002.

The improved method used in the preparation of (6), namely potassium acetate and 18-crown-6 in acetonitrile, was not satisfactory.

A significant increase in yield (up to 83%) was obtained by using potassium acetate (2 equiv.) in acetic acid (20 ml $mmol^{-1}$) and heating under reflux for 3 h. Partition between ethyl acetate and water, washing of the organic layer with $NaHCO_3$ (sat. aq.) drying over $MgSO_4$ and evaporation of the solvent gave (11) as a pale yellow oil.

Methyl 3-[(4'-methyl)phenyl]-3-methyldiphenylsilylpropanoate (8)

The procedure used for the preparation of (7) was used to give the *title compound* (8) as an oil (72%); ν_{max} (thin film), 3090-2840, 1745, 1515, 1430 cm^{-1}; δ_H (200 MHz, $CDCl_3$), 0.45 (3H, s, $SiCH_3$), 2.28 (3H, s, $ArCH_3$), 2.76 (2H, d, $SiCHCH_2$), 3.25 (1H, t, $SiCH$), 3.48 (3H, s, OCH_3), 6.80, 6.98 (4H, 2 x d, Ar), 7.25-7.58 (10H, m, Ph); *m/z* 374 (M^+, 53%), 197(100), HRMS 374.1711; $C_{24}H_{26}O_2Si$ requires 374.17020.

Methyl 3-[(4'-bromomethyl)phenyl]-3-methyldiphenylsilylpropanoate (10)

The procedure used for the preparation of (9) was used; the reaction was complete after 0.5 h to give the *title compound* (10) as an orange oil (quantitative); ν_{max} 3090-2940, 1740, 1510, 1430 cm^{-1}; δ_H (200 MHz, $CDCl_3$), 0.42 (3H, s, $SiCH_3$), 2.77 (2H, d, $SiCHCH_2$), 3.28 (1H, t, $SiCH$), 4.43 (2H, s, CH_2Br), 6.85-7.16 (4H, 2 x d, Ar), 7.23-7.58 (10H, m, Ph); *m/z* 454 (MH^+, 5%), 374(25). 198(100); HRMS 452.0804; $C_{24}H_{25}BrO_2Si$ requires 452.08076.

Methyl 3-[(4'-acetoxymethyl)phenyl]-3-methyldiphenylsilylpropionate (12)

The procedure for preparing compound (11) using potassium acetate in acetic acid at reflux was used, and gave the *title compound* (12) as an oil (83%); ν_{max} (thin film) 3090-2840, 1740, 1430, 1230 cm^{-1}, λ_{max} 270 nm; δ_H (80 MHz, $CDCl_3$), 0.46 (3H, s, $SiCH_3$), 2.11 (3H, s, CH_3CO), 2.80 (2H, d, $SiCHCH_2$), 3.32 (1H, t, $SiCH$), 3.48 (3H, s, CO_2CH_3), 5.05 (2H, s, OCH_2), 6.88, 7.15 (4H, 2 x d, Ar), 7.25-7.62 (10H, m, Ph); *m/z* 432 (4%), 373(4), 151(100), HRMS 432.17569, $C_{26}H_{28}O_4Si$ requires 432.17567.

Stability studies of the series (Me_3), (Me_2Ph), ($MePh_2$); (6), (11) and (12) respectively.

The silyl derivative under examination (1 mg) was dissolved in the appropriate solvent (each of DCM, CH_3CN, DMF) (10 ml) to give a stock solution. Aliquots of 0.5 ml were diluted to 10 ml and the fluoride reagent was introduced. Formation of the cinnamic acid derivative was monitored by calibrated U.V. absorption at 289 nm.

General degradation of the materials was followed on the 20 mg scale by infra red and n.m.r. at 80 MHz, and by t.l.c. (generally employing ether/hexane mixtures). Formal identification of the products of reaction with TBAF of both (11) and (12) were carried out on the 50-100 mg scale by isolation and chromatography. Isolated yields of the cinnamate (3) were 66% and 87% respectively.

Phenyl 3-[(4'-hydroxymethyl)phenyl]-3-trimethylsilylpropanoylglycinate (13)

To a solution of glycine phenyl ester tosylate salt (13.28 g, 41.1 mmol) in dichloromethane (160 ml) and DMF (80 ml) at 0°C was added acid (1) (10.16 g, 40.3 mmol) in DCM (48 ml) and DMF (12 ml) also at 0°C. Dicyclohexylcarbodiimide (8.72 g, 42.3 mmol) in DCM (40 ml) and N-methyl morpholine (4.65 ml, 42.4 mmol) were added successively at 5 min intervals. The reaction was then allowed to warm to room temperature and stirred for 12 h. Precipitated DCU was removed by filtration, the solvents were removed *in vacuo* and the residue was taken up in ethyl acetate. Any further precipitate of DCU was removed and discarded and the solution was washed successively with citric acid (5% aq, x 2) $NaHCO_3$ (sat, x 2), water and brine, dried ($MgSO_4$) and evaporated. The resulting yellow oil was purified by dry flash chromatography (silica gel 60 H, gradient from hexane to ether) to give the *title compound* (13) as a white powder (10.09 g, 65%); m.p. 79-81°C; (Found C, 65.75; H, 7.3; N, 3.7, $C_{21}H_{27}NO_4Si$ requires C, 65.4; H, 7.0; N, 3.6%); ν_{max} (CH_2Cl_2) 3610, 3440, 2960, 2900, 2880, 1765, 1675, 1510, 865, 845 cm^{-1}; δ_H (80 MHz, $CDCl_3$), 7.36-6.91 (9H, m, Ar), 6.04 (1H, bs, NH), 4.54 (2H, s, $-CH_2-O$), 4.08 (2H, d, αCH_2), 2.67 (4H, m, O*H*, Si*CHCH*$_2$), -0.05 (9H, s, $SiCH_3$); *m/z* 385 (M^+), 368, 292, 248, 205, 117, 73.

Phenyl 3-[4'-(N^{α}-9''-fluorenylmethoxycarbonylleucyloxymethyl)-phenyl]-3-trimethylsilylpropanoylglycinate (14). Fmoc.Leu.(1).Gly OPh.

To a solution of alcohol (13) (1.93 g, 5 mmol) in dry DCM (25 ml) was added Fmoc.Leu.OH (1.77 g, 5 mmol) in dry DMF (5 ml). After cooling to 0°C a solution of DCCI (1.04 g, 5 mmol) in DCM (5 ml) together with a catalytic amount of DMAP (5 mg) was added and the reaction was stirred at 0°C for 20 min. The temperature was allowed to rise to room temperature (~20°C) and the reaction was stirred for a further 2 h. Cooling to -20°C precipitated DCU which was removed by filtration; the solvent was removed *in vacuo* and the residue taken up in ethyl acetate (100 ml). This solution was washed with successive 20 ml portions of citric acid (5% aq, X3), water (X3) and brine (X3). Drying ($MgSO_4$) and evaporation gave the *title compound* (14) (3.38 g, 93%) as a foam; (C, 69.8; H, 6.7; N, 4.0, $C_{42}H_{48}N_2O_7Si$ requires C, 70.0; H, 6.7; N, 3.9%); ν_{max} (CH_2Cl_2) 3438, 1765, 1730, 1680, 1195, 1168, 840 cm^{-1}; λ_{max} 221 (ϵ = 28,200), 228(21,000), 258(16,380), 263(17,880), 266(18,180), 277(11,400); δ_H (200 MHz, $CDCl_3$), 7.80-6.95 (17H, m, FmocH, ArH, Ph), 5.93 (1H, t, GlyNH), 5.23 (1H, d, LeuNH), 5.09 (2H, s, OCH_2), 4.38 (3H, m, Fmoc *CHCH*$_2$), 4.25 (1H, m. LeuαCH), 4.15 (2H, m, GlyCH_2), 2.70 (3H, m, Si *CHCH*$_2$), 1.62 (3H, m,

LeuβCH_2, γCH), 0.90 (6H, m, 2 x LeuCH_3), -0.06 (9H, s, SiCH_3); δ_C (50 MHz, $CDCl_3$), 172.8(CO), 158.4(CO), 150.0(*ipso* OPh), 143.9, 143.7, 142.7, 141.2, 132.0, 129.4, 128.4, 127.6, 127.3, 127.0, 126.0, 125.0, 121.1, 119.9 (aromatic), 66.9 (Fmoc CH_2, CH_2Ar), 52.6 (LeuαC), 47.2 (Fmoc CH), 41.7 (GlyαC), 41.4 (LeuβC), 36.5 (Si$CHCH_2$), 32.6 (Si$CHCH_2$), 24.6 (LeuγC), 22.6 (LeuCH_3), 21.8 (LeuCH_3), -3.2 (SiCH_3); *m/z* 721 (MH^+), 742, 704, 628, 557, 498, 456, 368, 191, 177, 144, 131, 105, 91, 73; HRMS 721.3309, $C_{42}H_{48}N_2O_7Si$ requires 721.32641.

3-[4'-(N^{α}-Fluorenylmethoxycarbonylleucyloxymethyl)-phenyl]-3-trimethylsilylpropanoylglycine (15)

The phenyl ester (14) (3.18 g, 4.5 mmol) was dissolved in dioxan (20% aq., 8 ml) and adjusted to pH 10.5 with NaOH (1 M). Hydrogen peroxide (100 vol; 0.5 ml) was added and the pH was maintained at 10.5 by automatic titration of more hydroxide. After 16 min the reaction was complete. The pH was adjusted to 6.5 with citric acid (5% aq.), the dioxan was removed *in vacuo* and the pH of the remaining solution adjusted to 3.0 with more citric acid. The suspension was extracted into ethyl acetate (100 ml), washed with citric acid (2 x 25 ml) and brine (2 x 15 ml) and the organic layer was separated and dried ($MgSO_4$). Evaporation of the solvent gave the *title compound* (15) as a white foam (1.91 g, 67%); (Found: C, 66.7; H, 6.8; N, 4.4. $C_{36}H_{44}N_2O_7Si$ requires C, 67.05; H, 6.9; N, 4.3%); ν_{max} (CH_2Cl_2) 3412, 1730, 1665, 1215, 840 cm^{-1}; λ_{max} 221 (ϵ = 22,024), 228(16,915), 258(14,296), 263(15,370), 266(15,670), 276(10,175) nm; δ_H (200 MHz, $CDCl_3$), 7.80-7.00 (12H, m, FmocH, ArH), 5.75 (1H, m, GlyNH), 5.33 (1H, m, LeuNH), 5.06, 4.95 (2H, 2 x d, ArCH_2), 4.45 (3H, m, Fmoc$CHCH_2$), 4.33 (1H, m, LeuαCH), 3.80 (2H, m, GlyCH_2), 2.65 (3H, m, Si$CHCH_2$), 1.60 (3H, m, LeuγCH, LeuβCH_2), 0.93 (3H, d, Leu CH_3), 0.84 (3H, d, LeuCH_3), -0.04 (9H, s, SiCH_3); *m/z* 667 (MH^+), 445, 422, 403, 191, 179, 165; HRMS 644.2915; $C_{36}H_{44}N_2O_7Si$ requires 644.29176.

3-[4'(N^{α}-Fluorenylmethoxycarbonylleucyloxymethyl)-phenyl]-3-trimethylsilylpropanoylglycylaminomethyl resin (16), Fmoc.Leu.(1).Gly.NH.CH_2-Ph-[R]

To a solution of acid (15) (0.95 g, 1.5 mmol) in dry DCM (5 ml) was added HOBT (0.20 g, 1.5 mmol) in dry DMF (2 ml) at 0°C. DCCI (0.31 g, 1.5 mmol) in DCM (5 ml) was added to the reaction which was stored for 1 min. at 0°C and then 30 min. at room temperature. Removal of the precipitated DCU by filtration gave the HOBT ester of (15) in solution. Aminomethyl resin (1.0 g, 0.75 mmol) was swollen in DCM (20 ml) and agitated for 30 min. on the automated synthesiser with the above solution of active ester, washed with DCM and drained to give (16) ready for further reaction.

N^{α}-Diphenylphosphinylleucylvalylglycylphenylalanylalanylleucine (17), Dpp.Leu.Val.Gly.Phe.Ala.Leu.OH

The peptide was prepared from (16) using the following solid phase cycle: (1) DCM, 2 x 1 min; (2) 20% piperidine/DCM 1 x 12 min; (3) DCM, 3 x 1 min; (4) DMF, 2 x 1 min; (5) DCM, 3 x 1 min; (6) coupling Fmoc-amino acid Dpp-mixed anhdyrides, 1 x 30 min; (7) coupling Dpp.Leu.Dpp mixed anhydride, 1 x 30 min; (8) DCM, 3 x 1 min; (9) DMF, 2 x 1 min; (10) DCM, 3 x 1 min. Samples of resin were taken after step (6) and subjected to both the Kaiser test and amino acid analysis. Each coupling employed two equivalents (1.5 mmol) of activated amino acid and 2,6-lutidine (1.5 mmol) with respect to the amino resin. With the

exception of Fmoc-Val-OH all gave negative Kaiser results after 30 min. In the case of Fmoc-Val-OH reacylation was required with an additional equivalent (0.75 mmol) of Fmoc.Val.ODpp and 2,6-lutidine to give complete coupling after 1 h.

Cleavage of the peptide from the support was mediated by anhydrous TBAF (236 mg, 0.75 mmol) in dry DMF (20 ml). This was carried out four times as indicated in the cycle: (1) DMF, 3 x 1 min; (2) 1st TBAF, 1 x 1 min; (3) DMF, 2 x 1 min; (4) 2nd TABF, 1 x 2 min; (5) DMF< 2 x 1 min; (6) 3rd TBAF, 1 x 4 min; (7) DMF, 2 x 1 min; (8) 4th TBAF, 1 x 20 min; (9) DMF, 2 x 1 min. Each of the peptide containing washings in steps 3, 5, 7 and 9 were treated separately on work up. To each was added distilled water (10 ml) and the solvent removed *in vacuo* leaving a yellow oil. Addition of citric acid (5% aq., 10 ml) produced a white gum which was filtered and washed with distilled water. The quantities obtained were as follows: 1st cleavage (232 mg), 2nd cleavage (219 mg), 3rd cleavage (118 mg) and 4th cleavage (6 mg).

Purification of the crude peptides was *via* preparative h.p.l.c. using a Hypersil C18 reverse-phase column and acetonitrile/water as the eluent. Following lyophilisation of the hplc fractions the pure peptide (17) was obtained as a white solid (62% combined cleavage yield); $[\alpha]_D^{20}$ = -38°C (C=1 MeOH); ν_{max} (KBr) 3300 (NH), 1690 (C=O), 1630 (amide C=O) and 720 cm^{-1} (Ar); δ_H (360 MHz, $(CD_3)_2SO$), 12.52 (1H, bs, Leu CO_2H), 8.64 (1H, t, Gly NH). 8.14 (1H, d, Ala NH), 7.97 (1H, d, Leu NH), 7.96 (1H, d, Phe NH), 7.82-7.76 (5H, m, Dpp, Val NH), 7.58-7.45 (6H, m, Dpp), 7.22-7.13 (5H, m, Ph), 5.81 (1H, dd, Leu NH), 4.55 (1H, dt, Phe $^{\alpha}CH$), 4.30 (1H, dq, Ala $^{\alpha}CH$), 4.22 (1H, dt, Leu $^{\alpha}CH$), 4.12 (1H, dd, Val $^{\alpha}CH$), 3.80 (1H, dd, Gly $^{\alpha}CH$), 3.64 (1H, dd, Gly $^{\alpha}CH_2$), 3.32 (1H, m, Leu $^{\alpha}CH$), 3.02 (1H, dd, Phe βCH_2), 2.74 (1H, dd, Phe βCH_2), 2.09 (1H, m, Val βCH), 1.85 (1H, m, Leu γCH), 1.65-1.43 (5H, m, Leu γCH), 2 x Leu βCH_2), 1.21 (3H, d, Ala CH_3), 0.90-0.82 (15H, m, 3 x Leu CH_3, 2 x Val cH_3), 0.71 (3H, d, Leu CH_3); δ_P (24.2 MHz, $(CD_3)_2SO$) 21.93; m/z (820 (MH^+), 819, 688, 617, 579, 506, 385, 286, 218, 201, 141, 91 and 58; HRMS MH^+ 819.4210 Calc. for $C_{43}H_{59}N_6O_8P$ 819.4200; hplc H_2O:MeCN (50:50)-(0:100) (25 min), retention time 10.0 min.

Phenyl 3-[(2',2'-Bis*(4''-nitrophenyl)ethoxycarbonyl]-glycyloxymethyl)-phenyl-3-trimethylsilylpropanoylglycinate* (19), Bnpeoc.Gly. (1). Gly.OPh.

A solution of Bnpeoc.Gly.OH (9.67 g, 24.9 mmol) in DCM (80 ml) and DMF (20 ml) at 0°C was added to a solution of (15) (8.69 g, 22.6 mmol) in DCM (20 ml) cooled to 0°C. DCCI (5.00 g, 24.9 mmol) in DCM (20 ml) cooled to 0°C was then added after 5 min. followed by a catalytic amount of DMAP. The reaction mixture was left stirring at room temperature for 12 h. after which time, the precipitated DCU was filtered off, the filtrate was washed with citric acid (5% aq. x 2), sodium bicarbonate solution (x 2) water and brine, dried and evaporated to give the crude material as a yellow oil. This was purified by dry flash chromatography (x 2) (silica gel 60 H, hexane - ether ethyl acetate gradient) to give the *title compound* (19) as a white powder (14.92 g, 87%); m.p. 68-71°C; (Found: C, 59.9; H, 5.54; N, 7.11; $C_{38}H_{40}N_4O_{11}Si$ requires C, 60.3; H, 5.33; N, 7.40%); ν_{max} (CH_2Cl_2) 3450 (N-H), 2970, 2860 (aliphatic C-H), 1735 (urethane, benzyl ester, phenyl ester C=O), 1680 (amide C=O), 1525 (urethane, amide N-H; NO_2), 1350 (NO_2), 865 845 cm^{-1}, (aromatic C-H); λ_{max} (CH_3CN), 274 nm (ϵ = 34483), 230 (19655), 220 (19655); δ_H (200 MHz, $CDCl_3$) 8.11-6.91 (17H, m, Ar), 6.50 (1H, bs, (8) NH), 5.57 (1H, broad s, Bnpeoc Gly NH), 4.99 (2H, s, benzyl CH_2), 4.64-4.56 (3H, m, Bnpeoc CH, CH_2), 4.07-4.02 (2H, m, (8) Gly-α-CH_2), 3.83 (2H, d, J 5.3 Hz, Bnpeoc-Gly-α-CH_2), 2.68-2.60 (3H, m, Si CH-CH_2), -0.09 (9H, s, $SiCH_3$); δ_C (50 MHz, $CDCl_3$), 172.7 (amide C=O),

169.5 (Bnpeoc Gly C=O), 168.4 (phenyl ester C=O), 155.9 (Bnpeoc C=O), 150.2 (phenyl ester quat. Ar), 147.0 (Bnpeoc quat. Ar (X4)), 143.0 (8) C-1), 131.5 (8) C-4), 129.1-121.1 (aromatic CH (X17)), 66.8 (benzyl CH_2), 66.0 (Bnpeoc CH_2), 49.6 (Bnpeoc CH), 42.6 (Bnpeoc Gly CH_2), 41.4 (8) Gly-α-CH_2), 36.0 (SiCHCH_2), 32.4 (SiCH), -3.2 ($SiCH_3$); m/z (FAB) 757, 742, 664, 603, 442, 307, 226, 117; HRMS 757.2541, $C_{38}H_{41}N_4O_{11}Si$ (MH^+) requires 757. 2541.

3-[2',2'-Bis(4''-nitrophenyl)ethoxycarbonyl]glycyloxymethyl-phenyl-3-trimethylsilylpropanoylglycine (20), Bnpeoc.Gly.(1).Gly.OH.

Hydrogen peroxide (100 vol; 740.0 μl, 6.64 mmol) followed by a 0.1M solution of sodium hydroxide (32.90 ml, 3.31 mmol) was added to a solution of phenyl ester (19) (2.5 g, 3.31 mmol) in acetone (80% aq., 250 ml), and the reaction mixture was stirred at room temperature for 15 min. The reaction mixture was then diluted with water (150 ml) and the acetone was removed *in vacuo*. The phenol formed was removed by ether extraction, and the remaining aqueous solution was acidified to pH 2-3 with citric acid (sat. aq.) and then extracted with ethyl acetate. The organic layer was separated washed with water (x2) and brine (x2), dried ($MgSO_4$) and evaporated. Gel filtration on Sephadex LH20 eluting with methanol, followed by dry flash chromatography (silica gel 60H, ethyl acetate to methanol gradient with approximately 25 mm of silica) gave the *title compound* (20), after lyophilisation, as a white powder (1.825 g, 81%); m.p. 105-107°C; (Found: C, 56.2; H, 5.44; N, 7.86; $C_{32}H_{36}N_{11}Si$ requires C, 56.5; H, 5.33; N, 8.23%); ν_{max} (CH_2Cl_2) 3450 (N-H), 3400-2500 (O-H, aliphatic C-H), 1730 (urethane, ester, acid C=O), 1660 (amide C=O), 1525 (urethane, amide N-H; NO_2), 1350 (NO_2), 860 845 cm^{-1} (aromatic C-H); λ_{max} (CH_3CN) 274 nm (ϵ = 33333), 230 (18027), 220 (16667); δ_H (200 MHz, $CDCl_3$), 8.14 (4H, d, Bnpeoc Ar), 7.37 (4H, d, Bnpeoc Ar), 7.17 (2H, d, (8) Ar), 7.00 (2H, d, (8) Ar), 5.90 (1H, broad s, (8) Gly NH), 5.35 (1H, broad s, Bnpeoc-Gly NH), 5.05 (2H, s, benzyl CH_2), 4.66-4.57 (3H, m, Bnpeoc CH, CH_2), 3.87 (2H, d J 5.6 Hz, (8) Gly CH_2), 3.76 (2H, d, Bnpeoc-Gly CH_2), 2.66-2.57 (3H, m, Si CH, CH_2), -0.07 (9H, s, $SiCH_3$); δ_C (50 MHz, $CDCl_3$) 173.3 (acid C=O), 171.2 (amide C=O), 169.5 (Bnpeoc-Gly C=O), 155.8 (Bnpeoc C=O), 146.7, 146.6 (Bnpeoc quat. Ar (2X2)), 142.4 (8) C-1), 131.2 (8) C-4), 128.9, 123.5 (Bnpeoc Ar CH (2X4)), 128.1 (8) C-3, C-5), 127.1 (8) C-2, C-6), 66.5 (benzyl CH_2), 65.8 (Bnpeoc CH_2), 49.2 (Bnpeoc CH), 42.3 (Bnpeoc-Gly CH_2), 40.8 (8) Gly-α-CH_2), 35.6 (SiCH_2), 32.3 ($SiCH_2$), -3.6 ($SiCH_3$); m/z (FAB) 703, 681, 664, 527, 364, 308, 292, 278, 117; HRMS 681.2228, $C_{32}H_{37}N_4O_{11}Si$ (MH^+) requires 681.2228.

3-[4'-(2'',2''-Bis(4'''-nitrophenyl)ethoxycarbonyl)glycyloxymethyl)-phenyl]-3-trimethylsilylpropanoylglycylamino methyl resin (21), Bnpeoc.Gly. (1).Gly.NH.CH_2.Ph.[R]

To a solution of acid (20) (1.22 g, 1.80 mmol) in DCM (10 ml) was added a solution of HOBt (0.24 g, 1.80 mmol) in DMF (2 ml). This mixture was shaken for 2 min. after which time a solution of DCCI (0.37 g, 1.80 mmol) in DCM (5 ml) was added, and the resulting mixture shaken for a further 2 min. This solution was then added to aminomethyl resin (Peninsula; 3.00 g, 1.20 mmol) swollen in DCM (40 ml), and the reaction mixture was sonicated for 5 h, filtered, washed thoroughly with DMF then DCM, and dried. The coupling procedure was repeated twice in the same manner. Kaiser testing after the third coupling showed that complete functionalisation of the resin (3) had still not occurred, hence capping was performed by adding a solution of acetic anhydride (1.13 ml, 12.0 mmol) and triethylamine (1.67 ml, 12.0 mmol) in DCM (10 ml) to the product resin swollen in DCM (40 ml) and sonicating the mixture for 1 h. After

this time, Kaiser testing showed that capping was complete, thus the product (21) was washed thoroughly with DMF then DCM, and dried. Functionality = 0.167 mmol g^{-1}; ν_{max} (KBr disk) 3410, 3330 (n-H), 1735 (urethane, ester C=O), 1670 (amide C=O), 1520 (urethane, amide N-H; NO_2), 1345 (NO_2), 850 cm^{-1} (Ar C-H).

3-(4'-Glycyloxymethyl)phenyl-3-trimethylsilylpropanoylglycylamino-methyl resin (22), H.Gly.(1).Gly.NH.CH_2.Ph.[R]

Bnpeoc protected material (21) 3.73 g) was swollen in DMF (40 ml) and treated with a solution of DBU (267 μl, 1.78 mmol) and acetic acid (102 μl, 1.78 mmol) in DMF (10 ml). The mixture was sonicated for 2 h. filtered, washed with DMF then DCM, and dried to give the *title material* (23). Functionality 0.15 mmol g^{-1} by quantitative Kaiser testing; ν_{max} (KBr) 3410, 3310 (NH), 1745 (ester CO), 1665 (amide CO), 1515 (CO*NH*), 850 cm^{-1} (Ar).

per-(Bu^t-protected) Ubiquitin (1-35) (24).

Linker-extended amino resin (22) (1.50 g, 0.25 mmol) was placed in the reaction vessel of the automated peptide synthesiser. All the transient N^{α}-protection used was base-labile. The N-terminal methionine was Boc-protected; side-chain protection was as follows - Lys (Boc); Glu, Asp, Thr, Ser (t-Bu). Each residue was double coupled: firstly by the symmetrical anhydride method and, secondly, by the DIC/HOBt method except in the following cases. Single coupling only was employed for Fmoc.Gly.OH, the second HOBt active ester coupling being omitted. For Fmoc.Asn.OH and Fmoc.Gln.OH, both couplings employed the HOBt active ester protocol with the first coupling lasting for 1 h. and the second coupling lasting for 90 min. Cleavage of the fully protected peptide from the resin was effected using seven tetrabutylammonium fluoride (TBAF) treatments of 5 min. duration - four with one equivalent followed by three with two equivalents of TBAF. After several selected couplings, small samples of resin-bound product (23) were removed for quantitative determination of the dibenzofulvene piperidine adduct by ultraviolet absorbance. Amino acid analyses of the resin were carried out after the fourth, fifth, sixth, and seventh TBAF treatments. The product obtained from TBAF treatments (I)-(V) was a white solid, whereas that from (VI) and (VII) was a yellow oil, the crude yields being as follows: (I) 67 mg (5%), (II) 91 mg (7%), (III) 107 mg (8%), (IV) 135 mg (10%), (V) 300 mg (23%), (VI) 197 mg (15%), (VII) 159 mg (12%). TBAF treatments (I)-(IV) were combined as were fractions and (V)-(VII) the two pools were purified separately by gel filtration on Sephadex LH20 eluting with DMF to give the fully protected peptide (24), after trituration with ether, as a white powder (624 mg, 48%); amino acid analysis: Asx_3 3.06, Thr_5 4.71, Ser_1 0.93, Glx_6 6.40, Pro_1 1.13, Gly_2 1.97, Ala_1 1.05, Val_3 2.95, Met_1 0.59, Ile_4 3.84, Leu_2 1.95, Phe_1 0.80, Lys_5 5.03; δ_H (360 MHz, $(CD_3)_2SO$), 1.50-0.82 (indeterminate H, m, t-Bu and Boc); m/z (FAB) HMRS 5179.1716, $C_{250}H_{436}N_{43}O_{69}S$ (MH^+) requires 5179.1716; HPLC (III) (isocratic-0.1% isopropanol in DCM, 254 nm) RT = 2.75 min.

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