Tetrahedron Vol. 47, No. 32, pp. 6353-6370, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

An Acid Labile Arginine Derivative for Peptide Synthesis: N^G-2,2,5,7,8-Pentamethylchroman-6-sulphonyl-L-arginine

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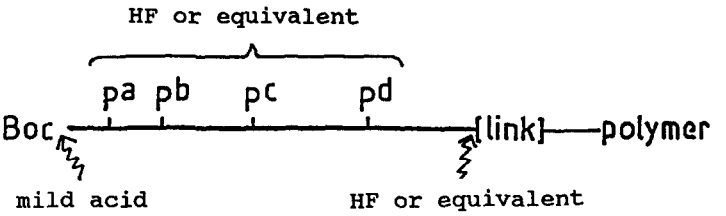
(*Received in UK* 30 *May* 1991)

Abstract

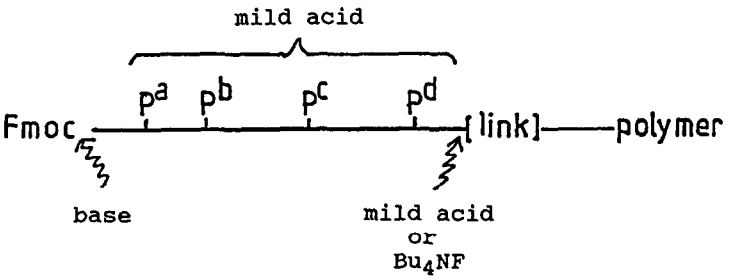
A trifluoroacetic acid (TFA) labile protecting group for the guanidine side chain function of arginine has been developed. N^G-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl-L-arginine is cleaved rapidly in TFA or 50% TFA in dichloromethane at room temperature. The preparations of Fmoc.Arg(Pmc).OH and Bnpeoc.Arg(Pmc).OH are described.

There are two main strategies for the synthesis of peptides according to the general concept of the Merrifield Solid Phase Peptide Synthesis (SPPS). The original protocol of Merrifield[1] employs activated N^{α}-t-butoxycarbonyl (Boc) amino acids for chain assembly with the consequent repetitive N^{α}-deprotection using mild acid (trifluoroacetic acid, TFA). It follows from this that the side chain protection strategy and design of the peptide linker attachment to the insoluble resin rely upon relatively more acid-stable groups which require strong acid (HF or CF_3SO_3H) or nucleophiles for final liberation of the peptide product (Scheme 1). With the introduction of the base- labile 9-fluorenylmethoxycarbonyl group (Fmoc) by Carpino[2] a complementary SPPS strategy emerged[3] in which N^{α}-Fmoc amino acids were incorporated in the chain assembly. A consequence of this change in N^{α}-protection to Fmoc, and the related Bnpeoc group,[4] is that the side chain protection and the peptide linker attachment to the resin may be cleaved by mild acid[5] (TFA) as illustrated in Scheme 2, thus limiting the acid treatment of the peptide products, many of which deteriorate at low pH on prolonged exposure. Furthermore recent studies on the design of linkers, which release the final *protected* peptide product by very mild acid[6] or fluoride ion,[7] add an extra dimension to the N^{α}-base-labile strategy which will undoubtedly be of great value in future syntheses of protected peptide fragments.

The majority of α-amino acid side chain protecting groups selected for the Fmoc strategy of SPPS are easily cleaved by TFA:H_2O (95:5) in *ca* 1 hour at room temperature, although the time for t-butyl ether deprotection of Ser and Thr residues may vary significantly according to sequence. For

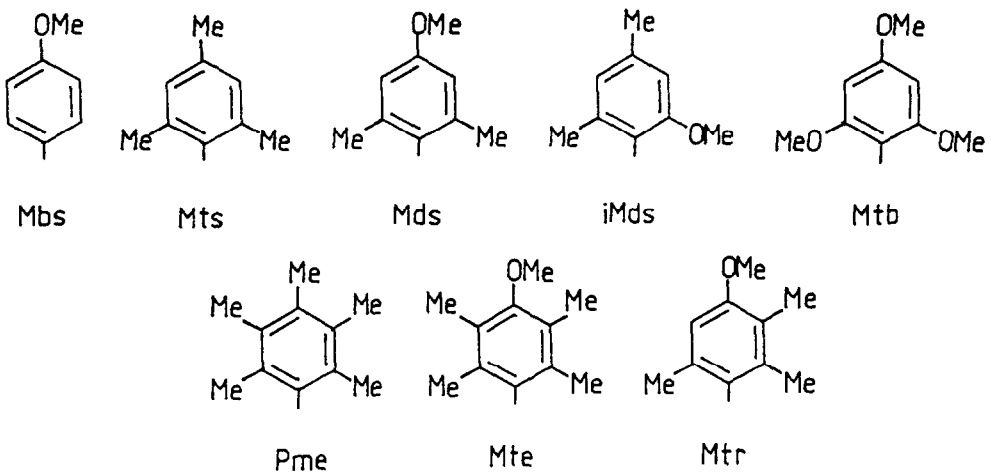


Scheme 1



Scheme 2

Aryl.SO_2- N^G-Protection of Arg



Scheme 3

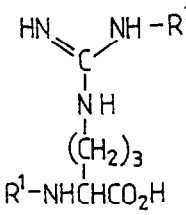
some time the guanidino group of arginine (Arg) (1) proved to be a problem, especially in sequences having many arginine residues, if the same mild acid conditions were set as constraints for guanidino protection/ deprotection of Arg.

Arginine is the most basic naturally occurring α-amino acid due to the resonance stabilised guanidinium cation (pK_1 1.82, pK_2 8.99 and pK_3 13.20). There have been four main approaches to the problem of Arg side chain protection of the guanidine group:

(1) preferential protonation,
(2) nitration,
(3) (di)urethane protection,
(4) arylsulphonyl protection.

Each of these options offers specific advantages and incurs disadvantages. At the onset of this work we sought to design a new N^G-Arg protecting group *with respect to use with the base-labile N^α protection strategy* for SPPS. The following criteria were considered essential:

(1) reagent availability,
(2) stability to basic conditions required for Fmoc deprotection,
(3) removal by TFA:H_2O (95:5) and TFA:CH_2Cl_2 (50:50) within 1 hour at room temperature.



1 $R^1 = R^2 = H$

2 R^1 = Fmoc ; R^2 = Mtr

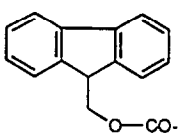
3 R^1 = Fmoc ; R^2 = Pmc

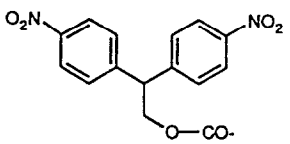
4 R^1 = Bnpeoc; R^2 = Pmc

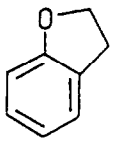
5 R^1 = Z ; R^2 = H

6 R^1 = Z ; R^2 = Pmc

7 R^1 = H ; R^2 = Pmc

Fmoc = 

Bnpeoc = 



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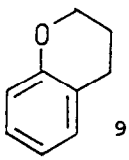

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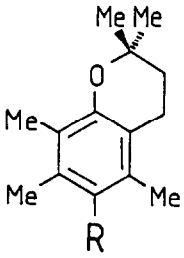


10 R = SO_2.Cl
11 R = SO_2.NHPh
12 R = H

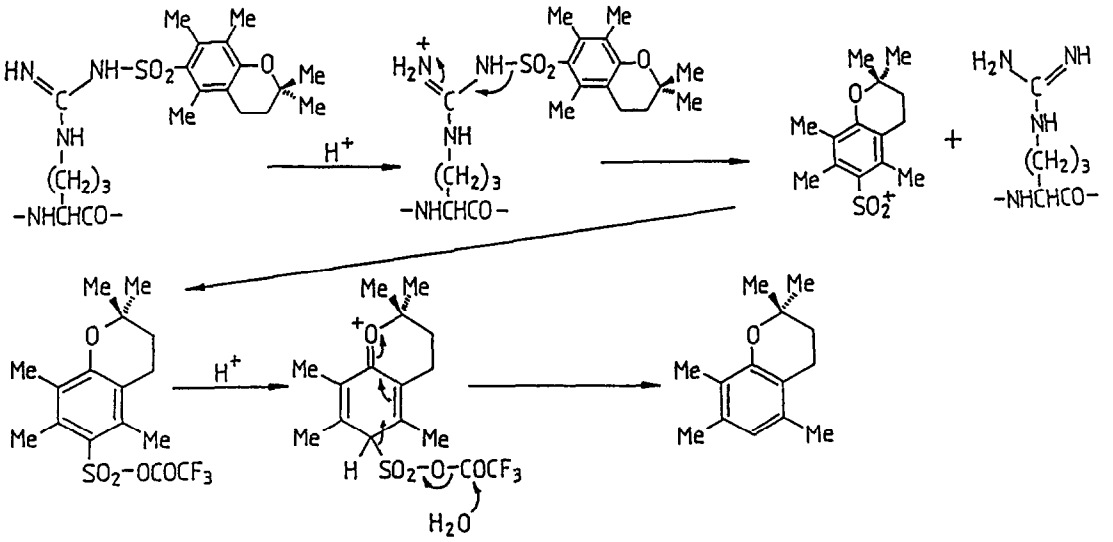
Design of Arylsulphonyl Groups for Guanidino Protection

The 4-toluenesulphonyl (Tos) group was originally introduced by Emil Fischer[8] and has since found wide use in peptide synthesis for the protection of α-amino functions, the side chain amino groups of lysine and

ornithine and, also, for the side chain functionalities of histidine, tryptophan and arginine. Although the N^G-Tos derivative of Arg is too acid-stable for application in the Fmoc protocol for SPPS, the benzene ring offers the opportunity for design changes to remedy this disadvantage. Attempts to increase the lability of N^G-arylsulphonyl protecting groups led Nishimura and Fujino[9] to examine the 4-methoxybenzenesulphonyl (Mbs) group (Scheme 3) in the syntheses of bradykinin and tuftsin when the final deprotections were achieved by methanesulphonic acid (MSA) in 40 min at room temperature. This represented a significant improvement over the N^G-Tos derivative. Yajima[10] and coworkers prepared and applied the mesitylene-2-sulphonyl (Mts) group in the synthesis of substance P when the final deprotection was achieved using MSA-anisole for 30 min at 0°C. An unfortunate consequence of the mechanism implicit in the acid-deprotection of such arylsulphonyl derivatives is that scavengers are required to minimise untoward side reactions of amino acid side chain functions with reactive intermediates produced during the deprotection reaction (Scheme 4). A comprehensive examination of the arylsulphonyl protection strategy was elegantly carried out by Fujino[11] in which a carefully selected series of multisubstituted N^G-arylsulphonyl Arg derivatives were compared. In addition two aspartyl peptides were studied representing another problem required to be overcome in peptide synthesis which is the $\alpha \rightarrow \beta$ rearrangement of Asp.X and Asn.X sequences under basic or acidic conditions. In this latter respect both MSA and HBr-HOAc can cause such side reactions which are minimised by use of TFA. The series of N^G-protecting groups shown in Scheme 3 were compared for acid stability in TFA-thioanisole (9:1) and from these results a further comparison of the five most acid labile groups in 100% TFA revealed the same order of lability; however much longer reaction times were required for complete cleavage. The N^G-protected derivatives were treated with TFA at 25°C for 1 hour and the percentage of regenerated Arg assessed by amino acid analysis to give the following results:

Mtr (52%) Mds (22.3%) Mtb (19.7%) Pme (2.0%) Mte (1.6%)

It was demonstrated that the Mtr group could be cleaved from the guanidino function by TFA within 4-6 hours at room temperature or in 1 hour in TFA-thioanisole (9:1) at the same temperature. The Mtr was therefore selected for use in N^G-protection of Arg and Fmoc.Arg(Mtr).OH (2) was accordingly made available commercially for peptide synthesis. Such protection of one Arg residue in a peptide sequence may be acceptable but when several Arg residues are present then the total deprotection of Mtr groups can be too slow under TFA conditions.



TFA Deprotection of N^G-Pmc Group

Scheme 4

Development of 2,2,5,7,8-Pentamethylchroman-6-sulphonyl N^G-Protection of Arginine

Consideration of the admirable results of Fujino clearly indicated that electron donation by inductive (effect of methyl substitution on the benzene ring) or resonance (effect of the 4-methoxy substituent) effects were important for acid lability of the N^G-Arg derivative. In a preliminary study we found that CF_3 substitution rendered the arylsulphonyl derivative very stable to acid. Comparison of the data for the Mbs and Mds groups suggested that the large increase in acid lability of the latter could not all be attributed to methyl inductive effects but was largely due to the steric buttressing effects of the C-methyl groups upon the conformation of the aryl-SO_2 bond. This could optimise the π aryl-d sulphone electronic transmission of substituent effects from the benzene ring and hence directly effect the rate of cleavage of the SO_2-N bond according to the mechanism in Scheme 4. The most significant comparison proved to be the relative rates of TFA cleavage of the Mtr and Mte groups where the introduction of one extra methyl group led to a 33 fold decrease in acid

lability. The obvious conclusion to be derived from this is that there is a conflict between the favourable CH_3 inductive effect and the steric implications of this additional substituent. It is known[12] that the methoxy groups of sterically unhindered anisoles are coplanar with the benzene ring with a CH_3-O-C(aryl) bond angle of 117-118° which would represent an sp^2 hybridised oxygen. This is the optimal situation with respect to transmission of the oxygen non bonding p-electrons to the aromatic π system, however in the case of the Mte group the flanking methyl groups prevent the CH_3-O-C(aryl) coplanarity.

From this analysis specific parameters may be incorporated into the design of a new arylsulphonyl reagent for protection of the guanidine function of Arg.

1. Maximum substitution of the benzene ring with electron donating substituents.
2. A 4-alkoxyl substitutent is favoured with control of stereochemistry to allow maximum p-π overlap.
3. Methyl substitution at 2- and 6-positions.

Two bicyclic systems (8) and (9) were first considered in the light of these criteria. A search of X-ray crystallographic data proved decisive in that for 2,3-dihydrofuran compounds (8) the C-O-C(aryl) bond angles varied between 106-111°, being typically 108° *ie*, consistent with an sp^3 hybridised oxygen. For the chroman system (9) the C-O-C(aryl) bond angle was typically 117°, indicating the desired sp^2 hybridised oxygen and thus optimal p-π interaction. Having taken the decision to progress with the chroman system we then took synthetic expediency into account and selected the reagent 2,2,5,7,8-pentamethylchroman-6-sulphonyl chloride (Pmc.Cl) (10) for N^G-protection of Arg. In order to confirm structural features of the design, (10) was converted to the crystalline anilide (11) which was subjected to X-ray structure elucidation. Crystals suitable for analysis by *X*-ray diffraction were grown from ethyl acetate/*n*-hexane.

Crystal Data:- $C_{20}H_{25}NO_3S$, M = 359.49, monoclinic, space group $P2_1/c$, a = 10.868(4), b = 9.271(5), c = 18.626(10)Å, β = 90.54(4)°, V = 1877Å^3 [from setting angles of 9 reflections with 2θ = 13-26°, λ = 0.71073Å], Z = 4, D_{calc} = 1.272 g cm^{-3}, T = 295K, colourless columnar crystal, 0.23 x 0.23 x 0.96 mm, μ = 0.18 mm^{-1}, F(000) = 768.

Data Collection and Processing:- Stoë STADI-4 four-circle diffractometer, graphite-monochromated Mo-K_α *X*-radiation, T = 295K, ω-2θ scans with ω scan width (0.8 + 0.347tanθ)°, 2736 data measured ($2\theta_{max}$ 45°, h -11 → 11, k 0 → 9, l 0 → 20), 2148 unique (R_{int} 0.040), giving 1225 reflections with $F > 4\sigma(F)$ for use in all calculations. No significant crystal decay or movement was observed.

Structure Solution and Refinement:- Automatic direct methods[13] located all non-H atoms, which were then refined (by least-squares on F[14]) with

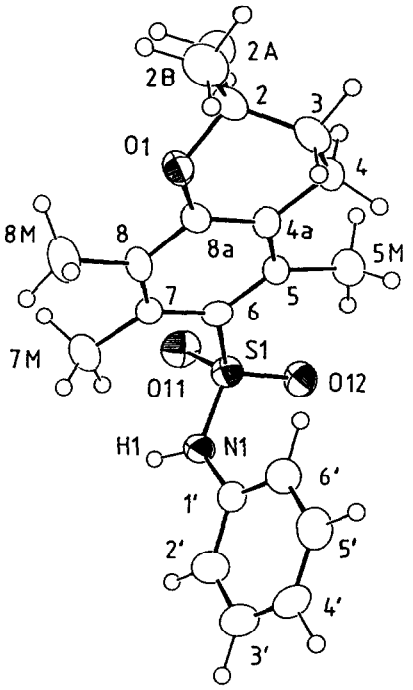


Figure 1

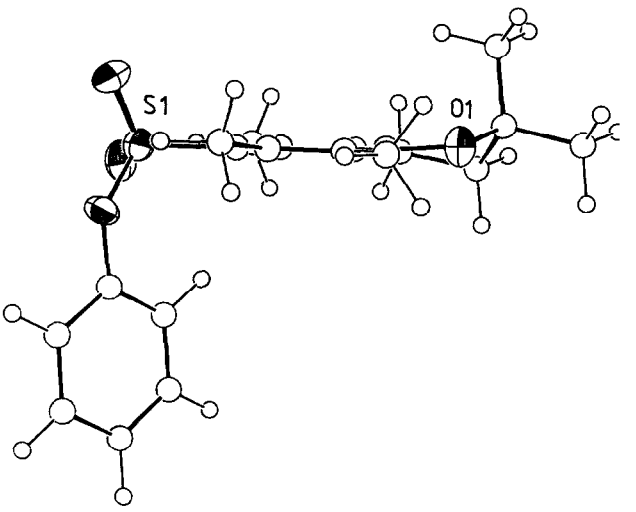


Figure 2

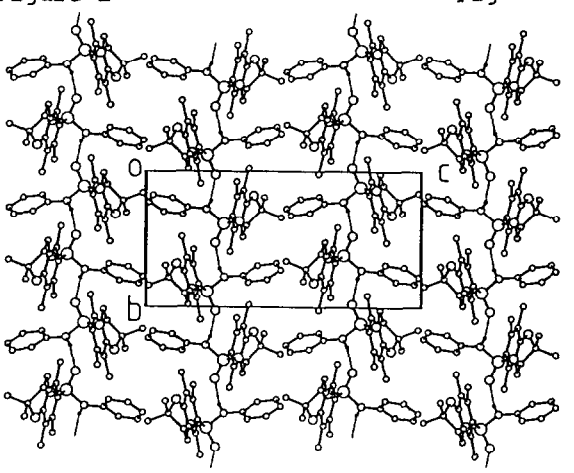


Figure 3

Figure 1

A general view of the molecule showing the crystallographic numbering scheme. Thermal ellipsoids are drawn at the 30% probability level, excepting those of H atoms which have arbitrary radii of 0.1 Å for clarity.

Figure 2

Complementary view of the molecule showing co-planarity of the aryl ring and O(1).

Figure 3

A view of the structure along the a axis showing molecules related by the 2_1 screw axis linked into infinite helices by N-H··· O=S hydrogen bonding. The H-bonding parameters are H(1)···O(12) 2.12(6)Å, N(1)H(1)...O(12) 161(5)°, H(1)···O(12)S(1) 130(2)°.

anisotropic thermal parameters. H atoms were included in fixed, calculated positions, with the exception of H(1) which was constrained to lie 1.00(1)A from N(1). The phenyl ring was refined with idealised D_{6h} symmetry. At final convergence R, R_w = 0.0572, 0.0679 respectively, S = 1.168 for 217 refined parameters and the final ΔF synthesis showed no $\Delta\rho$ above 0.34 eA^{-3}. The weighting scheme $w^{-1} = \sigma^2(F) + 0.00191F^2$ gave satisfactory agreement analyses and in the final cycle $(\Delta/\sigma)_{max}$ was 0.026.
Atomic scattering factors were inlaid[14], molecular geometry calculations utilised CALC[15] and the Figure was produced by ORTEPII[16].

The results of the crystallographic study are shown in Figures 1, 2 and 3. As predicted above the C-O-C(aryl) angle was found to be 118.3° (5) and Figure 2 shows clearly the planarity of the aryl ring with O(1).

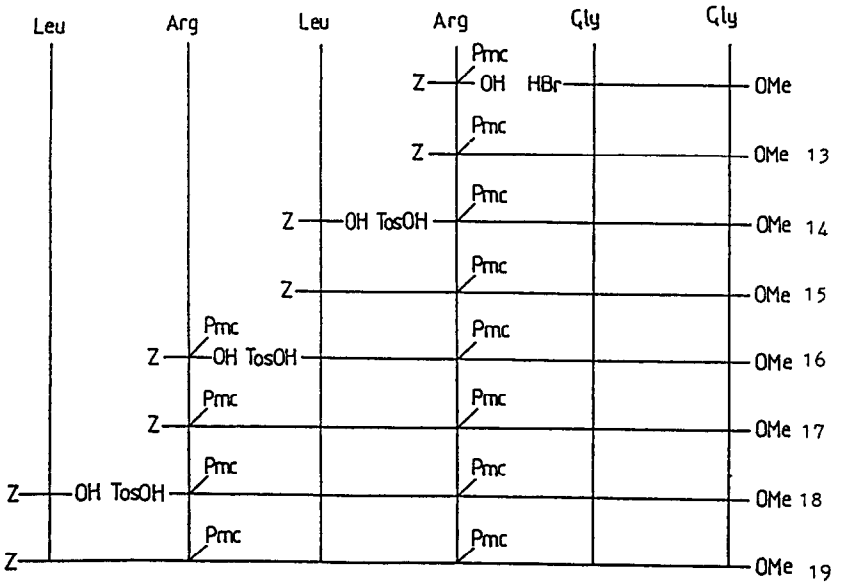
Synthesis and Application of FmocArg(Pmc).OH (3) and Bnpeoc.Arg(Pmc).OH (4)

The construction of 2,2,5,7,8-pentamethylchroman (12) was achieved[17] in a single step from 2,3,5-trimethylphenol and isoprene, using $ZnCl_2$ as catalyst in HOAc. Chlorosulphonation of (12) afforded the required reagent, Pmc.Cl, (10) which was reacted with N^{α}-Z.Arg.OH (5), under the usual alkaline conditions to give N^{α}-benzyloxycarbonyl-N^{G}-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginine (Z.Arg(Pmc).OH) (6). Purification of (6) was achieved by crystallisation of the cyclohexylamine salt. Re- conversion of this salt to (6) and subsequent hydrogenolysis (10% Pd/C) gave N^{G}-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginine (H.Arg(Pmc).OH) (7).

Application of (7) to the ion exchange column of an amino acid analyser gave a broad peak, however it was possible to study a series of qualitative deprotection reactions leading to the formation of arginine (1). Complete deprotection of (7) was observed after 2 hours at room temperature using TFMSA-thioanisole (10:1), TFA-thioanisole (10:1), TFA and 45% HBr in HOAc. In order to place these rsults in perspective, it is noteworthy that H.Arg(Mtr).OH was deprotected in 4-6 hours using TFA. The by-product from acid deprotection of the N^{G}-Pmc group is the chroman (12).

A series of arginine-containing peptides (Scheme 5) representing the C-terminal sequence of ubiquitin were synthesised using solution phase methodology. In each case diphenylphosphinyl chloride (Dpp.Cl)[18] was used for carboxyl activation, whilst the Z group was removed by hydrogenolysis. This series of peptides containing 1 and 2 N^{G}-Pmc groups were assessed with respect to acid deprotection whereupon it was found that the Pmc groups were cleaved by 100% TFA at room tempearture within 20 mins whilst further exposure to this reagent for periods >60 mins caused partial cleavage of the Z group. In contrast to other arylsulphonyl groups, the use of 10% thioanisole afforded no apparent increase in the rates of deprotection, which suggests that cleavage of the S-N bond is rapid following protonation

and that involvement of thioanisole occurs subsequent to this event (Scheme 4). It was found early in these studies that 100% TFA led to product contamination and thus deprotection of the Pmc group should be associated with the presence of scavengers as was found in the case of the Mtr group.[19] This observation was subsequently corroborated by other workers.[20] The usual acid medium used by us for Boc deprotection is TFA:H_2O (95:5) containing anisole (3%), ethanedithiol (1%) and ethylmethylsulphide (1%) and, in these first studies, it was found that the inclusion of H_2O had no deleterious effect upon the deprotection rate. In each model deprotection experiment, the peptidic product was authenticated by ^1H and ^{19}F n.m.r. and by FAB MS. The most interesting feature of these experiments was the finding that N^G-Pmc deprotection could be achieved by 50% TFA in CH_2Cl_2 within 1 hour which is compatible with the rate of removal of Bu^t-derived protecting groups. Indeed, in our experience gained in many SPPS syntheses of Arg peptides we have found that the rate limiting deprotection is not the Arg(Pmc) group but rather the t-butyl ether deprotection of Ser, Thr and Hyp residues. It was also found that 45% HBr in HOAc at room temperature caused a rapid (<5 min) deprotection of (13) to Br^- H_2^+Arg(HBr). Gly.Gly.OMe whereas (13) was unaffected by 4.5M HCl in methanol over a period of 2 hours.



Z.Leu.Arg(Pmc).Leu.Arg(Pmc).Gly.Gly.OMe

Scheme 5

These results augured well for the programme and it proved possible to convert H.Arg(Pmc).OH (7) to both Fmoc.Arg(Pmc).OH (3) and Bnpeoc.Arg(Pmc).OH (4) using the succinimidyl carbonates of the corresponding alcohols. The reagent Fmoc.Arg(Pmc).OH (3) has been extensively exemplified by us[21] using Fmoc/Bu^t SPPS methodology and it has proven to be remarkably satisfactory in all respects. Probably the most significant early application was in the synthesis of the (48-76) sequence of ubiquitin (20) which contains 3 Arg residues and a very sensitive C-terminal Gly.Gly sequence. Chain assembly and subsequent deprotection, using TFA:H_2O (95:5) in the presence of thioanisole, afforded the required ubiquitin sequence. Subsequently we have successfully applied Fmoc.Arg(Pmc).OH (3) to many syntheses, including that of the protein ubiquitin.[22]

48 76
K Q L E D G R T L S D Y N I Q K E S T L H L V L R L R G G
20

EXPERIMENTAL

All amino acids were purchased from the SAS group of companies and were used as supplied. Z-amino acid derivatives were prepared by literature methods. Melting points were recorded in open capillaries on a Buchi MP10 melting point apparatus and are uncorrected. Optical rotations were measured on an AA1000 polarimeter (Optical Activity Ltd) using a 10 cm cell. Thin layer chromatography (t.l.c.) was carried out on plastic sheets precoated with silica gel 60GF-254 (Merck 5735) in the following systems: (A) $CHCl_3$-MeOH (9:1), (B) $CHCl_3$-MeOH-AcOH (9:1:0.5), (C) *n*-BuOH-AcOH-H_2O (3:1:1), (D) 40-60 Petrol-EtOAc (4:1). Visualisation of the compounds was achieved by a suitable combination of the following methods: iodine vapour, uv absorption at 254 nm, chlorine-starch-potassium iodide, ninhydrin for peptides with free amino groups, and Sakaguchi reagent for peptides containing free arginine side chains. High performance liquid chromatography was carried out using a Waters system, *ie* two 6000A pumps, a U6K model injector, a 660 automatic gradient controller and a Waters uv detector (Model 441) operating at 254 or 229 nm. Analytical separations were executed on an ODS 5μ Hypersil column (3.9 x 300 mm) using a gradient over 25 minutes between solvent A (0.05% TFA in water) and solvent B (0.05% in TFA in acetonitrile), eluted at 1 ml/min. Amino acid analyses were carried out on an LKB 4150 alpha amino analyser following sealed tube hydrolyses in constant boiling hydrochloric acid at 110°C for 18 hours. Infrared spectra were recorded on a Perkin Elmer 781 spectrophotometer in the solvent indicated, or by the bromoform mull technique using polystyrene as the standard (1603 cm^{-1}). Resin samples were recorded using the KBr disc technique. Ultraviolet spectra were recorded in distilled methanol on a Pye-Unicam SP8-400 spectrophotometer. Mass spectra were measured on a Kratos MS50TC machine. ^1H n.m.r. spectra were recorded on either Bruker WP80 (80 MJHz), WP200 (200 MHz), or WH360 (360 MHz) machines in the solvent indicated, using tetramethylsilane (TMS) as the external standard (δ = 0.00). Carbon-13 n.m.r. spectra were recorded on either a Bruker WP200 machine operating at 50.1 MHz or a Bruker WH360 machine operating at 90 MHz. Samples were dissolved in the solvent indicated and chemical shifts were measured relative to TMS assigned at zero. Phosphorus-31 n.m.r. spectra were recorded on a Jeol FX60Q machine operating at 24.1 MHz. All chemical shift values were measured relative to external 85% aqueous phosphoric acid assigned at zero. Elemental analyses were carried out on a Carlo Erba elemental analyser model 1106.

All solvents were distilled before use and the following were dried using the reagents given in parentheses when required: acetic acid (acetic

anhydride), acetonitrile (calcium hydride), *n*-butanol (magnesium-iodine), chloroform (phosphorus pentoxide), dichloromethane (calcium hydride), diethyl ether (sodium wire), *N,N*-dimethylformamide (calcium hydride or 4A molecular sieves), and methanol (magnesium-iodine). SPPS were performed using an applied Biosystems 430A instrument.

2,2,5,6,8-Pentamethylchroman (12)

2,3,5-Trimethylphenol (200 g, 1.47 mol) and fused zinc chloride (23.5 g, 0.17 mol) were stirred together with anhydrous acetic acid (180 ml) and isoprene (147 ml, 1.47 mol) for 12 h at 23°C and then warmed gradually whereupon the isoprene began to reflux and the solution became clear. The solution was then refluxed for 7 h on an oil bath (temperature 150°C) and the solution turned black. After allowing to cool to room temperature, water (1000 ml) was added and the oil separated. The aqueous solution was extracted with 40-60 petrol (3 x 800 ml) and the combined oil and extracts washed with Claisen's alkali (3 x 700 ml), water (3 x 1000 ml) and brine (2 x 800 ml). The solution was dried over $CaCl_2$ and the solvent was then evaporated under reduced pressure. The residue was distilled at 0.3 mm Hg affording the product as a pale yellow liquid that solidified on cooling (131.6 g, 44%); b.p. 99-108°C (0.3 mm Hg); m.p. 32-38°C (lit.,[17] 40-41°C, after recrystallisation from MeOH); (Found: C, 82.0; H, 9.96. Calc. for $C_{14}H_{20}O$: C, 82.3; H, 9.87%); t.l.c.-D R_F 0.67; ν_{max} (CH_2Cl_2) 2940 (CH_3), 1455, 1315, 1165, 1125, 1100 cm^{-1}; λ_{max} 284 nm (ϵ 1490 dm^3 mol^{-1} cm^{-1}), 218, (1420), 275 (1360); δ_H ($CDCl_3$, 80 MHz) 6.59 (1H, s, aromatic H), 2.64 (2H, t, *J* 7.0 Hz, CH_2), 2.24, 2.20, 2.12 (3H each, 3 x s, 3 x aromatic-CH_3), 1.83 (2H, t, *J* 7.0 Hz, CH_2), 1.35 (6H, s, CH_3's); δ_C ($CDCl_3$, 50 MHz) 151.7 (aromatic C-9), 134.5, 133.2, 121.9, 116.5 (quaternary aromatic C's), 122.2 (aromatic C-6), 72.9 (C-2), 32.8 (C-4), 26.8 (2 x CH_3's on C-2), 20.4 (C-3), 19.5, 18.6, 11.2 (aromatic-CH_3's); *m/z* (EI) 204, 189, 149. HRMS 204.1514. Calc. for $C_{14}H_{20}O$, 204.1515.

2,2,5,7,8-Pentamethylchroman-6-sulphonyl chloride, Pmc-Cl (10)

2,2,5,7,8-Pentamethylchroman (12) (51.7 g, 0.25 mol) was dissolved in anhydrous chloroform (1000 ml) and cooled to -5°C. A solution of chlorosulphonic acid (70 ml, 1.05 mol) in dry chloroform (800 ml) was added maintaining the temperature at -5°C. After addition was complete the reaction was left to stir for 15 minutes at the low temperature and for a further hour with the cooling bath removed. The dark brown solution was then poured onto crushed ice and the organic layer separated and washed with 5% Na_2CO_3 (1500 ml), saturated $NaHCO_3$ (1500 ml), water (1500 ml) and brine (1500 ml) before drying over $MgSO_4$. The solution was then stirred with activated charcoal to decolourise and after filtration through Kieselguhr and evaporation of the solvent, the residue was crystallised from 40-60 petrol (40.5 g, 53%) m.p. 79-82°C; (Found: C, 55.3; H, 6.33; $C_{14}H_{19}ClO_3S$ requires C, 55.5; H, 6.32%), t.l.c.-D R_F 0.50; ν_{max} (CH_2Cl_2) 1550, 1450, 1360 (S=O asymmetric), 1300, 1170 (S=O symmetric), 1125, 1110 cm^{-1}; δ_H ($CDCl_3$, 200 MHz) 2.68 (2H, t, *J* 6.9 Hz, CH_2), 2.63 and 2.61 (6H, 2 x s, CH_3's), 2.14 (3H, s, CH_3), 1.85 (2H, t, *J* 6.8 Hz, CH_2), 1.34 (6H, s, CH_3's); δ_C ($CDCl_3$, 50 MHz) 156.7 (C-6), 137.3, 135.6, 125.3, 118.7 (aromatic C's), 74.9 (C-2), 32.4 (C-4), 26.7 (2 x CH_3's on C-2), 21.2 (C-3), 18.5, 17.5, 12.0 (aromatic-CH_3's); *m/z* (EI) 304, 302, 267, 249, 247, 147. HRMS 302.0744, $C_{14}H_{19}{}^{35}ClO_3S$ (M^+) requires 302.0745.

N^{α}-Benzyloxycarbonyl-N^{G}-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginine cyclohexylamine salt, Z.Arg(Pmc).OH (6) CHA

Z.Arg.OH (34.79 g, 113 mmol) was dissolved in 3.2M sodium hydroxide solution (146 ml) and acetone (400 ml) and cooled to 0°C. To this was added a solution of 2,2,5,7,8-pentamethylchroman-6-sulphonyl chloride (54.92 g, 181 mmol) in acetone (250 ml) and the mixture was stirred at 0°C for 2 h and a further 2 h at room temperature. After acidification to pH 6.5 with saturated citric acid the acetone was removed under reduced pressure. The remaining solution was further acidified with saturated citric acid to pH 3 and diluted with water (500 ml), and was extracted with ethyl acetate (3 x 500 ml). The combined extracts were filtered to remove the insoluble PmcO-Na+ by-product. The ethyl acetate solution was then

washed with water (2 x 700 ml) and brine (2 x 700 ml) before drying over $MgSO_4$. The solution was then concentrated *in vacuo* to a total volume of *ca* 500 ml, cooled in an ice-water bath and cyclohexylamine (12.9 ml, 113 mmol) was added. Addition of anhydrous ether gave a thick white gum which solidified on standing overnight at 4°C. Recrystallisation from methanol-diethylether gave the desired product as a white crystalline solid (76.05 g, 59%), (NB extra material obtainable by chromatography of filtrates); m.p. 156°C; (Found: C, 60.3; H, 7.68; N, 10.5. $C_{34}H_{51}N_5O_7S$ requires: C, 60.6; H, 7.63; N, 10.4%), $[\alpha]_D^{25}$ +6.6° (*c* 1 in MeOH); t.l.c.-B R_F 0.41; ν_{max} ($CHBr_3$) 3450, 3350, 3290 (NH, OH), 1710 (C=O), 1300 cm^{-1} (S=O asymmetric); λ_{max} 251 nm (ϵ 13600 dm^3 mol^{-1} cm^{-1}); δ_H [$(CD_3)_2SO$, 200 MHz] 7.7 and 6.9 (3H, br, s, guanidino NH's), 7.33 (5H, s, *Z* aromatic), 6.57 (1H, d, $^3J_{NH-\alpha CH}$ 7.2 Hz, α-NH), 4.99 (2H, s, Z-CH_2), 3.38 (1H, m, α-CH), 2.99 (3H, m, Arg δ-CH_2 and CHA CH), 2.55 (2H, t, CH_2), 2.48 (6H, S 2 x CH_3's *o* to -SO_2-), 2.03 (3H, s, CH_3 *m* to -SO_2-), 2.0-1.1 (20H, m, Arg β-CH_2 and γCH_2, 5 x CHA CH_2's and 2 x Pmc (CH_3's); δ_C [$(CD_3)_2SO$, 50 MHz] 174.7 (Arg CO), 156.3 (urethane CO), 155.4 (guanidino C), 152.3 (C-6), 137.3 (Z-C1), 134.7-117.7 (aromatic C's), 73.3 (C2), 65.0 (Z-CH_2), 55.3 (Arg α-C), 49.2 (CHA CH), 40.8 (Arg δ-C), 32.3 (C4), 30.9 (CHA CH_2's), 30.0 (Arg β-C), 26.4 (2 x CH_3 on C2), 25.5 (Arg γ-C), 24.6 and 23.8 (CHA CH_2's), 20.7 (C3), 18.0 and 17.0 (2 x CH_3's *o* to -SO_2-), 11.8 (CH_3 *m* to -SO_2-); *m/z* (FAB) 575, 531, 441, 309, 203, 92. HRMS $C_{28}H_{33}N_4O_7S$ (MH+) requires 575.2539, found 575.2539.

N^G-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl)-arginine, H.Arg(Pmc)OH (7)

Z.Arg(Pmc)OH.CHA (33.57 g, 49.8 mmol) was converted to the free acid, Z.Arg(Pmc)OH (6), as a foam which was taken up in methanol (250 ml), 10% palladium on charcoal (3.05 g) was added under an atmosphere of nitrogen and the resulting mixture was hydrogenated overnight. The catalyst was removed by filtration through Kieselguhr and following evaporation of the solvent, addition of ether to the methanol solution gave (7) as a white powder (18.67 g, 85%), m.p. 95°C, then 145°C; (Found: C, 53.5; H, 7.68; N, 12.0. $C_{20}H_{32}N_4O_5S.CH_3OH$ requires C, 53.4; H, 7.68; N, 11.9%), $[\alpha]_D^{25}$ -4.2° (c 1 in MeOH); t.l.c.-C R_F 0.42; ν_{max} ($CHBr_3$) 3700-2400, 3450, 3340 (NH, OH), 1300 cm^{-1} (S=O asymmetric); λ_{max} 252 nm (ϵ 12700 dm^3 mol^{-1} cm^{-1}); δ_H ($(CD_3)_2SO$, 200 MHz) 8.0-6.7 (6H, br, NH_3^+ and guanidino NH's), 3.27 (1H, m, Arg α-CH), 3.06 (2H, m, Arg δ-CH_2), 2.58 (2H, t, *J* 6.8 Hz, Pmc CH_2), 2.49 (6H, s, Pmc, CH_3's), 2.03 (3H, s, Pmc CH_3), 1.75 (2H, t *J* 6.6 Hz, Pmc CH_2), 1.5 (4H, br m, Arg β,γ-CH_2's), 1.26 (6H, s Pmc CH_3's); δ_C [$(CD_3)_2SO$, 50 MHz] 171.5 (ArgCO), 156.4 (guanidino C), 152.3-117.7) Pmc aromatics), 73.3 (C2), 53.7 (Arg α-C), 39.8 (Arg δ-C), 32.1 (C4), 28.3 (Arg β-C), 26.4) Pmc CH_3's on C2), 25.1 (Arg γ-C), 20.7 (C3), 18.0, 17.0, 11.8 (Pmc CH_3's); *m/z* (FAB) 441, 203, 179, 147. HRMS found 441.2172, $C_{20}H_{33}N_4O_5S$ (MH+) requires 441.2172.

N^α-Fluorenylmethoxycarbonyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginine, Fmoc.Arg(Pmc)OH (3)

N^G-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl)-arginine (7) (2.49 g, 5.65 mmol) was dissolved in 6% aqueous sodium carbonate (21 ml) and the solution cooled to 0°C. A solution of 9-fluorenylmethyl succinimidyl carbonate (1.92 g, 5.66 mmol) in DMF (10 ml) was added dropwise and the reaction left to stir for one hour with the ice bath removed. The solution was diluted with water (100 ml) and washed with ether (2 x 50 ml) before acidification with saturated citric acid (30 ml). The solution was extracted with ethyl acetate (3 x 100 ml) and the combined extracts were washed with water (x 2) and brine, before drying over $MgSO_4$. The dried solution was concentrated *in vacuo* and the desired product precipitated by the addition of *n*-hexane (3.33 g, 89%); m.p. 80-93°C (Found: C, 63.5; H, 6.74; N, 8.02. $C_{35}H_{42}N_4O_7S$ requires C, 63.4; H, 6.39; N, 8.45%), $[\alpha]_D^{25}$ +3.6° (*c* 1 in $CHCl_3$); t.l.c.-B R_F 0.32; ν_{max} (CH_2Cl_2) 3430, 3350 (NH), 1720 (C=O), 1625, 1550, 1110 cm^{-1}; λ_{max} 300 nm (ϵ 5600 dm^3 mol^{-1} cm^{-1}), 289 (5000), 255 (26600), 221 (46000); δ_H [$(CD_3)_2CO$, 200 MHz] 7.87-7.28 (8H, m, Fmoc aromatics), 6.75 (1H, d, *J* 8.7 Hz, urethane NH), 6.6-6.3 (3H, br, guanidino NH's), 4.33 (4H, m, Arg α-CH and Fmoc CH, CH_2), 3.26 (2H, m, Arg δ-CH_2), 2.64 (2H, t, *J* 6.9 Hz, Pmc CH_2), 2.58 (6H, 2 x s, Pmc CH_3's),

2.0 (Pmc CH_3 obscured by d_5-acetone), 2.81 (2H, t, Pmc CH_2), 1.69 (4H, br m, Arg β,γ CH_2's), 1.29 (6H, s, Pmc CH_3's); δ_C [$(CD_3)_2CO$, 50 MHz] 172.1 (Arg CO), 155.5 (gaunidino C), 152.1 (urethane CO), 143.4-117.0 (aromatic C's), 72.6 (Pmc C-2), 65.6 (Fmoc CH_2), 52.8 (Arg α-C), 46.4 (Fmoc CH), 39.5 (Arg δ-C), 31.8 (Pmc C-4), 27.9 (Arg β-C), 25.3 (Pmc CH_3's on C-2), 25.0 (Arg γ-C), 20.3 (Pmc C-3), 17.0, 16.0, 10.5 (Pmc CH_3's); *m/z* (FAB) 663, 397, 203, 179, 147. HRMS 663.2853, $C_{35}H_{43}N_4O_7S$ (MH+) requires 663.2852.

N^{α}-2,2-Bis(4-nitrophenyl)ethyloxycarbonyl-N^{G}-(2,2,5,7,8-pentamethylchroman-6 sulphonyl)-arginine, Bnpeoc.Arg(Pmc)OH (4)

N^{G}-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl)-arginine (7) (0.182 g, 0.41 mmol) was dissolved in 5% aqueous sodium carbonate (2 ml) and the solution cooled to 0°C. A solution of 2,2-bis-(4-nitrophenyl)ethyl succinimidyl carbonate[4] (0.182 g, 0.42 mmol) in DMF (1 ml) was added in a single portion and the reaction stirred for 1 hour at room temperature during which time a precipitate formed. The reaction was acidified with 20% citric acid solution (5 ml) and extracted with ethyl acetate (3 x 10 ml) and the combined extracts were washed with water (x 2) and brine, before drying over Na_2SO_4. The dried solution was concentrated *in vacuo* and the desired product precipitated as an amorphous powder by the addition of light petroleum, (0.204 g, 65%); m.p. *ca* 140°C (Bnpeoc.Arg(Pmc)OH,CHA salt, m.p. 137°C); (Found: C, 55.6; H, 5.79; N, 10.8. $C_{35}H_{42}N_6O_{11}S$ requires C, 55.7; H, 5.61; N, 11.1%), $[\alpha]_D^{25}$ +5.2° (*c* 1 in $CHCl_3$), $[\alpha]_D^{28}$ +2.8° (*c* 1 in DMF); t.l.c.-B R_F 0.33; ν_{max} ($CHCl_3$) 1740 (CO), 1540 (NO_2), 1365 (NO_2), 910 cm^{-1}; λ_{max} 257 nm (ϵ 25500 dm^3 mol^{-1} cm^{-1}); δ_H [$(CD_3)_2CO$, 200 MHz] 8.20 and 7.67 (8H, 2 x d, *J* 8.8 Hz, Bnpeoc aromatics); 6.60 (1H, d, *J* 7.5 Hz, urethane NH), 6.5 (3H, m, guanidino NH's), 4.75 (3H, m, Bnpeoc CH and CH_2), 4.15 (1H, m, Arg α-CH), 3.2 (2H, m, Arg δ-CH_2), 2.65 (2H, t, *J* 6.7 Hz, Pmc CH_2), 2.57 (6H, 2 x s, 2 x Pmc CH_3's), 2.1 (Pmc CH_3 obscured by d_5 acetone), 1.81 (2H, t, *J* 6.8 Hz, Pmc CH_2), 1.8-1.5 (4H, m, Arg β,γ-CH_2's), 1.29 (6H, s, Pmc CH_3's); δ_C [$(CD_3)_2CO$, 50 MHz] 172.1 (Arg CO), 155.6 (urethane CO), 155.0 (guanidino CO), 152.2-117.1 (aromatic C's), 72.6 (Pmc C-2), 64.9 (Bnpeoc CH_2), 52.9 (Arg α-C), 49.0 (Bnpeoc CH), 39.6 (Arg δ-C), 31.8 (Pmc C-4), 29.9 Arg β-C), 25.0 (Pmc CH_3's), 24.7 (Arg γ-C), 20.3 (Pmc C-3), 17.1, 16.0, 10.6 (Pmc CH_3's); *m/z* (FAB) 755, 219, 203. HRMS 755.2710, $C_{35}H_{43}N_6O_{11}S$ (MH+) requires 755.2710.

N^{α}-Benzyloxycarbonyl-N^{G}-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginyl-glycylglycine Methyl Ester, Z.Arg(Pmc).Gly.GlyOMe (13)

Z.Arg(Pmc)OH.CHA (6.828 g, 10.13 mmol) was stirred with ethyl acetate (60 ml) and saturated citric acid (60 ml) for 1 h and the organic phase was then washed with saturated citric acid (x 2), water (x 2) and brine (x 1). The washings were consecutively re-extracted with ethyl acetate (60 ml) and the extracts combined and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave Z.Arg(Pmc)OH as a foam (4.908 g, 84% recovery).

Z.Arg(Pmc)OH (4.908 g, 8.54 mmol) was dissolved in dry DCM (25 ml) and cooled to -10°C. A solution of DppCl (2.021 g, 8.54 mmol) in dry DCM (6.6 ml) was added followed by NMM (0.94 ml, 8.54 mmol), maintaining the temperature at -3°C. After stirring for 2 minutes a solution of $Br^{-}H_2^{+}$Gly.GlyOMe (2.34 g, 10.3 mmol) in DMF (10 ml) was added, followed by NMM (1.13 ml, 10.3 mmol) and 2,6-lutidine (1.20 ml, 10.3 mmol). The reaction was left to stir at -10°C for 1 h, followed by 1 h with the cooling bath removed. The reaction solvents were then removed *in vacuo* and the residue partitioned between ethyl acetate (80 ml) and water (80 ml). The organic phase was washed with saturated $NaHCO_3$ (x 2), water, 5% citric acid, water, saturated $NaHCO_3$ (x 2), water, and brine, and dried over $MgSO_4$. The solution was then concentrated and the product precipitated as a gum by the addition of *n*-hexane. The supernatant was decanted and the gum triturated under *n*-hexane to give a white powder (5.329 g, 89%), m.p. *ca* 85°C; (Found: C, 56.3; H, 6.75; N, 11.6. $C_{33}H_{46}N_6O_9S$ requires C, 56.4; H, 6.60; N, 12.0%); $[\alpha]_D^{25}$ -5.0° (*c* 1 in $CHCl_3$); t.l.c.-B R_F 0.40; amino acid analysis Arg 1.01, Gly 1.99; ν_{max} (CH_2Cl_2) 3350 (NH), 2940 (CH), 1780 (ester CO), 1545, 1115 cm^{-1}; λ_{max} 252 nm (ϵ 14100 dm^3 mol^{-1} cm^{-1}), 222 nm (34000); δ_H ($CDCl_3$, 200 MHz) 7.86 (1H, br t, Gly NH), 7.50 (1H, br t, Gly NH), 7.28 (5H, s, Z aromatic), 6.45-6.13

(4H, m, urethane and guanidino NH's), 5.01 (2H, s, Z CH_2), 4.24 (1H, m, Arg α-CH), 3.9 (4H, m, Gly CH_2's), 3.63 (3H, s, ester CH_3), 3.18 (2H, m, Arg δ-CH_2), 2.59 (2H, obscured t, Pmc CH_2), 2.54 and 2.51 (6H, s, Pmc *o*-CH_3's), 2.08 (3H, s, Pmc *m*-CH_3), 1.78 (2H, t, *J* 5Hz, Pmc CH_2), 1.5 (4H, m, Arg β-γ-CH_2's), 1.30 (6H, s, Pmc CH_3's); δ_C ($CDCl_3$, 50 MHz) 173.2, 170.2, 170.0 (Arg CO and Gly CO's), 156.6, 156.4 (guanidino C and urethane CO), 153.6 (Pmc C-6), 136.2-117.9 (aromatic C's), 73.6 (Pmc C-2), 66.9 (Z-CH_2), 54.8 (Arg α-C), 52.1 (ester CH_3), 42.9 and 41.0 (Gly α-C's), 40.1 (Arg δ-C), 32.8 (Pmc C-4), 29.2 (Arg β-C), 26.7 (Pmc CH_3's on C-2), 25.3 (Arg γ-C), 21.3 (Pmc C-3), 18.3 and 17.3 (Pmc CH_3's *o* to -SO_2-), 11.9 (CH_3 *m* to -SO_2-); *m/z* (FAB) 703, 569, 437. HRMS 7033.3125, $C_{33}H_{47}N_6O_9S$ (MH+) requires 703.3125.

N^G-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl)-arginylglycylglycine Methyl Ester Tosylate, $TosO^-H_2^+$Arg(Pmc).Gly.GlyOMe (14)

Z.Arg(Pmc).Gly.GlyOMe (4.461 g, 6.35 mmol) was dissolved in methanol (25 ml) together with 4-toluenesulphonic acid (1.214 g, 6.38 mmol) and the solution hydrogenolysed for 18 h in the presence of 10% palladium on charcoal (0.63 g). The solution was filtered through Kieselguhr and concentrated *in vacuo*. Addition of anhydrous ether gave a thick gum which was triturated under ether to give a white powder (4.210 g, 90%), m.p. *ca* 90°C; (found: C, 50.5; H, 6.70; N, 10.8. $C_{32}H_{48}N_6O_{10}S_2.H_2O$ requires C, 50.6; H, 6.64; N, 11.1%), $[\alpha]_D^{25}$ +13.8° (c 1 in MeOH); t.l.c.-C R_F 0.40; amino acid analysis Arg 1.03, Gly 1.94; ν_{max} ($CHBr_3$) 3700-2500 (NH), 1740 (C=O), 1300 (S=O asymmetric), 1035, 1010 cm^{-1}. λ_{max} 253 nm (ϵ 15400 dm^3 mol^{-1} cm^{-1}), 223 (37400), δ_H [$(CD_3)_2SO$, 200 MHz] 8.75 (1H, t, *J* 6.2 Hz, Gly NH), 8.43 (1H, t *J* 6.2 Hz, Gly NH), 8.13 (3H, br s, Arg NH_3+), 7.50 and 7.12 (4H, 2 x d, *J* 8.0 Hz, tosylate aromatic), 6.5-7.0 (3H, br s, guanidino NH's), 3.85 (5H, m, Arg α-CH and Gly CH_2's), 3.63 (3H, s, ester CH_3), 3.08 (2H, m, Arg δ-CH_2), 2.59 (2H, t, *J* 6 Hz, Pmc CH_2), 2.49 (6H, s, Pmc CH_3's), 2,29 (3H, s, tosylate CH_3), 2.04 (3H, s, Pmc CH_3), 1.77 (2H, t, 7 Hz, Pmc CH_2), 1.74 and 1.50 (4H, m, Arg β- and γ-CH_2's), 1.26 (6H, s, Pmc CH_3's); δ_C [$(CH_3)_2SO$, 50 MHz] 170.0, 168.7, 168.6 (Arg CO and Gly CO's), 156.0 (gaunidino C), 152.4 (Pmc C-6), 145.3-117.7 (aromatic C's), 73.4 (Pmc C-2), 52.1 (Arg α-C), 51.6 (ester CH_3), 41.7, 40.4 (Gly α-C's), 39.7 (Arg δ-C), 32.2 (Pmc C-4), 28.5 (Arg β-C), 26.4 (Pmc CH_3's on C-2), 24.4 (Arg γ-C), 20.7 (tosylate CH_3 and Pmc C-3), 18.0, 17.0, 11.8 (Pmc CH_3's); *m/z* (FAB) 569, 303, 203. HRMS 569.2757, $C_{26}H_{41}N_6O_7S$ (MH+) requires 569.2757.

N^α-Benzyloxycarbonylleucinyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl-arginylglycylglycine Methyl Ester, Z.Leu.Arg(Pmc).Gly.Gly.OMe (15)

Z.LeuOH (oil) (1.800 g, 6.79 mmol) was dissolved in dry DCM (20 ml) and cooled to -6°C. A solution of DppCl (1.606 g, 6.79 mmol) in DCM (4.2 ml) was added followed by NMM (0.75 ml, 6.79 mmol), and the solution was stirred at -60°C for 10 minutes. A solution of $TosO^-H_2^+$Arg(Pmc).Gly.Gly OMe (3.983 g, 5.38 mmol) in DMF (15 ml) was added followed by NMM (0.59 ml, 5.38 mmol) and 2,6-lutidine (0.79 ml, 6.78 mmol) and the reaction was left stirring for 1 h at -4°C and for a further 1.5 h with the cooling bath removed. The reaction was worked up as described for (13) to give the desired product (15) as a white powder (3.347 g, 76%); m.p. 90-95°C (Found: C, 56.9; H, 7.04; N, 11.8. $C_{39}H_{57}N_7O_{10}S$ requires C, 57.4; H, 7.04; N, 12.0%), $[\alpha]_D^{27}$ -15.9° (*c* 1 in $CHCl_3$); t.l.c.-A R_F 0.32; ν_{max} ($CHCl_3$) 3600-2670, 3440, 3320 (NH), 2960, 2930 (CH), 1750 (ester C=O), 1670, 1665 (amide C=O), 1370, 1170 cm^{-1} (S=O); λ_{max} 252 nm (ϵ 14200 dm^3 mol^{-1} cm^{-1}), 223 (30200); δ_H ($CDCl_3$, 360 MHz) 7.86 (1H, br, Gly NH), 7.77 (1H, br, Arg NH), 7.53 (1H, br t, Gly NH), 7.25 (5H, s, Z aromatic), 6.4-6.0 (3H, br, guanidino NH's), 6.06 (1H, d *J* 7.5 Hz, Leu NH), 5.00 (2H, ABq, *J* 12.3 Hz, Z CH_2), 4.49 (1H, m, Arg α-CH), 4.26)1H, m, Leu α-CH), 3.95 (2H, br d, Gly CH_2), 3.90 (2H, d, $^3J_{NH-\alpha CH}$ 5.6 Hz, Gly CH_2), 3.61 (3H, s, ester CH_3) 3.13 (2H, br m, Arg δ-CH_2), 2.57 (2H, t, *J* 6.6 Hz, Pmc CH_2), 2.51 (6H, 2 x s, 2 x Pmc CH_3's), 2.06 (3H, s, Pmc CH_3), 1.87 (1H, m, Leu β-CH_2), 1.76 (2H, t, 6.8 Hz, Pmc CH_2), 1.66-1.50 (7H, m, Leu β-CH_2, γ-CH, Arg β,γ-CH_2's), 1.27 (6H, s, 2 x Pmc CH_3's), 0.85 (6H, 2 x d, *J* 6.4 Hz, Leu δ-CH_3's); δ_C ($CDCl_3$, 50 MHz) 173.6, 172.5, 170.1, 169.9 (Leu CO, Arg CO, 2 x Gly CO's), 156.6, 156.4 (guanidino C and urethane CO), 153.6 (Pmc C-6),

136.2-117.9 (aromatic C's), 73.5 (Pmc C-2), 66.9 (Z CH_2), 54.1, 53.0 (Leu α-C, Arg α-C), 52.0 (ester CH_3), 42.9, 41.2, 41.0 (Leu β-C, Gly α-C's), 40.3 (Arg δ-C), 32.8 (Pmc C-4), 28.9 (Arg B-C), 26.7 (Pmc CH_3's on C-2), 25.3 (Arg γ-C), 24.7 (Leu γ-C), 22.9, 21.5 (Leu δ-C's), 21.3 (Pmc C-3), 18.3, 17.3, 11.9 (Pmc CH_3's); *m/z* (FAB) 817, 551, 203. HRMS 816.3965, $C_{39}H_{58}N_7O_{10}S$ (MH+) requires 816.3966.

Leucinyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginylglycylglycine Methyl Ester Tosylate, $TosO^-H_2{}^+$Leu.Arg(Pmc).Gly.GlyOMe (16)

Z.Leu.Arg(Pmc).Gly.GlyOMe (15) (2.356 g, 2.89 mmol) was dissolved in methanol (25 ml) with 4-toluenesulphonic acid monohydrate (0.575 g, 3.02 mmol) and hydrogenolysed in the presence of 10% palladium on charcoal (0.44 g) overnight. The catalyst was removed by filtration through Kieselguhr and the solution concentrated under reduced pressure. Addition of anhydrous ether precipitated the product as a gum which was triturated under ether to give a white powder (2.323 g, 94%), m.p. indefinite, 80-140°C; (Found: C, 51.9; H, 7.08; N, 11.0. $C_{38}H_{59}N_7O_{11}S_2.H_2O$ requires: C, 52.3; H, 7.05; N, 11.2%), $[\alpha]_D^{25}$ +6.9° (*c* 1 in MeOH); t.l.c.-C R_F 0.49; ν_{max} ($CHBr_3$) 3700-2800, 3430 (NH), 1300 cm^{-1} (S=O asymmetric); λ_{max} 253 nm (ε 14000 dm^3 mol^{-1} cm^{-1}), 223 (32000); δ_H [$(CD_3)_2SO$, 360 MHz] 8.63 (1H, d, $^3J_{NH-\alpha CH}$ 7.8 Hz, Arg NH), 8.30 (1H, t, $^3J_{NH-\alpha CH2}$ 5.9 Hz, Gly NH), 8.27 (1H, t, $^3J_{NH-\alpha CH2}$ 5.9 Hz, Gly NH), 8.06 (3H, br s, Leu NH_3^+), 7.50 and 7.11 (4H, 2 x d, *J* 8.0 Hz, tosylate aromatics), 7.0-6.5 (3H, br, guanidino NH's), 4.35 (1H, m, Arg α-CH), 3.86 (2H, d, $^3J_{NH-CH2}$ 5.8 Hz, Gly CH_2), 3.82 (1H, m, Leu α-CH), 3.76 (2H, d, $^3J_{NH-\alpha CH2}$ 6.1 Hz, Gly CH_2), 3.62 (3H, s, ester CH_3), 3.06 (2H, m, Arg δ-CH_2), 2.58 (2H, t, *J* 6.7 Hz, Pmc CH_2), 2.48 (6H, 2 x s, 2 x Pmc CH_3's), 2.29 (3H, s, tosylate CH_3), 2.04 (3H, s, Pmc CH_3), 1.77 (2H, t, *J* 6.8 Hz, Pmc CH_2), 1.73-1.43 (7H, m, Leu β-CH_2, γ-CH, Arg β,γ-CH_2's), 1.26 (6H, s, Pmc CH_3's), 0.88 (6H, 2 x d, *J* 6.1 Hz, Leu δ-CH_2's); δ_C [$(CD_3)_2SO$, 50 MHz] 171.0, 170.0, 169.0, 168.8 (Leu CO, Arg CO, Gly CO's), 156.0 (guanidino C), 152.4 (Pmc C-6), 145.3-117.7 (aromatic C's), 73.4 (Pmc C-2), 52.5, 51.8, 50.8, (Leu α-C, Arg α-C, ester CH_3), 41.7, 40.4, 40.1, 39.9 (Leu β-C, Arg δ-C, 2 x Gly α-C's), 32.2 (Pmc C-4), 29.3 (Arg β-C), 26.4 (Pmc CH_3's), 25.3 (Arg γ-C), 23.4 (Leu γ-C), 22.6, 21.8 (Leu δ-C's), 20.7 (tosylate CH_3 and Pmc C-3), 18.0, 16.9, 11.8 (Pmc CH_3's); *m/z* (FAB) 682, 416, 203. HRMS 682.3598, $C_{31}H_{52}N_7O_8S$ requires 682.3598.

N^α-Benzyloxycarbonyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl-arginyl-leucinyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginylglycylglycine Methyl Ester, Z.Arg(Pmc)Leu.Arg(Pmc).Gly.Gly.OMe (17)

Z.Arg(Pmc)OH.CHA (2.162 g, 3.21 mmol) was converted to the free amino acid (7) (1.500 g, 81% recovery). Z.Arg(Pmc)OH (1.490 g, 2.59 mmol) was dissolved in dry DCM (20 ml) and cooled to -5°C. A solution of DppCl (0.613 g, 2.59 mmol) in dry DCM (6.6 ml) was added followed by NMM (0.285 ml, 2.59 mmol), and the solution stirred for 2 minutes at -5°C. A solution of $TosO^-H_2{}^+$Leu.Arg(Pmc).Gly.GlyOMe (1.943 g, 2.28 mmol) in DMF (10 ml) was added, followed by NMM (0.250 ml, 2.28 mmol) and 2,6-lutidine (0.304 ml, 2.61 mmol). The solution was left to stir at -10°C for 1 h, followed by 3 h gradually warming to room temperature and a further hour at room temperature. The reaction solvents were then removed *in vacuo* and the residue taken up in DMF and a solid precipitated by the addition of dilute sodium bicarbonate solution. The solid was reprecipitated as before, then finally purified in two approximately equal portions by gel filtration on Sephadex LH20 eluted with DMF. The peptide containing fractions were pooled and concentrated and the pure product obtained as a white powder by the addition of water (2.032 g, 72%), m.p. indefinite, 90-125°C; (Found: C, 57.1: H, 7.25; N, 12.2. $C_{59}H_{87}N_{11}O_{14}S_2$ requires C, 57.2; H, 7.08; N, 12.4%), $[\alpha]_D^{24}$ -11.8° (*c* 1 in $CHCl_3$); t.l.c.-B R_F 0.48; ν_{max} ($CHCl_3$) 3440, 3340 (NH), 1670 (amide CO's), 1550, 1300 (S=O asymmetric), 1110 cm^{-1}; λ_{max} 253 nm (ε 28160 dm^3 mol^{-1} cm^{-1}, 221 (69100); δ_H [$(CD_3)_2SO$, 360 MHz] 8.24 (1H, t, $^3J_{NH-\alpha CH2}$ 5.8 Hz, Gly NH), 8.18 (1H, t, $^3J_{NH-\alpha CH2}$ 5.4 Hz, Gly NH), 7.98 (1H, d, $^3J_{NH-\alpha CH2}$ 7.2 Hz, Leu NH), 7.92 (1H, d, $^3J_{NH-\alpha CH2}$ 8.0 Hz, Arg NH), 7.41 (1H, d, $^3J_{NH-\alpha CH}$ 8.0 Hz, Arg α-CH), 7.34 (5H, s, Z aromatic H's), 7.0-6.3 (6H, br, guanidino NH's), 5.01 (2H,

s, Z CH_2), 4.29 (1H, dt, $^3J_{NH-\alpha CH}$ 7.5 Hz, Arg α-CH), 4.21 (1H, dt, $^3J_{NH-\alpha CH}$ 6.0 Hz, Leu α-CH), 3.98 (1H, m, Arg α-CH), 3.85 (2H, dd, $^3J_{NH-\alpha CH2}$ 5.9 Hz, Gly CH_2), 3.73 (2H, overlapping dd, $^3J_{NH-\alpha CH2}$ 5.7 Hz, Gly CH_2), 3.61 (3H, s, ester CH_3), 3.01 (4H, br, Arg δ-CH_2's), 2.57 (4H, t, $^3J_{NH-\alpha-CH}$ 6.5 Hz, Pmc CH_2's), 2.47 (12H, s, 4 x Pmc CH_3's), 2.02 (6H, s, 2 x Pmc CH_3's), 1.76 (4H, m, 2 x Pmc CH_2's), 1.7-1.3 (11H, m, Arg β,γ-CH_2's, Leu β-CH_2, γ-CH), 1.25 (12H, s, 4 x Pmc CH_3's), 0.86, 0.82 (6H, 2 x d $^3J_{\alpha CH-\alpha-CH3}$ 6.2 Hz, Leu δ-CH_3's); δ_C ($CDCl_3$, 50 MHz), 173.4, 172.4, 170.1 (Arg CO's, Leu CO, Gly Co's overlapping), 156.5, 156.3, 156.1 (guanidino C's, urethane CO), 153.5 (Pmc C-6's), 136.3-117.9 (aromatic C's), 73.5 (Pmc C-2's), 54.0, 53.2, 51.0 (Arg α-C's, Leu α-C, ester CH_3), 42.7, 41.0, 40.4, 39.8 (Arg δ-C's, Leu β-C's Gly α-C's), 32.7 (Pmc C-4's), 29.3, 28.6 (Arg β-C's), 26.6 (Pmc C(2)-CH_3's), 25.2 (Arg γ-C's), 24.6 (Leu γ-C), 22.6, 21.4 (Leu δ-C's), 21.3 (Pmc C-3's), 18.3, 17.3, 11.9 (Pmc CH_3's); *m/z* (FAB) 1240, 1238, 972, 203. HRMS 1238.5953, $C_{59}H_{88}N_{11}O_{14}S_2$ (MH+) requires 1238.5953.

N^G-(2,2,5,7,8-Pentamethylchroman-6-sulphonyl)-arginylleucinyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginylglycylglycine Methyl Ester Tosylate, $TosO^-H_2^+$Arg(Pmc).Leu.Arg(Pmc).Gly.GlyOMe (18)

Z.Arg(Pmc)Leu.Arg(Pmc).Gly.GlyOMe (17) (1.112 g, 0.90 mmol) was dissolved in methanol (12 ml) with 4-toluenesulphonic acid monohydrate (0.174 g, 0.92 mmol) and hydrogenolysed in the presence of 10% palladium on charcoal (0.18 g) over 24 h. The catalyst was removed by filtration through Kieselguhr and the solution concentrated *in vacuo*. Addition of anhydrous ether precipitated the product as a gum which was triturated under ether to give a white powder (1.010 g, 88%), m.p. indefinite, 88-133°C; $[\alpha]_D^{25}$ +3.9° (*c* 1 in MeOH); t.l.c.-C R_F 0.62; ν_{max} ($CHBr_3$) 3700-2700, 3320 (NH), 1660 (amide CO), 1545, 1300 (S=O asymmetric), 1035, 1010 cm^{-1}; λ_{max} 253 nm (ε 29100 dm^3 mol^{-1} cm^{-1}), 221 (82200); δ_H [$(CD_3)_2SO$, 360 MHz] 8.47 (1H, d, $^3J_{NH-\alpha CH}$ 7.7 Hz, Arg NH), 8.27 (1H, t, $^3J_{NH-\alpha CH2}$ 5.8 Hz, Gly NH), 8.16 (2H, m, Gly NH and Leu NH), 8.08 (3H, br, Arg NH_3^+), 7.48 and 7.11 (4H, 2 x d, *J* 8.1 Hz, tosylate aromatic), 7.0-6.3 (6H, br, guanidino NH's), 4.35 (1H, dt, $^3J_{NH-\alpha CH}$ 7.5 Hz, Arg α-CH), 4.23 (1H, dt, $^3J_{NH-\alpha CH}$ 6.0 Hz, Leu α-CH), 3.85 (2H, dd, $^3J_{NH-\alpha CH2}$ 5.9 Hz, Gly CH_2), 3.76 (3H, m, Gly CH_2 and Arg α-CH), 3.62 (3H, s, ester CH_3), 3.04 (4H, t, *J* 6.2 Hz, Arg α-CH_2's), 2.57 (4H, t, *J* 6.5 Hz, Pmc CH_2's), 2.47 (12H, s, Pmc CH_3's), 2.02 (6H, s, Pmc CH_3's), 1.76 (2 x 2H, t, *J* 6.6 Hz, Pmc CH_2's), 1.7-1.4 (1H, m, Arg β,γ-CH_2's, Leu β-CH_2, γ-CH), 1.25 (12H, s, Pmc CH_3's), 0.89, 0.87 (6H, 2 x d, $^3J_{\gamma CH-\delta CH3}$ 6.6 Hz, Leu δ-CH_3's); *m/z* (FAB) 1104, 838, 203. HRMS 1104.5586, $C_{51}H_{82}N_{11}O_{12}S_2$ (M+) requires 1104.5585.

Nα-Benzyloxycarbonylleucinyl-N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-arginylleucinyl-N^G-(2,5,7,8-pentamethylchroman-6-sulphonyl)-arginylglycyl-glycine Methyl Ester, Z.Leu.Arg(Pmc).Leu.Arg(Pmc),Gly.GlyOMe (19)

Z.LeuOH (oil) (0.239 g, 0.90 mmol) was dissolved in dry DCM (10 ml) and cooled to -5°C. A solution of DppCl (0.214 g, 0.90 mmol) in DCM (2.1 ml) was added followd by NMM (99 μl, 0.90 mmol) and the reaction left to stir for 5 minutes at -5°C. A solution of $TosO^-H_2^+$Arg(Pmc).Leu.Arg(Pmc).Gly.GlyOMe (18) (0.900 g, 0.71 mmol) in DMF (10 ml) was added followed by NMM (77.5 μl, 0.70 mmol) and 2,6-lutidine (105 μl, 0.90 mmol) and the reaction left to stir for 1 hour at -5°C and for a further hour at room temperature. The solvents were then removed under reduced pressure and the residue taken up in DMF. Addition of dilute sodium bicarbonate solution precipitated an off-white solid. Reprecipitation from DMF with dilute $NaHCO_3$ offered no significant improvement in purity. Pure product was obtained by 'wet flash' chromatography on a short silica column using 8% methanol in chloroform as eluant. The contaminated fractions were pooled and subjected to gel filtration on Sephadex LH20 eluted with DMF. All fractions containing pure compound from both columns were evaporated, then taken up in chloroform and precipitated by the addition of *n*-hexane (0.582 g, 61%), m.p. indefinite, 92-123°C; (Found: C, 57.0; H, 7.38; N, 12.1. $C_{65}H_{98}N_{12}O_{15}S_2.H_2O$ requires C, 57.0; H, 7.36; N, 12.3%), $[\alpha]_D^{27}$ +21.0° (*c* 1 in $CHCl_3$); t.l.c.-B R_F 0.46; ν_{max} ($CHCl_3$) 3440, 3320 (NH), 1650 (amide CO), 1545, 1300 (S=O asymmetric), 1115 cm^{-1}; λ_{max} 252 nm (ε

28100 dm^3 mol^{-1} cm^{-1}), 223 (58200); δ_H [$(CD_3)_2SO$, 360 MHz] 8.24 (1H, t, $^3J_{NH-\alpha CH2}$ 6.0 Hz, Gly NH), 8.19 (1H, t, $^3J_{NH-\alpha CH2}$ 5.7 Hz, Gly NH), 7.99 (1H, d, $^3J_{NH-\alpha CH}$ 7.8 Hz, Arg NH), 7.95 (1H, d, $^3J_{NH-\alpha CH}$ 7.3 Hz, Leu NH), 7.88 (1H, d, $^3J_{NH-\alpha CH}$ 7.8 Hz, Arg NH), 7.41 (1H, d, $^3J_{NH-\alpha CH}$ 8.0 Hz, Leu NH), 7.34 (5H, s, Z aromatics), 7.0-6.3 (6H, br, guanidino NH's), 5.01 (2H, Abq, *J* 12.6 Hz, Z CH_2), 4.22 (3H, m, 2 x Arg α-CH and Leu α-CH), 4.04 (1H, m, Leu α-CH), 3.85 (2H, dd, $^3J_{NH-\alpha CH2}$ 5.8 Hz, Gly CH_2), 3.74 (2H, overlapping dd, $^3J_{NH-\alpha CH2}$ 6,7 Hz, Gly CH_2), 3.62 (3H, s, ester CH_3), 3.03 (4H, m, Arg δ-CH_2's), 2.57 (4H, t, *J* 6.3 Hz, Pmc CH_2's), 2.47 (12H, s, 4 x Pmc CH_3's), 2.02 (6H, s, Pmc CH_3's), 1.76 (2 x 2H, overlapping t, 2 x Pmc CH_2's), 1.7-1.3 (14H, m, 2 x Arg β,γ-CH_2's, 2 x Leu β-CH_2, γ-CH), 1.25 (12H, s, Pmc CH_3's), 0.95-0.80 (12H, m, Leu δ-CH_3's); *m/z* (FAB) 1351, 1086, 203. HRMS 1351.6794, $C_{65}H_{99}N_{12}O_{15}S_2$ (MH+) requires 1351.6794.

Nα-9-Fluorenylmethoxycarbonylglycyl p-Alkoxybenzyl Alcohol Resin

p-Alkoxybenzyl alcohol resin (Bachem, 0.65 mmol/g) (2.222 g, 1.44 mmol) was swollen in dry DCM (10 ml) and then treated with a solution of Fmoc.GlyCl (2.5 mmol)[23] in DCM (10 ml), NMM (275 μl), and a solution of 4-dimethylaminopyridine (10 mg, 80 μmol) in DCM (1 ml). The reaction mixture was stirred for 2 h, then filtered, and washed with DCM. The acylation was then repeated in the same manner, but using DMF as the solvent. The resin was filtered after 2 h, washed with DMF and DCM, and dried. Elemental and infrared analysis indicated complete derivatisation at this stage, but the resin was capped by swelling in DCM (10 ml) and treatment with acetic anhydride (1 ml) and pyridine (1 ml) for 30 minutes. The resin was finally washed with DCM, and dried (2.479 g); (Found: N, 0.77%. Fmoc.Gly-Resin (0.55 mmol/g) requires N, 0.77%); ν_{max} (KBr disc) 3430 (NH), 1730 cm^{-1} (ester CO and urethane CO); *cf.* *p*-alkoxybenzyl alcohol resin, ν_{max} (KBr disc) 3560 cm^{-1} (OH).

Solid Phase Synthesis of Ubiquitin Fragment 48-76

Nα-9-Fluorenylmethoxycarbonylglycyl *p*-alkoxybenzyl alcohol resin (0.90 g, 0.5 mmol) was placed in the reaction vessel of an Applied Biosystems model 430A automated peptide synthesiser. Preformed symmetrical anhydrides were prepared from Fmoc amino acids (2.0 mmol) and diisopropyl carbodiimide (DIC) (1.0 mmol) in dimethylacetamide (DMA, Aldrich h.p.l.c. grade) and allowed to react together for 30 minutes, whilst simultaneous deprotection of the Fmoc-peptide-resin was achieved using 20% piperidine in DMA. The symmetrical anhydride was added to the resin-bound peptide and allowed to react for 1 h. Each Fmoc amino acid (1 mmol) was subsequently activated using DIC (1 mmol) and 1-hydroxybenzotriazole (HOBt) (1 mmol) over 30 minutes before addition to the resin and allowing to react for a further hour. The resin was thoroughly washed in preparation for the next cycle. Fmoc.AsnOH, Fmoc.GlnOH, and Fmoc.Arg(Pmc)OH were each coupled twice using only the DIC-HOBt procedure. After each complete coupling cycle a resin sample (*ca* 10 mg) was automatically removed for quantitative analysis (Figure 3.23). After completion of 28 cycles, approximately 10% (0.5 g) of peptide-resin was removed. Amino acid analysis: Asx_3, Thr_2 1.97, Ser_2 1.85, Glx_4 4.24, Gly_3 3.09, Val_1, 0.96, Ile_1 1.05, Leu_6 5.59*, Tyr_1 0.89, His_1 1.31, Lys_2 1.93, Arg_3 3.08. *Leucine analysis off scale

To the dried resin (0.5 g) was added thioanisole (2.5 ml) and the resulting slurry stirred for 10 minutes to allow permeation of the resin. TFA:H_2O (95:5) (50 ml) was added and the reaction stirred at room temperature for 1.5 h during which time the mixture turned yellow. The cleaved resin was removed by filtration and the solution evaporated under reduced pressure. The residue was partitioned between 10% aqueous acetic acid and ethyl acetate, which gave an emulsion. Washing was achieved by addition of ether and careful decantation**. The aqueous solution was lyophilised to give a white powder (230 mg) which was divided into 4 equal portions and partially purified by gel filtration on Sephadex G-15 eluted with 30% aqueous acetic acid. Product-containing fractions were identified by u.v. detection (254 nm, absorbance range 2.0) and optical rotation (589 nm). All fractions containing optically active product were evaporated *in vacuo* and subsequently lyophilised. Further purification was

achieved by preparative h.p.l.c. on a Partisil ODS-3 10 μ column (10 x 250 mm) (see general experimental section) to yield the pure product (*ca* 30 mg) *m/z* (FAB) (after gel filtration) 3564 (MH+), 3532 (M+-Gly.Gly). HRMS 3562.8852, $^{12}C_{158}{}^{13}C_1H_{254}N_{45}O_{48}$ requires 3562.8850. ** In this respect it is probably more convenient to precipitate the peptide by addition of ether.

ACKNOWLEDGMENT

We thank Wendstone Chemicals, Laporte Industries Ltd and SERC for support of JG. We acknowledge the assistance of Kevin Shaw and Brian Whigham with solid phase peptide synthesis and amino acid analysis. We thank Dr C.P. Ashton for his advice and Applied Biosystems Inc. for generous support.

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