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(54) **Peptides, pharmaceutical compositions containing the peptides and a process for the preparation of the peptides.**

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*** Abstract ***

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*** Abstract ***

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*** Abstract ***

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Peptides, pharmaceutical compositions containing the peptides, and a process for the preparation of the peptides

This invention relates to certain peptides which may be used in desensitisation therapy, and to pharmaceutical compositions containing them.

It is known that allergic reactions in allergic humans are largely caused by the release of histamine from mast cells. This release is taken to be caused by the cross-linking of IgE antibodies attached to mast cells by allergen, which cross-linking is believed to distort the antibodies thereby bringing basic portions of the antibodies into proximity with the cell surface, which in turn causes release of histamine from the cell. It is also known that this histamine release from mast cells can be minimized by the use of antificial liberators, such as melittin, ACTH and fragments thereof (D.R. Stanworth, "Immediate Hypersensitivity", Chapter 8, North Holland Publishing Company, London, 1973, and B. Jasam and D.R. Stanworth Int. Arch. Allergy 45,74—81 (1973)).

In Belgian Patent No. 840193, one method for treating allergies is proposed. This is the use of a short amino acid sequence from the Fc portion of IgE to block IgE receptor sites on mast cells. In this way it is proposed that the allergen caused IgE cross-linking reaction, leading to histamine reiease from the mast cell to which the IgE is bound, maybe prevented or inhibited, as IgE molecules will be blocked from binding to mast cells by the presence on the mast cells of the said short amino acid sequences.

To put the disclosure of this Belgian Patent into perspective, it should be noted that although in our hands some success was obtained in confirming some aspects of the proposed system (D.R. Stanworth *et al*, Int. Arch Allergy appl. Immuno 56:409—415 (1978)) with the preferred penta peptide, other workers generally accepted as among the world's leading experts in the field were unable to demonstrate *any* of the claimed activity with the preferred penta peptide (Bennich, Ragnarsson, Johansson, K. Ishizaha, T. Ishizaka, Levy and Lichtenstein, Int. Archs Allergy appl. Immuno 53:459—468 (1977)).

A class of peptides has now been discovered which can be used in the desensitisation therapy of allergic humans. In complete contrast these peptides act by releasing histamine from mast cells, not by inhibiting or preventing such histamine release as alleged for the peptides disclosed in the said Belgian Patent.

Accordingly the present invention provides a peptide or a salt thereof, characterised by containing 6 to 12 naturally occuring amino acid residues in a sequence $-[-R_1—R_2—R_3-]-$, wherein, R_1 consists of a residue of a basic amino acid, optionally linked to one or more residue of a neutral non-hydrophobic amino acid and/or to one or more further residue of a basic amino acid; R_2 consists of one or more residue of a neutral non-hydrophobic amino acid; and R_3 consists of a residue of a hydrophobic amino acid, optionally linked to one or more residue of a neutral non-hydrophobic amino acid and/or to one or more further residue of a hydrophobic amino acid; the said basic amino acid residues are selected from arginyl, lysyl and ornithyl; the said neutral non-hydrophobic amino acid residues are selected from glycyl, alanyl, seryl and threonyl; and the said hydrophobic amino acid residues are selected from phenylalanyl, valyl and leucyl; said peptide having the formula (I):

$$X—R-[-R_1—R_2—R_3-]-Y \qquad (I)$$

wherein the sequence $-[-R_1—R_2—R_3-]-$ is as defined and; X is hydrogen, or a N-protecting group; Y is hydroxy, or a C-terminal protecting group; and R is an optionally present group, capable of confering on a peptide resistance to enzyme breakdown.

Unless otherwise stated, the amino acids referred to hereafter are in the L-configuration.

When R is present, it is a group capable of confering on a peptide resistance to enzyme breakdown. Examples of suitable groups R are given in J. Rudinger, "The Design of Peptide Hormone Analogues", Chapter 9 in Drug Design, Volume (II) edited by E. J. Ariëns, Academic Press, New York and London, 1971.

Thus suitable examples of R, when present, include prolyl, hydroxyprolyl, the D— form of a common amino acid residue or an amino acid residue with omission of the terminal amino group.

Particularly suitable examples of R_1 include Lys-Thr-Lys and Arg-Lys-Thr-Lys. Normally R_1 will consist of 1 to 5 amino acid residues, suitably 3 to 5 residues. R_1 will often contain at least two basic amino acid residues and at least one neutral non-hydrophobic amino acid residue.

A particularly suitable example of R_2 is Gly-Ser-Gly. Preferably R_2 consists of 1 to 5 amino acid residues, for example 3 amino acid residues.

Particularly suitable examples of R_3 include Phe-Phe and Phe-Phe-Val-Phe. Preferably R_3 consists of 1 to 4 amino acid residues, for example 2 or 4 residues.

X is hydrogen or a N-protecting group. Suitable examples of N-protecting groups X include those conventionally known for this use in peptide chemistry. Examples of such groups include carboxylic acid groups such as acetyl, chloroacetyl, trifluoroacetyl, butyryl, benzoyl, phenylacetyl, pyridine-carbonyl; or an acid group derived from carbonic acid such as ethoxycarbonyl, benzyloxycarbonyl, t-butyloxy-carbonyl, biphenylisopropoxycarbonyl, p-methoxy-benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-phenylazobenzyloxycarbonyl, p-(p'-methoxyphenylazo)-benzyloxy-

carbonyl, t-amyloxycarbonyl; or an acid group derived from a sulphonic or p-toluene-sulphonic acid; or other groups such as benzyl, trityl, formyl, phthaloyl, o-nitrophenylsulphenyl, benzylidene or nitro. Preferred N-protecting groups X include t-butyloxycarbonyl or benzyloxycarbonyl.

Suitable C— terminal protecting groups Y include ester residues, for example residues of C_{1-6} alkyl esters such as methoxy, ethoxy and *t*-butoxy, benzyloxy, *p*-nitrobenzyloxy, *p*-methoxybenzyloxy; residues of trimethylsilyl esters; and residues of amides, substituted amides (e.g. amides substituted by one or two C_{1-6} alkyl groups, or by a C_{1-6} acyl group), and hydrazino residues. Preferred groups Y include hydroxyl and methoxy.

The peptides of the invention have 6 to 12 amino acid residues in the $-[-R_1-R_2-R_3-]-$ sequence. Preferably they have 8 to 10 amino acid residues in this sequence.

One particularly suitable group of peptides is of formula (II):

$$X—R—[b\text{-}c\text{-}d\text{-}e]—[f\text{-}g\text{-}h]—[i\text{-}j\text{-}k\text{-}l]—Y \qquad (II)$$

wherein X, Y and R are as defined; c and e are lysyl, arginyl or ornithyl; d is threonyl or seryl; b is an optionally present arginyl; lysyl or ornithyl; f and h are glycyl or alanyl; g is seryl or threonyl; i and j are phenylalanyl, valyl or leucyl; and k and l are optionally present phenylalanyl, valyl or leucyl; and salts thereof.

Preferably in formula (II) X is hydrogen and Y is hydroxyl, $—NH_2$ or C_{1-4} alkoxy such as methoxy, and, when R is present, it is prolyl or hydroxyprolyl.

Examples of peptides within the scope of the invention are:—

Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1

wherein Y^1 is hydroxyl, $—NH_2$ or methoxy.

The peptides of this invention may be prepared by methods known in the art of peptide synthesis comprising the sequential coupling of the amino acids from which the peptide is derived.

Methods of sequential coupling of amino acids to form peptides by forming amide links are well known in the art. In general the amino acids, provided with protecting groups where necessary, are coupled in the correct order, or smaller peptides are combined into larger units. The amide linkage is usually prepared by condensing an amino acid, or peptide, having a protected α-amino group and a free or activated terminal carboxyl group, with an amino acid or peptide with a protected carboxyl group and a free α-amino group.

Activation of the carboxyl group can be effected, for example, by converting the carboxyl group into an acid halide, an azide, anhydride or imidazolide, or into an activated ester such as the cyanomethyl ester, p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, pentachlorophenyl ester, N-hydroxysuccinimide ester or benztriazole ester.

The most widely used methods of condensation of amino acids or peptides include the carbodiimide method, the azide method, the anhydride method, and the activated esters method, as described, for example, by Schroder and Lubke in "The Peptides", Volume 1 (1969), (Academic Press). An alternative method is the solid phase method of Merrifield (J. Am. Chem. Soc., 85, 2149 (1963)).

Any reactive groups in the amino acid or peptide which are not to take part in the condensation reaction should be protected by any of the N-protecting groups or carboxyl protecting groups described above which can be readily removed after the condensation.

The removal of the protecting group(s) present in the resultant peptide may be effected by an appropriate procedure depending upon the kind(s) of the protective group(s). Some typical procedures are as follows: hydrogenation in the presence of palladium catalyst (e.g. palladium carbon, palladium black) for benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromo-benzyloxycarbonyl, p-phenylazo-benzyloxycarbonyl, p-(p'-methoxyphenylazo)-benzyloxycarbonyl and trityl groups protecting the amino end; treatment with hydrogen bromide in glacial acetic acid for benzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-phenylazobenzyloxycarbonyl and t-butyloxycarbonyl groups protecting the amino end; treatment with metallic sodium in liquid ammonia for benzyloxycarbonyl, p-bromobenzyloxycarbonyl and tosyl groups protecting the amino end; treatment with hydrochloric acid and/or acetic acid for trityl, t-butyloxycarbonyl, formyl and benzylidene groups protecting the amino end; treatment with alkali for methyl, ethyl and benzyl esters protecting the carboxyl end; treatment with acid for methyl, ethyl, benzyl, p-methoxybenzyl and t-butyl esters protecting the carboxyl end; and hydrogenation in the presence of palladium catalyst for benzyl and p-nitrobenzyl esters protecting the carboxyl end.

Acid addition salts of compounds of formula (I) are included within this invention, for example the salts of pharmaceutically acceptable acids as a hydrohalide, especially the hydrochloride or hydrobromide; or the phosphate, acetate, phenylpropionate, maleate, tartrate and citrate.

The peptides and salts of the present invention may be employed as the active agents in desensitisation vaccines. Such vaccines are well known to those skilled in the art and comprise a sterile liquid vehicle in which the active agent is dissolved or suspended. If suspended, the particles of active agent should be small enough not to block the orifice of an injection needle. Certain adjuvants such as tyrosine are often included in such vaccine compositions and are believed to provide a support and prolonged slow release of active material *in vivo.* Usually a patient receiving treatment with such desensitisation vaccines is administered a number of injections, spread over a period of weeks or days, each injection containing a higher concentration of active agent than the preceding one. In this way the patient is desensitised such that his allergic reaction to allergens is reduced or eliminated.

An alternative mode of administration for desensitisation agents is by application to the nasal mucosa as a liquid spray or as a dry powder snuff.

Yet another possible route of administration would be by application to the buccal mucosa, again as a liquid or dry composition.

Accordingly, the present invention includes a pharmaceutical composition for use in desensitisation therapy, comprising a peptide or pharmaceutically acceptable salt of formula (I) together with a pharmaceutically acceptable carrier suitable for parenteral, intra-nasal or buccal administration.

The compositions of the invention may be administered in conventional manner for desensitisation therapy.

The invention also provides a peptide of the formula (I) as defined, or a salt thereof, for use in the desensitisation therapy of allergies.

The preparation and properties of some of the peptides of this invention are illustrated by the following examples.

Peptides were synthesized by classical methods of peptide synthesis described in the literature of peptide chemistry, for example by means of classical solution synthesis or solid phase peptide synthesis (SPPS), or by use of a combination of these methods.

Where appropriate amino acids refer to the L-configuration unless otherwise stated, and the following abbreviations are used:

BOC	Tert-Butyloxycarbonyl	BAW	*n*-Butanol:Acetic Acid:water
Bzl	Benzyl	$CHCL_3$	Chloroform
Z	Benzyloxycarbonyl (carbobenzoxy)	MES	Morpholine Ethyl Sulphonic Acid
OTcP	2,4,5 Trichlorophenyl ester	EtAc	Ethyl Acetate
DMF	Dimethylformamide	MeOH	Methanol
Et_3N	Triethylamine	EtOH	Ethanol
DCCI	Dicyclohexylcarbodiimide	—OMe	Methyl ester
THF	Tetrahydrofuran	AcOH	Acetic Acid
OSu	N hydroxysuccinimide ester	HCL	Hydrochloric Acid
OBz	Benzyl ester	TLC	Thin Layer Chromatography
MDC	Methylene dichloride	M.P.	Melting Point
Rf	Ratio of product distance:solvent front distance, from point of application	NMR	Nuclear Magnetic Resonance
		TFA	Trifluoroacetic acid
C	Number of grams per 100 mls	p.Tsa	p-Toluensulphonate
$[\alpha]_D^{25}$	Specific rotation at 25°C using sodium light (the D line)	R.T.	Room Temperature

Example 1

The preparation of LysThrLysGlySerGlyPhePheOMe

The octapeptide methyl ester was prepared by a 4 + 4 fragment condensation strategy, one fragment (I) being prepared by solid phase peptide synthesis (SPPS) (according to SPPS Manual by J. M. Stewart and J. D. Young Freeman and Company, San Francisco, 1969) and the other fragment (II)

by classical solution synthesis. Combination of I and II gave fully protected octapeptide (III) which on deprotection afforded the desired product (V).

(I) *BOC Lys(Z)Thr(Bzl)Lys(Z)Gly N_2H_3*

This intermediate was prepared by SPPS, employing standard DCCI mediated coupling procedures using a 0.47 mM/g glycine substituted Merrifield Resin. The fully protected tetrapeptide-resin was cleaved by treatment with 100 equivalents of hydrazine hydrate in DMF at room temperature for 3 days. Standard work-up gave I in good yield. This was crystallised from EtOH/water and then EtAc; m.p. 132—134°C; TLC homogeneous in 9:1 $CHCl_3$:MeOH/I_2 stain with Rf 0.44; NMR consistent with structure; $[\alpha]_D^{25} = -1.3°$ (C = 1, DMF); amino acid analysis:
required: 1.00 Thr: 1.00 Gly: 2.00 Lys.
found: 1.00 Thr: 1.05 Gly: 2.01 Lys.

(II) *Ser(Bzl)GlyPhePheOMe.HCl*

The tetrapeptide methyl ester hydrochloride was prepared by solution synthesis in six stages.

(i) *BOC-Phe.Phe.OMe:*

BOC-Phe-OSu (5.25 g, 0.0145 M) was coupled to Phe.OMe. HCL (3.13 g, 0.0145 M) in DMF (25 ml) in the presence of 1 equivalent of Et_3N (2.03 ml) at room temperature over 3 days. The reaction mixture was poured into water (250 ml) and the product extracted into EtAc (100 ml). It was isolated in 81% (5.00 g) yield and crystallisation from petrol (b.pt. 80—100°C) gave a m.p. of 123—124°C; $[\alpha]_D^{25} = -11.0°$ (C = 1, DMF).

(ii) *Phe.Phe.Ome.HCl:*

The intermediate (i) (4.65 g) was BOC-deprotected using a solution of 2*N* HCL in EtAc (30 ml) over 2 hours at room temperature. The product precipitated from solution in 78% yield (3.10 g) and had m.p. 205°C; $[\alpha]_D^{25} = 43.3°$ (C = 1, AcOH).

(iii) *BOC.Gly.Phe.Phe.OMe:*

BOC.Gly.OSu (2.18 g, 0.008 M) was coupled to (ii) (2.90 g, 0.008 M) in DMF in the presence of 1 equivalent of Et_3N (1.12 ml) at room temperature over 3 days. Similar work-up described for isolation of (i), gave the product in 67% yield (2.60 g); m.p. 159—161°C, after crystallisation from EtAc (40 ml) $[\alpha]_D^{25} = -9.5°$ (C = 1, DMF); amino acid analysis:
required: 1.00 Gly: 2.00 Phe.
found: 1.00 Gly: 2.02 Phe.

(iv) *GlyPhePheOMe.HCl:*

The intermediate (iii) (2.60 g) was BOC-deprotected in a similar manner to that described for (ii). The product deposited as an oil which was triturated with ether to give a white crystalline solid in almost quantitative yield. The material was purified further on Sephadex LH20 column eluting with water and had m.p. 196—199°C; TLC in 9:1 $CHCl_3$:MeOH showed one spot with I_2 stain at Rf. 0.22. Amino acid analysis:
required: 1.00 Gly: 2.00 Phe.
found: 1.00 Gly: 1.94 Phe.

(v) *BOC.Ser(Bzl)GlyPhePheOMe:*

BOC.Ser(Bzl)OH (1.66 g, 0.0056 M) was coupled to (iv) (2.36 g, 0.0056 M) in MDC (20 ml) at 0°C using DCCI (1.16 g, 0.0056 M) and Et_3N (0.79 ml: 1 equivalent). The reaction mixture was stirred at 0°C for $\frac{1}{2}$ hour, room temperature for 2 hours, filtered and filtrate evaporated *in vacuo.* Crystallisation of the residue from EtAc/petrol (80—100°C) afforded a 67% yield (2.50 g) of product, m.p. 163—167°C. TLC in 9:1 $CHCl_3$:MeOH (I_2 stain) showed product at Rf 0.68; $[\alpha]_D^{25} = -13.0°$ (C = 1, DMF). The NMR spectrum was consistent with structure. Amino acid analysis:
required: 1.00 Ser: 1.00 Gly: 2.00 Me.
found: 0.94 Ser: 1.12 Gly: 2.00 Phe.

(vi) *Ser(Bzl)GlyPhePheOMe.HCl (III):*

Intermediate (v) (1.75 g) above was BOC-deprotected in a similar manner to that described for (ii). Addition of ether to the reaction mixture gave the product as a solid in 96% yield (1.52 g), $[\alpha]_D^{25} = 21.0°$ (C = 1, AcOH). It was purified on Sephadex LH20 eluting with 1M AcOH, $[\alpha]_D^{25} = 21.0°$ (C = 1, AcOH) TLC examination in 9:1 $CHCl_3$:MeOH (I_2 stain) showed product (acetate salt) as one spot at Rf 0.27. The NMR spectrum was consistent with structure. Amino acid analysis:
required: 1.00 Ser: 1.00 Gly: 2.00 Phe.
found: 1.00 Ser: 1.11 Gly: 1.92 Phe.

(III) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheOMe*

Tertiary-butyl nitrite (0.32 ml, 0.00266 M) was added with vigorous stirring to a solution of (I) (1.60 g, 0.00177 M) in DMF (30 ml) containing 60 equivalents 2*N* HCL in THF (5.5 ml, 0.0011 M) at −20°C. After 30 minutes, (II) (1.05 g, 0.00177 M) in DMF (5 ml) with sufficient Et_3N (2.11 ml) present to neutralise all HCl present, was added, and the reaction mixture stirred for 18 hours at 4°C, filtered and filtrate concentrated *in vacuo.* Addition of cold water gave the product which was obtained in 50% yield (1.28 g) after crystallisation from EtOH. TLC in 9:1 $CHCl_3$:MeOH (I_2 stain) was homogeneous and showed product at Rf 0.6. M.p. 202—203°C; $[\alpha]_D^{25} = -5.9°$ (C = 1, DMF). The NMR spectrum was consistent with structure. Amino acid analysis:
required: 2.00 Lys: 2.00 Phe: 2.00 Gly: 1.00 Thr: 1.00 Ser.
found: 2.00 Lys: 1.94 Phe: 2.06 Gly: 0.99 Ser: 0.96 Thr.

(IV) *Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheOMe.HCl*

Fully protected octapeptide (III) (1.20 g) was BOC-deprotected in 2*N* HCl solution in a 6:14 DMF/EtAc solvent mixture (20 ml). Prolonged reaction time of 4 hours was used at room temperature and addition of ether deposited the product. Recrystallisation from MeOH/ether gave a 52% yield (0.60 g) of product. TLC in 9:1 $CHCl_3$:MeOH (I_2 stain) showed one major spot at Rf 0.4; $[\alpha]_D^{25} = 10.1°$ (C = 1, AcOH). Amino acid analysis:
required: 1.00 Thr: 1.00 Ser: 2.00 Gly: 2.00 Phe: 2.00 Lys.
found: 1.00 Thr: 1.00 Ser: 2.17 Gly: 2.19 Phe: 2.32 Lys.

(V) *LysThrLysGlySerGlyPhePheOMe*

Partially protected octapeptide (IV) (0.10 g) was hydrogenated in 85% AcOH (70 ml) with 10% Pd/C catalyst (0.20 g) over a steady stream of hydrogen for 20 hours. The mixture was filtered, evaporated *in vacuo* and residue filtered on Sephadex LH20 eluting with water to give the desired octapeptide methyl ester (V) (0.03 g, 46% yield). TLC examination showed 1 spot at Rf 0.2 in 5:2:2 BAW (t-BuOCl/KI-starch stain) and Rf 0.5 in 5:2:3 BAW (Ninhydrin stain). Amino acid analysis:
required: 1.00 Ser: 1.00 Thr: 2.00 Gly: 2.00 Phe: 2.00 Lys.
found: 1.00 Ser: 1.03 Thr: 2.02 Gly: 2.06 Phe: 1.98 Lys.

Isotachophoretic examination showed one band in >95% amount (leading electrolyte 10 mM KOH + MES pH 6.0 and terminating electrolyte 10 mM β-alanine and HCl pH 4.23). The NMR 80 MHz FT spectrum was consistent with structure.

Example 2

The preparation of ArgLysThrLysGlySerGlyPhePheOMe

This nonapeptide was prepared by coupling of (IV) above with $Z.Arg(Z)_2.OSu$, followed by hydrogenolysis of the resultant fully protected nonapeptide.

(i) *$Z.Arg(Z)_2Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheOMe$:*

To octapeptide (IV) (0.344 g, 0.30025 M) above in DMF (3 ml) at 0°C was added (1 equivalent) Et_3N (0.025 g in 1 ml DMF) and $Z\text{-}Arg(Z)_2OSu$ (0.17 g, 0.00025 M in 2 ml DMF). The solution was left at 4°C for 65 hours, diluted with water (8 ml) and the deposited product filtered off and dried (0.37 g, 78% yield). Crystallisation from DMF/EtOH gave product with m.p. 204—210°C (decomposition). TLC examination in 9:1 $CHCl_3$:MeOH (I_2 stain) showed on U.V. visualisation 1 spot at Rf 0.69. The NMR spectrum was consistent with structure. Amino acid analysis:
required: 1.00 Thr: 1.00 Ser: 2.00 Gly: 2.00 Phe: 2.00 Lys: 1.00 Arg.
found: 1.00 Thr: 1.00 Ser: 2.10 Gly: 2.01 Phe: 2.04 Lys: 1.04 Arg.

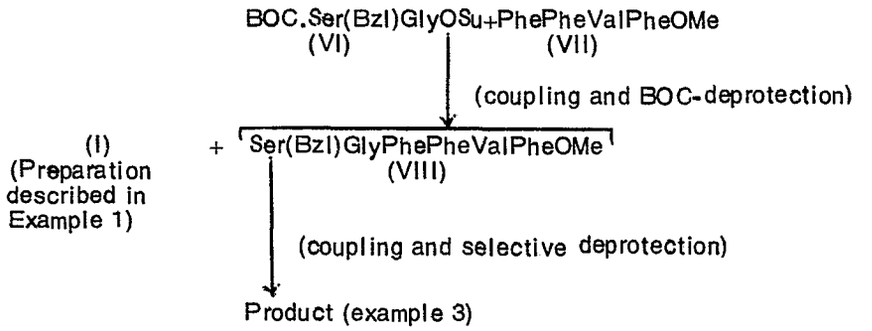
(ii) *ArgLysThrLysGlySerGlyPhePheOMe*

Fully protected nonapeptide (i) (0.07 g) above was dissolved in a minimum amount of DMF and 5 times the volume of AcOH added. The mixture was hydrogenated in the presence of 10% Pd/C catalyst (2.5 times weight of compound) for 19 hours at room temperature using a steady stream of hydrogen. Water was added to give a 15% aqueous solution and the mixture hydrogenated for a further 3 hours. Filtration and evaporation *in vacuo* at 45°C gave product as a glassy solid. Purification was performed on a Sephadex LH20 column eluting with 1M AcOH and product isolated in 26% yield (0.018 g). TLC in 5:3:5 BAW (ninhydrin stain) showed product at Rf 0.34. Amino acid analysis:
required: 1.00 Thr: 1.00 Ser: 2.00 Gly: 2.00 Phe: 2.00 Lys: 1.00 Arg.
found: 1.00 Thr: 1.01 Ser: 2.07 Gly: 2.00 Phe: 2.07 Lys: 1.00 Arg.

Example 3

The preparation of LysThrLysGlySerGlyPhePheValPheOMe

The decapeptide methyl ester was synthesised by a 4 + 2 + 4 fragment condensation strategy as follows:—



(VI) *BOC.Ser(Bzl)Gly.OSu* Prepared in three stages:—

(i) *BOC.Ser(Bzl)GlyOMe*

BOC.Ser(Bzl)OH (5.0 g, 0.017 M) was coupled to Gly.OMe.HCl (2.13 g, 0.017 M) in M.D.C. (100 ml) at R.T. for $3\frac{1}{2}$ hours in the presence of 1 equivalent of Et_3N and using DCCI (3.5 g, 0.017 M) as the condensing agent. The precipitate was filtered off and the solution washed X 2 with water, aqueous $NaHCO_3$, water, dried and evaporated *in vacuo* to leave an oil (7.1 g).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.64; $[\alpha]_D^{25°C} = 4.7°$ (C = 1, MeOH).

(ii) *BOC.Ser(Bzl)GlyOH*

Compound (i) above (7.0 g) was dissolved in dioxan (25 ml) and treated with an equal volume of 1*N* NaOH (25 ml) and the solution stirred for $\frac{1}{2}$ hour at R.T. *N* HCl (25 ml) was added to a slight excess and the oil that formed extracted into EtAc. The organic layer was back-extracted into $NaHCO_3$ and acidified to pH 3.8 with 20% citric acid, extracted with EtAc, the organic layer washed with water, brine, dried and evaporated to leave the product as a syrup (4.0 g).

TLC 1:1 $CHCl_3$/EtOH (I_2 stain) showed product at Rf 0.59. The NMR spectrum was consistent with structure.

(iii) *BOC.Ser(Bzl)GlyOSu*

Compound (ii) above (4 g, 0.01135 M) was treated with HOSu (1.3 g, 0.011 M) and DCCI (2.34 g, 0.011 M) in dioxan (50 ml) at R.T. overnight. The precipitate that formed was filtered off, solvent removed and the product crystallised from I.P.A. (100 ml) in 59% yield (3.00 g).

TLC 9:1 $CHCl_3$/MeOH (I_2 stain) showed one major spot Rf 0.57; M.P. 132—134°C; $[\alpha]_D^{25} = -1.8°$ (C = 1, MeOH).

(VII) *PhePheValPheOMe* Prepared in six steps:—

(i) *BOC.ValPheOMe*

BOC.ValOSu (10.0 g, 0.0328 M) was coupled to PheOMe.HCl (6.85 g 0.0318 M) in toluene (2.00 ml) at room temperature overnight and in the presence of Et_3N (1 equivalent). The mixture was filtered and filtrate washed with 1N HCl, saturated NaCl solution, dried and evaporated *in vacuo* to give the product (11.31 g) as a white crystalline compound in 94% yield.

TLC in 9:1 ($CHCl_3$:MeOH) (I_2 stain) shows one spot at Rf 0.77 $[\alpha]_D^{25} = -27.4°$ (C = 1, MeOH).

(ii) *ValPheOMe.HCl*

Compound (i) (9.25 g) was BOC-deprotected in 2N HCl in EtAc (100 ml) for 24 hours at room temperature when the product precipitated. The mix was diluted with dry EtAc and product filtered off in 78% yield (6.0 g). The product was finally purified on Sephadex LH20. M.P. 193—193.5°.

TLC 9:1 $CHCl_3$/MeOH (I_2 stain) shows one spot at Rf 0.60. $[\alpha]_D^{25} = 63.3°$ (C = 1, AcOH).

(iii) *BOC.PheValPheOMe*

Compound (ii) (5.34 g, 0.017 M) was coupled to BOC.PheOSu (6.15 g, 0.017 M) in 25% DMF in toluene (250 ml) at room temperature for 65 hours in the presence of Et_3N (1 equiv.). The mixture was then filtered, solvent removed *in vacuo* and the syrup quenched with water. The white precipitate (8.5 g) was filtered off and recrystallised from EtAc/80—100° petrol; yield 80%.

TLC 9:1 $CHCl_3$/MeOH (I_2 stain) shows one spot at Rf 0.69 $[\alpha]_D^{25} = -31.0°$ (C = 1, MeOH). NMR consistent with structure.

(iv) *PheValPheOMe.HCl*

Compound (iii) (6.87 g) was BOC-deprotected in 2*N* HCl in EtAc (100 ml) for 2 hours at room temperature when a white solid precipitate (5.84 g) representing 97% yield of product. M.P. 243—245° (decomposition).

TLC 9:1 $CHCl_3$/MeOH (I_2 stain) shows one spot at Rf 0.59. $[\alpha]_D^{25°} = 8.8°$ (C = 1, AcOH).

(v) *BOC.PhePheValPheOMe*

BOC.PheOSu (4.30 g, 0.0119 M) was coupled to compound (iv) (5.5 g, 0.0119 M) in toluene (100 ml) at room temperature for 65 hours in the presence of sufficient DMF to produce solution, and also in the presence of Et_3N (1 equivalent). The solvent was evaporated *in vacuo* and the syrup quenched with water and product filtered off. The product was then triturated with hot ethanol, cooled and collected (6.38 g, 80% yield). M.P. 218—219°C.

TLC in 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.62. $[\alpha]_D^{25} = -15.9°$ (C = 1, DMF). The NMR spectrum was consistent with structure.

(vi) *PhePheValPheOMe.Tfa*

Compound (v) (5 g) was BOC-deprotected in T.F.A. (25 ml) at 0°C for $\frac{1}{2}$ hour, and at room temperature for $\frac{1}{2}$ hour. The solution was then quenched with ether (75 ml) and the product filtered off (4.48 g, 88% yield). M.P. 224—226° (decomposition).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.49. $[\alpha]_D^{25} = 10.6°$ (C = 1, AcOH). The NMR spectrum was consistent with structure.

(VIII) *Ser(Bzl)GlyPhePheValPhe.OMe* Prepared in two stages:—

(i) *BOC.Ser(Bzl)GlyPhePheValPheOMe*

Intermediate VI (2.37 g, 0.00528 M) was coupled to intermediate VII (3.62 g, 0.00528 M) in toluene (500 ml) overnight at room temperature in the presence of Et_3N (0.74 ml, 0.00528 M). The mixture was washed with water, and solvent evaporated *in vacuo.* The solid obtained was triturated with water, dried and recrystallized from EtOH (4.42 g, yield 93%).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.61. $[\alpha]_D^{25°} = -13.5°$ (C = 1 DMF). The NMR spectrum was consistent with structure.

(ii) *Ser(Bzl)GlyPhePheValPheOMe*

Compound (i) (2.8 g) was BOC-deprotected in T.F.A. (30 ml) for 40 minutes at 0°C. The solution was quenched with ether (200 ml) and the precipitated product obtained in quantitative yield.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.2. M.P. 214—216°C (decomposition). $[\alpha]_D^{25°} = 3.7°$ (C = 1, AcOH). The NMR spectrum was consistent with structure.

BOC.Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheValPheOMe

Peptide I (2.25 g) (see Example 1) was coupled to VIII (2.19 g) by the Honzl-Rudinger modification of the azide method, as previously described for the octapeptide. The product was recrystallized from EtOH and obtained in (2.5 g, 61% yield). M.P. 243—244°.

Amino acid analysis: calculated: 1.00 Thr: 1.00 Ser: 2.00 Gly: 1.00 Val: 3.00 Phe: 2.00 Lys.
found: 1.13 Thr: 1.11 Ser: 2.15 Gly: 1.00 Val: 3.04 Phe: 1.98 Lys.

$[\alpha]_D^{25°} = -7.5°$ (C = 1, DMF). The NMR spectrum was consistent with structure.

LysThrLysGlySerGlyPhePheValPheOMe

The intermediate above (0.18 g) was BOC, Z and Bzl-deprotected by treatment with 33% HBr in dioxan (5 ml) at room temperature for 1 hour when a precipitate formed. Additional HBr/dioxan (5 ml) and water (1 ml) was then added which effected solution and reaction continued for a further $\frac{1}{2}$ hour. Acetone (50 ml) was then added and the solution quenched with ether (100 ml). The supernatent was decanted and solid dissolved in water (7 ml) and freeze-dried to give 0.145 g product as the trihydrobromide salt.

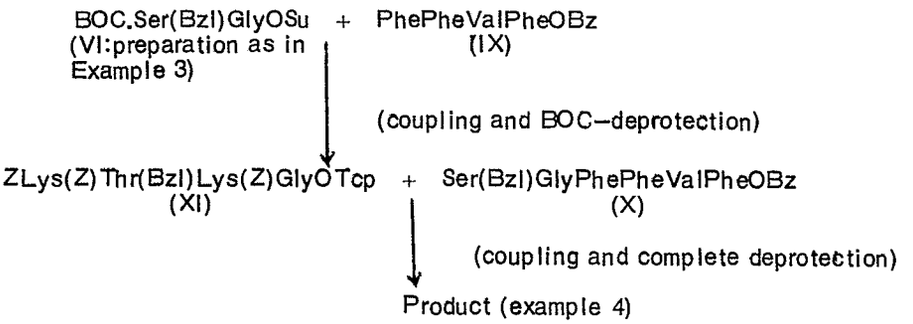
Amino acid analysis: required: 1.00 Thr: 1.00 Ser: 2.00 Gly: 1.00 Val: 3.00 Phe: 2.00 Lys.
found: 1.05 Thr: 1.00 Ser: 2.14 Gly: 1.02 Val: 3.13 Phe: 2.00 Lys.

An aliquot of product was purified on Sephadex LH20 eluting with water, to a one-spot material with Rf 0.384 (BAW 5:2:2, ninhydrin spray).

Example 4

The preparation of LysThrLysGlySerGlyPhePheVal.PheOH

The decapeptide free acid was synthesised by a 4 + 2 + 4 fragment condensation strategy as follows:—



(IX) *PhePheValPheOBz* Prepared in six stages:—

(i) *BOC.ValPheOBz*

BOC.ValOSu (15.7 g, 0.050 M) was coupled to PheOBz.pTsa (21.35 g, 0.050 M) in dioxan (200 ml) at R.T. for 4½ hours in the presence of 1 equivalent of Et_3N. The reaction mixture was evaporated at reduced pressure and the resulting residue dissolved in EtAc and the solution washed with water, dried and evaporated *in vacuo* to leave a crystalline solid (21.3 g).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.93. $[\alpha]_D^{25°} = -31.8°$ (C = 1, MeOH).

(ii) *ValPheOBz.HCl*

Compound (i) (21.3 g) was BOC-deprotected in 2*N* HCl in EtAc (240 ml) for 4½ hours at R.T. when the product precipitated. The mix was diluted with dry ether and product filtered off in 78% yield (15.25 g). M.P. 180—182°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.44. $[\alpha]_D^{25°} = 24.4°$ (C = 1, AcOH).

(iii) *BOC.PheValPheOBz*

Compound (ii) (15.25 g, 0.039 M) was coupled to BOC.PheOSu (14.13 g, 0.039 M) in 50% dioxan/DMF (450 ml) at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The mixture was poured into iced water and the resulting white precipitate (20.0 g) was filtered off and recrystallised from EtAc/40—60° petrol; yield 85%. M.P. 160—162°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.72. $[\alpha]_D^{25°} = -36.0°$ (C = 1, MeOH).

(iv) *PheValPheOBz.HCl*

Compound (iii) (20.0 g, 0.033M) was BOC-deprotected in 2*N* HCl in EtAc (240ml) for 2 hours at R.T. when a white solid precipitated (15.23 g) representing 85% yield of product. M.P. 228—229° (decomposition).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.63. $[\alpha]_D^{25°} = -6.9°$ (C = 1, AcOH).

(v) *BOC.PhePheValPheOBz*

BOC.PheOSu (10.26 g, 0.0283 M) was coupled to compound (iv) (15.23 g, 0.0283 M) in 50% dioxan/DMF (250 ml) at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The mixture was poured into iced water and the resulting white precipitate filtered off and recrystallised from EtAc/40—60° petrol in quantitative yield (21.41 g). M.P. 191—193°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.72. $[\alpha]_D^{25°} = -12.8°$ (C = 1, DMF).

(vi) *PhePheValPheOBz.HCl*

Compound (v) (21.15 g, 0.028 M) was BOC-deprotected in 2*N* HCl in EtAc (500 ML) for 2 hours at R.T. The product (17.9 g) was precipitated in 92% yield upon addition of dry ether. M.P. 242° (decomposition).

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.74. $[\alpha]_D^{25°} = -5.9°$ (C = 1, AcOH). The NMR spectrum was consistent with structure.

(X) *Ser(Bzl)GlyPhePheValPheOBz* Prepared in two stages:—

(i) *BOC.Ser(Bzl)GlyPhePheValPheOBz*

Intermediate (VI) (4.49 g, 0.010 M) was coupled to intermediate (IX) (6.85 g, 0.010 M) in 35% DMF/dioxan (75 ml) at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The mixture was poured

into iced water and the precipitated product (9.39 g) recrystallised from methanol in 91% yield. M.P. 226—228°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.74. $[\alpha]_D^{25°} = -13.0°$ (C = 1, DMF). The F.T. 'H NMR was consistent with structure.

(ii) *Ser(Bzl)GlyPhePheValPheOBz.HCl*

Compound (i) (5.0 g, 0.0051 M) was BOC-deprotected in 2*N* HCl in EtAc (150 ml) for 2 hours at R.T. The product (4.42 g) was precipitated in 94% yield upon addition of dry ether. M.P. 232—234° (decomposition).
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) shows one spot at Rf 0.44. $[\alpha]_D^{25°} = -4.3°$ (C = 1, AcOH). The NMR was consistent with structure.

(XI) *ZLys(Z)Thr(Bzl)Lys(Z)GlyOTcp* Prepared in seven stages:—

(i) *BOC.Lys(Z)GlyOMe*

BOC.Lys(Z)OSu (23.85 g, 0.050 M) was coupled to GlyOMe. HCl (6.25 g, 0.050 M) in 50% dioxan/DMF at R.T. for $4\frac{1}{2}$ hours in the presence of 1 equivalent of Et_3N. The reaction mixture was evaporated *in vacuo* and the residue dissolved in EtAc. The solution was washed, dried, filtered and evaporated to a colourless oil which solidified on standing in 89% yield.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.54.

(ii) *Lys(Z)GlyOMe.HCl*

Compound (i) (20.00 g, 0.0443 M) was BOC-deprotected in 2*N* HCl in EtAc (250 ml) for 2 hours at R.T. when the product precipitated. The mixture was diluted with dry ether and the product filtered off in 96% yield (15.67 g). M.P. 158—159°.
TLC EtAc (I_2 stain) showed one spot at Rf 0.52.

(iii) *BOC.Thr(Bzl)Lys(Z)GlyOMe*

BOC.Thr(Bzl)OH (6.18 g, 0.020 M) was coupled to compound (ii) (7.76 g, 0.020 M) in 30% DMF/dioxan (75 ml) in iced water for 1 hour then at R.T. for a further 2 hours in the presence of DCCI (1 equivalent) and Et_3N (1 equivalent). The reaction mixture was filtered and evaporated *in vacuo* and the residue purified by silica column chromatography eluting with chloroform. The product was isolated as a colourless solid in 49% yield. M.P. 135—136°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.57. The F.T. ^{13}C NMR was consistent with structure.

(iv) *Thr(Bzl)Lys(Z)GlyOMe.HCl*

Compound (iii) (3.48 g, 0.0054 M) was BOC-deprotected in 2*N* HCl in EtAc (100 ml) for 2 hours at R.T. The product (2.88 g) was precipitated in 91% yield upon addition of dry ether. M.P. 100—101°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.52. $[\alpha]_D^{25°} = -13.5°$ (C = 1, AcOH).

(v) *ZLys(Z)Thr(Bzl)Lys(Z)GlyOMe*

ZLys (Z)OTcp (1.80 g, 0.003 M) was coupled to compound (iv) (1.72 g, 0.003 M) in dioxan (45 ml) at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The product was filtered off, washed with water and dried *in vacuo* (1.36 g, 50% yield). M.P. 185—188°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.76.

(vi) *ZLys(Z)Thr(Bzl)Lys(Z)GlyOH*

A solution of compound (v) (0.92 g, 0.001 M) in 50% DMF/methanol was treated with 1*N* NaOH solution (2.5 ml) and stirred at R.T. for 1 hour. Upon acidification the precipitated product (0.45 g) was recrystallised from methanol in 49% yield. M.P. 171—173°.
TLC 2:1 $CHCl_3$:MeOH (t.butyl chloroformate/NaI — starch spray) showed one spot at Rf 0.50. $[\alpha]_D^{25°} = -5.4°$ (C = 1, AcOH). The F.T. ^{13}C NMR was consistent with structure.

(vii) *ZLys(Z)Thr(Bzl)Lys(Z)GlyOTcp*

A solution of TcpOH (0.10 g, 0.0005 M) and compound (vi) (0.46 g, 0.0005 M) in DMF was treated with DCCI (0.11 g, 0.0005 M) and stirred at 5° for 1 hour then at R.T. overnight. The reaction mixture was filtered and the product (0.60 g) isolated as a crispy solid upon evaporation *in vacuo*. M.P. 176—178°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.71. $[\alpha]_D^{25°} = -6.0°$ (C = 1, AcOH).

ZLys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheValPheOBz

Peptide (XI) (0.55 g, 0.0005 M) was coupled to compound (X) (0.46 g, 0.0005 M) in DMF at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The reaction mixture was poured into iced water and

the resulting precipitate filtered off and dried *in vacuo*. Purification by silica column chromatography, eluting with $CHCl_3$, gave the product (0.92 g) in 85% yield.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.34. The F.T. ^{13}C NMR was consistent with structure.

LysThrLysGlySerGlyPhePheValPheOH

The intermediate above (0.76 g, 0.0003 M) was deprotected by continuous hydrogenation in 85% acetic acid with 1*N* HCl (1 mM) for 18 hours in the presence of 10% Pd/charcoal (0.80 g). The product was purified on a Biogel P2 column eluting with 1 M ammonium acetate and subsequently on a CM32 cellulose column eluting with 0.1 M ammonium acetate pH5. Final isolation of the product in 23% yield was by lyopholisation.

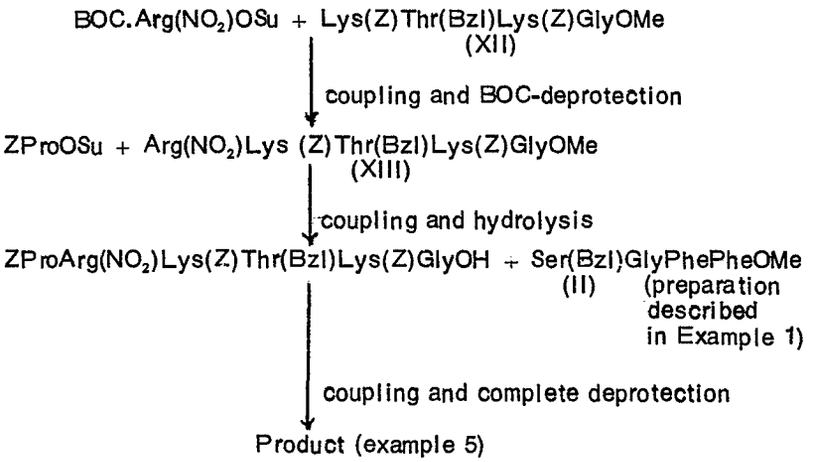
TLC butanol/acetic acid/water (5:2:2) (ninhydrin spray) showed one spot at Rf 0.22. Amino acid analysis:

calculated: 2.00 Lys: 1.00 Thr: 2.00 Gly: 1.00 Ser: 3.00 Phe: 1.00 Val.
found: 1.80 Lys: 0.92 Thr: 2.00 Gly: 1.07 Ser: 3.24 Phe: 0.91 Val.

Example 5

The preparation of ProArgLysThrLysGlySerGlyPhePheOMe

This decapeptide was synthesised by a 1 + 1 + 4 + 4 fragment condensation strategy as follows:—



(XII) *Lys(Z)Thr(Bzl)Lys(Z)GlyOMe* Prepared in two steps from Thr(Bzl)Lys(Z)GlyOMe described in example 4.

(i) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOMe*

BOC.Lys(Z)OSu (2.38 g, 0.005 M) was coupled to Thr(Bzl)Lys(Z)GlyOMe.HCl (2.87 g, 0.005 M) in dioxan (60 ml) at R.T. for 4 hours in the presence of Et_3N (1 equivalent). The reaction mixture was poured into iced water to give the required product (3.80 g) as a crystalline white solid in 84% yield. M.P. 103—105°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.55. $[\alpha]_D^{25°} = -10.4°$ (C = 1, AcOH).

(ii) *Lys(Z)Thr(Bzl)Lys(Z)GlyOMe.HCl*

Compound (i) (3.80 g, 0.0042 M) was BOC-deprotected in 2*N* HCl in EtAc (100 ml) for 2 hours at R.T. The product (3.30 g) was precipitated in 93% yield upon addition of dry ether. M.P. 184—186°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.30. $[\alpha]_D^{25°} = 5.6°$ (C = 1, AcOH).

(XIII) *Arg(NO$_2$)Lys(Z)Thr(Bzl)Lys(Z)GlyOMe* Prepared in two stages:—

(i) *BOC.Arg(NO$_2$)Lys(Z)Thr(Bzl)Lys(Z)GlyOMe*

BOC.Arg(NO_2)OSu (1.40 g, 0.0033 M) was coupled to compound (XII) (3.30 g, 0.004 M) in 10% DMF/dioxan (55 ml) at R.T. for 3 hours in the presence of Et_3N (1 equivalent). Unreacted (XII) was filtered off and the reaction mixture poured into iced water, extracted with EtAc to give the product (2.60 g) which was recrystallised from IPA in 71% yield. M.P. 133—135°.

TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.42. $[\alpha]_D^{25°} = -6.7°$ (C = 1, AcOH).

(ii) *Arg(NO_2)Lys(Z)Thr(Bzl)Lys(Z)GlyOMe.HCl*

Compound (i) (2.0 g, 0.0018 M) was BOC-deprotected in 2*N* HCl in EtAc (50 ml) for 2 hours at R.T. The product (1.75 g) was precipitated in 92% yield upon addition of dry ether. M.P. 157° (decomposition).

TLC 2:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.53. $[\alpha]_D^{25°} = -5.6°$ (C = 1, MeOH).

(XIV) *ZProArg(NO_2)Lys(Z)Thr(Bzl)Lys(Z)GlyOH* Prepared in two stages:—

(i) *ZProArg(NO_2)Lys(Z)Thr(Bzl)Lys(Z)GlyOMe*

ZProOSu (0.57 g, 0.0016 M) was coupled to compound (XIII) (1.71 g, 0.0016 M) in 20% DMF/dioxan (30 ml) at R.T. for 2 hours in the presence of Et_3N (1 equivalent). The reaction mixture was poured into iced water and extracted with EtAc to give the product (1.11 g) in 54% yields.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.36.

(ii) *ZProArg(NO_2)Lys(Z)Thr(Bzl)Lys(Z)GlyOH*

A solution of compound (i) (0.87 g, 0.0007 M) in 50% DMF/methanol (30 ml) was treated with 1*N* NaOH solution (1.7 ml) and stirred at R.T. for 2 hours. Acidification precipitated the product (0.43 g) in 50% yield.
TLC 2:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.77. $[\alpha]_D^{25°} = -4.1°$ (C = 1, AcOH). The NMR was consistent with structure.

ZProArg(NO_2)Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheOMe

Peptide (XIV) (0.40 g, 0.0033 M) was coupled to compound (II) (0.20 g, 0.0034 M) in DMF (5 ml) in the presence of Et_3N (1 equivalent), DCCI (0.07 g, 0.0035 M) and hydroxybenzotriazole (0.044 g, 0.0035 M) at 5° for 1 hour then at R.T. for 1 hour. The precipitated urea was filtered off and the required product (0.50 g) isolated by pouring the reaction mixture into iced water and isolating by filtration in 88% yield.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.52. The NMR was consistent with structure.

ProArgLysThrLysGlySerGlyPhePheOMe

The intermediate above (0.40 g, 0.0022 M) was deprotected by continuous hydrogenation in 85% acetic acid for 18 hours in the presence of 10% Pd/charcoal catalyst (0.40 g). The product was purified on a Biogel P2 column eluting with water and subsequently on an LH20 Sephadex column again with aqueous elution. Final isolation of the product in 34% yield was by lyopholisation.
TLC BAW (5:2:2) (ninhydrin spray) showed one spot at Rf 0.34. Amino acid analysis:

calculated: 1.00 Pro: 1.00 Arg: 2.00 Lys: 1.00 Thr: 2.00 Gly: 1.00 Ser: 2.00 Phe.
found: 0.95 Pro: 0.99 Arg: 1.88 Lys: 1.00 Thr: 2.00 Gly: 1.01 Ser: 1.96 Phe.

Example 6

The preparation of LysThrLysGlySerGlyPhePheOH

The octapeptide free acid was synthesized by a 4 + 4 fragment condensation strategy as follows:—

BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOSu (XVI) + Ser(Bzl)GlyPhePheOBz (XV)

↓ (coupling and complete deprotection)

Product (example 6)

(XV) *Ser(Bzl)GlyPhePheOBz* Prepared in six stages:—

(i) *BOCPhePheOBz*

BOCPheOH (11.88 g, 0.045 M) was coupled to PheOBz.pTsa 19.4 g, 0.045 M) in MDC (200 ml) at 0° for 1 hour then at R.T. overnight in the presence of Et_3N (1 equivalent) and DCCI (1 equivalent). The reaction mixture was filtered and the product (14.92 g) isolated in 64% yield upon evaporation *in vacuo* and recrystallisation from EtOAc/80—100° petrol (14.92 g). M.P. 123.5—124.5°.
TLC 1:1 EtAC: 80—100° petrol (I_2 stain) showed one spot at Rf 0.68. $[\alpha]_D^{25°} = -16.7°$ (C = 1, MeOH).

(ii) *PhePheOBz.Tfa*

Compound (i) (14.0 g, 0.028 M) was BOC-deprotected in 50% TFA in MDC (100 ml) for ½ hour at 0°. The solution was quenched with dry ether and the product (14.23 g) filtered off in quantitative yield. M.P. 180° (decomposition).
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.73. $[\alpha]_D^{25°} = 17.5°$ (C = 1, AcOH).

(iii) *BOCGlyPhePheOBz*

BOC.GlyOSu (9.6 g, 0.0353 M) was coupled to compound (ii) (18.20 g, 0.0353 M) in toluene, MDC and DMF (125 ml) at R.T. overnight in the presence of Et_3N (1 equivalent). The reaction mixture was evaporated at reduced pressure and the resulting residue dissolved in EtAc, washed, dried and evaporated *in vacuo* to leave a crystalline solid (19.71 g) in quantitative yield. M.P. 127—130°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.81. $[\alpha]_D^{25°} = -17.4°$ (C = 1, MeOH).

(iv) *GlyPhePheOBz.Tfa*

Compound (iii) (19.3 g, 0.0346 M) was BOC-deprotected in 50% TFA in MDC (130 ml) for 1½ hours at 0°. The solution was quenched with ether and the product (17.79 g) filtered off in 90% yield.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed a single spot at Rf 0.35. $[\alpha]_D^{25°} = 6.0°$ (C = 1, AcOH).

(v) *BOC.Ser(Bzl)GlyPhePheOBz*

BOC.Ser(Bzl)OSu (11.03 g, 0.0282 M) was coupled to compound (iv) (16.13 g, 0.0282 M) in 15% DMF/toluene (350 ml) at R.T. overnight in the presence of Et_3N (1 equivalent). The reaction mixture was evaporated at reduced pressure and the resulting residue dissolved in EtAc, washed, dried and evaporated *in vacuo* to give the product (14.88 g) in 72% yield upon recrystallisation from EtAc/petrol. M.P. 149—151°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed a single spot at Rf 0.65. $[\alpha]_D^{25°} = -11.2°$ (C = 1, MeOH).

(vi) *Ser(Bzl)GlyPhePheOBz*

Compound (v) (14.45 g, 0.0196 M) was BOC-deprotected in 50% TFA in MDC (140 ml) for 1 hour at 0°. The solution was quenched with ether and the product (13.1 g) filtered off in 89% yield. M.P. 185—187° (decomposition).
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.43. $[\alpha]_D^{25°} = 10.1°$ (C = 1, AcOH).

(XVI) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOSu* Prepared in four stages from BOC.Thr(Bzl)Lys(Z)GlyOMe described in example 4.

(i) *Thr(Bzl)Lys(Z)GlyOMe.Tfa*

BOC.Thr(Bzl)Lys(Z)GlyOMe (2.87 g, 0.0045 M) was BOC-deprotected in 50% TFA in MDC (50 ml) for 1 hour at 0°. The solution was quenched with ether and the product (2.10 g) filtered off in 72% yield.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.26.

(ii) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOMe*

BOC.Lys(Z)OSu (2.00 g, 0.003 M) was coupled to compound (i) (1.45 g, 0.003 M) in 10% DMF/toluene at R.T. overnight. The reaction mixture was evaporated at reduced pressure and the resulting residue dissolved in EtAc, washed, dried and evaporated *in vacuo* to give the product (2.04 g) in 74% yield upon recrystallisation from EtAc/petrol. M.P. 117—119°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.60.

(iii) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOH*

A solution of compound (ii) (1.98 g, 0.0022 M) in DMSO (30 ml) was treated with 1*N* NaOH solution (1½ equivalents) and stirred at R.T. for 1 hour. Acidification gave the product (1.82 g) in quantitative yield.
TLC 5:1 $CHCl_3$:MeOH (I_2 stain) showed compound just above baseline.

(iv) *BOC.Lys(Z)Thr(Bzl)Lys(Z)GlyOSu*

HOSu (0.24 g, 0.002 M) was coupled to compound (iii) (1.82 g, 0.002 M) in dioxan (25 ml) at R.T. for 4 hours in the presence of DCCI (1 equivalent). The reaction mixture was filtered and the filtrate evaporated at reduced pressure. Recrystallisation of the residue from EtOH gave the product (0.30 g) in 15% yield. M.P. 118—122°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.50. $[\alpha]_D^{25°} = -4.0°$ (C = 1, DMF). The NMR was consistent with structure.

BOC.Lys(Z)Thr(Bzl)Lys(Z)GlySer(Bzl)GlyPhePheOBz

Peptide (XVI) (0.22 g, 0.0023 M) was coupled to compound (XV) (0.17 g, 0.0023 M) in 5% DMF/toluene (21 ml) at R.T. overnight in the presence of Et_3N (1 equivalent). The reaction mixture was evaporated at reduced pressure and the residue recrystallised from EtOH to give the product (0.29 g) in 85% yield. M.P. 195—199°.
TLC 9:1 $CHCl_3$:MeOH (I_2 stain) showed one spot at Rf 0.67. The NMR was consistent with structure.

LysThrLysGlySerGlyPhePheOH

The intermediate above (0.25 g, 0.0017 M) was dissolved in TFA (10 ml) and deprotected by bubbling through HBr at R.T. for 1 hour. The solution was quenched with dry ether and the product dried *in vacuo* over P_2O_5 and KOH. The product was purified on a Biogel P2 column eluting with water and subsequently on a CM32 cellulose column eluting with a linear ionic strength gradient of ammonium acetate pH5 which gave separation of the free acid and some benzyl ester contaminant. Final isolation of the product was by lyopholisation.

TLC BAW (5:2:2) (ninhydrin spray) showed one spot at Rf 0.20. Amino acid analysis:

calculated: 2.00 Lys: 1.00 Thr: 2.00 Gly: 1.00 Ser: 2.00 Phe.
found: 1.77 Lys: 0.96 Thr: 2.00 Gly: 1.06 Ser: 2.10 Phe.

Biological Activity

Biological results obtained for Examples 1—6 in three different assay systems are presented in Tables 1 and 2.

As is apparent from Table 1, the peptides were capable of releasing histamine selectively from rat mast cells *in vitro*, and producing histamine release effects in rat and baboon skin *in vivo*. In the latter case in particular (primate tissue) activity was unusually high.

Table 2 demonstrates cross-desensitisation in rat mast cells *in vitro* between the peptides of Example 3 and an antigen.

TABLE 1

Histamine Release Effects of Synthetic Peptides (Examples 1–6)

Peptide	System	No. of Exps.	Activity	LDH/^{51}Cr Release	Inhibition of Activity
LysThrLysGlySer-GlyPhePheOMe (Example No. 1)	Rat Mast Cells *in vitro*[1]	7	7.5×10^{-6}M	None at 10^{-6}M	50% Inhibition by Intal (10^{-4}M)
	Rat Skin *in vivo*[2]	2	1.0×10^{-4}M		
	Baboon Skin *in vivo*[3]	2	5.0×10^{-7}M		
ArgLysThrLysGly-SerGlyPhePheOMe (Example No. 2)	Rat Mast Cells *in vitro*[1]	7	1.5×10^{-7}M		
	Rat Skin *in vivo*[2]	2	1.0×10^{-4}M		
	Baboon Skin *in vivo*[3]	2	1.0×10^{-7}M		
LysThrLysGlySerGly-PhePheValPheOMe (Example No. 3)	Rat Mast Cells *in vitro*[1]	10	1.0×10^{-6}M	None at 10^{-4}M peptide	
	Rat Skin *in vivo*[2]	5	1.0×10^{-5}M		
	Baboon Skin *in vivo*[3]	14	1.0×10^{-8}M		Partial Inhibition by Antihistamine or Intal-like drug
LysThrLysGlySer-GlyPhePheValPheOH (Example No. 4)	Rat Mast Cells *in vitro*[1]	2	3.3×10^{-5}M		
	Rat Skin *in vivo*[2]	4	1.0×10^{-4}M		
	Baboon Skin *in vivo*[3]	2	1.0×10^{-5}M		

(Intal is a Registered Trade Mark, at least in the United Kingdom).

TABLE 1 (Continued)

Peptide	System	No. of Exps.	Activity	LDH/^{51}Cr Release	Inhibition of Activity
ProArgLysThrLysGly-SerGlyPhePheOMe (Example No. 5)	Rat Mast Cells *in vitro*[1]	2	9.2×10^{-6}M		
	Rat Skin *in vivo*[2]	4	1.0×10^{-4}M		
	Baboon Skin *in vivo*[3]	2	1.0×10^{-7}M		
LysThrLysGly-SerGlyPhePheOH (Example No. 6)	Rat Mast Cells *in vitro*[1]	4	1.0×10^{-5}M		
	Rat Skin *in vivo*[2]	4	1.0×10^{-4}M		
	Baboon Skin *in vivo*[3]	3	1.0×10^{-5}M		

[1]The average concentration of peptide to give approximately 50% release of available histamine from rat mast cells in a number of experiments.

[2]The average concentration of peptide to give an end point in intradermal titrations in rat skin in a number of experiments.

[3]The average concentration of peptide to give an end point in intradermal titrations in baboon skin in a number of experiments.

TABLE 2

Cross Desensitisation between Antigen and the Peptide of Example 3 in the Rat Mast Cell System

Desensitiser	Desensitisation Steps 1	2	3	4	Challenge	Total average % histamine release
Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	4
	Buffer	Buffer	Buffer	Buffer	Peptide (10^{-5}M)	47
	Buffer	Buffer	Buffer	Buffer	XOA 1μg/ml	56
Peptide of Example 3	5×10^{-8}M	10^{-7}M	5×10^{-7}M	10^{-6}M	Buffer	6
	5×10^{-8}M	10^{-7}M	5×10^{-7}M	10^{-6}M	Peptide (10^{-5}M)	6
	5×10^{-8}M	10^{-7}M	5×10^{-7}M	10^{-6}M	XOA 1μg/ml	17

Methods

(a) (1) Histamine, (2) Cr^{51} and (3) Lactic Dehydrogenase Release from Rat Peritoneal Mast Cells (Rat Mast Cell *in vitro* test)

Mast cells, derived from the peritoneal washings of three male, outbred Wistar rates (250—300 g), were purified by the procedure according to Cooper and Stanworth (Preparative Biochem. 4(2), 105, 1975).

The purified cells were washed twice in Dulbecco's incomplete (i.e. free from mineral salts) buffer and then resuspended in Dulbecco's medium to the required volume. In a typical experiment, sufficient cells were available for 30 duplicate challenges, i.e. 60 samples and in this case the resuspension volume employed was 6.1 mls. 0.1 ml of cell suspension were taken for estimating the cell count.

(1) *Histamine release:*

One third of the cell suspension was employed. To 0.9 ml duplicate aliquots of challenge solution, prepared in complete Dulbecco's medium and prewarmed to 37°C, was added 0.1 ml of cell suspensions. The solutions were then shaken gently, and allowed to incubate for 5 minutes at 37°C. The reaction tubes were then quickly removed from the incubator and placed in an ice bath. Supernatants were then separated from the cell population following centrifugation for 3 minutes at 1000 r.p.m. The cell residues were then treated with 2 mls of 0.4 N perchloric acid and allowed to stand for approximately 30 minutes at ambient centrifugation and the supernatant solutions set aside for histamine analysis. The original supernatant solutions were treated with 1.0 ml of 0.8 N perchlorate and then treated in a similar manner to the cell residues. Histamine was measured by the method according to Evans, Lewis and Thompson (Life Sciences, *12*, 327, 1973) using a Technicon Auto-analyser*. Histamine release was calculated as a percentage of total histamine available in each challenge solution.

(2) *Cr^{51} release:*

One third of the cell suspension was employed. To approximately 2.0 ml of cell suspension in Dulbecco's medium was added 0.1 ml of a solution of Cr^{51} labelled sodium chromate. Approximately 50—100 μCi Cr^{51} was employed (specific activity: 300—500 μCi/mg Cr). The cells were allowed to stand for 30 minutes at ambient temperature and then excess chromium was removed by washing the cells three times in Dulbecco's buffer. The cell pellet was finally resuspended in the same buffer and 0.1 ml of cell suspension was then added to 0.9 ml of each challenge solution, prewarmed to 37°C. After 5 minutes' incubation the cell suspensions were removed from the water bath and the supernatants separated by centrifugation. Activity present in the whole recovered supernatants was measured using a Tracer Laboratory Spectromatic γ counter. The percentage of Cr^{51} released was assessed in relation to the values obtained for the positive and negative control solutions.

(3) *LDH measurement:*

One third of the cell suspension was employed. The incubation procedure was identical to that described above and carried out simultaneously until the challenge solution supernatants were separated from the cell residues. Lactic dehydrogenase activity was then estimated directly in the supernatant solutions by the method according to Johnson and Erdös (Proc. Soc. Exp. Biol. Med. *142*. 1252. 1973). To 0.5 ml of supernatant was added 0.5 ml of NAD (1 mM in 0.2 M Tris buffer, pH 8.5). 0.5 ml of this solution was then taken and treated with 50 μl of lactic acid (50 mM in 0.2 M Tris buffer, pH 8.5); as control, 50 μl of 0.2 M Tris buffer (pH 8.5) was added to a second aliquot (0.5 ml) of the NAD solution. The solutions were incubated at ambient temperature for 20 minutes and the fluorescence emission was then measured. The excitation and emission wave lengths used were 340 and 460 nm respectively. All measurements were carried out using a Baird Atomic automatic spectro-fluorimeter (Fluoripoint). The LDH activity was assessed in terms of the increase of fluorescence over control due to NADH formation following lactate addition. The percentage of LDH released was assessed in relation to the fluorescence intensity obtained in the positive control challenge solution supernatants (i.e. Triton X 100 challenge).

(b) Skin Test Method

Skin tests were carried out in the shaved backs of animals (rats and baboons) immediately after intravenous injection of pontamine sky blue (5%) in aqueous sodium chloride solution (0.9%) at a dose of 0.1 ml per kilogram of body weight in the base of rats and 5 ml per animal in the case of baboons.

Peptide in aqueous sodium chloride solution (0.9%), or saline control, were injected intradermally in 0.05 ml or 0.10 ml volumes. Skin reactions were read 20 minutes after intradermal challenge.

* (Technicon is a Registered Trade Mark, at least in the United Kingdom)

(c) Cross Desensitisation in the vitro rat mast cell system between antigen and peptide

Brown Norway rats were immunised intraperitoneally with 100 μg of ovalbumen (XOA) in 1 mg 'alum'. On day 27, peritoneal mast cells were removed, bulked and washed. Aliquots of cells were desensitised by the addition of 4 x 5 minute incubations with various peptide concentrations or buffer alone. The cells were then submitted to an optimal histamine releasing challenge of peptide, XOA, or challenged with buffer alone.

Claims

1. A peptide, or a salt thereof, characterised by containing 6 to 12 naturally occuring amino acid residues in a sequence $-[-R_1-R_2-R_3]-$, wherein,

R_1 consists of a residue of a basic amino acid, optionally linked to one or more residue of a neutral non-hydrophobic amino acid and/or to one or more further residue of a basic amino acid;

R_2 consists of one or more residue of a neutral non-hydrophobic amino acid; and

R_3 consists of a residue of a hydrophobic amino acid, optionally linked to one or more residue of a neutral non-hydrophobic amino acid and/or to one or more further residue of a hydrophobic amino acid;

the said basic amino acid residues are selected from arginyl, lysyl and ornithyl;

the said neutral non-hydrophobic amino acid residues are selected from glycyl, alanyl, seryl and threonyl; and

the said hydrophobic amino acid residues are selected from phenylalanyl, valyl and leucyl said peptide having the formula (I):

$$X-R-[-R_1-R_2-R_3]-Y \qquad (I)$$

wherein the sequence $-[-R_1-R_2-R_3]-$ is as defined and;

X is hydrogen, or a N-protecting group;

Y is hydroxy, or a C-terminal protecting group; and

R is an optionally present group, capable of confering on a peptide resistance to enzyme breakdown.

2. A peptide according to claim 1, wherein R is not present, X is hydrogen and Y is hydroxy, amino or methoxy.

3. A peptide according to claim 1 or claim 2, wherein the $-[-R_1-R_2-R_3]-$ sequence has from 8 to 10 amino acid residues.

4. A peptide according to claim 1, 2 or 3, wherein R_3 only contains hydrophobic amino acid residues.

5. A peptide according to claim 1 having the structure

$$X-R-[b\text{-}c\text{-}d\text{-}e]-[f\text{-}g\text{-}h]-[i\text{-}j\text{-}k\text{-}l]-Y$$

wherein X, Y and R are as defined in claim 1; c and e are lysyl, arginyl or ornithyl; d is threonyl or seryl; b is an optionally present arginyl, lysyl or ornithyl; f and h are glycyl or alanyl; g is seryl or threonyl; i and j are phenylalanyl, valyl or leucyl; and k and l are optionally present phenylalanyl, valyl or leucyl; or a salt thereof.

6. A peptide according to claim 5, wherein X is hydrogen, Y is hydroxyl, amino, or methoxy, and R is not present.

7. A peptide as claimed in claim 1 selected from:

Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1

wherein Y^1 is hydroxyl, $-NH_2$ or methoxy, or a salt thereof.

8. Lys Thr Lys Gly Ser Gly Phe Phe Val PheOH, or a salt thereof.

9. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe NH_2, or a salt thereof.

10. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe OCH_3, or a salt thereof.

11. A pharmaceutical composition, for use in desensitisation therapy, comprising a peptide according to any one of the preceding claims or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier, suitable for parenteral, intra-nasal or buccal administration.

12. A peptide, or a salt thereof, as defined in any one of the claims 1 to 10, for use in desensitisation therapy of allergies.

13. A process for the preparation of a peptide, or a salt thereof, as defined in any one of the claims 1 to 10, which process comprises the coupling of an optionally protected smaller peptide with either an optionally protected amino acid or with another optionally protected smaller peptide; and thereafter if

desired removing any protecting group present, and if desired forming a salt of the peptide.

Revendications

1. Peptide ou sel de celui-ci, caractérisé en ce qu'il renferme de 6 à 12 restes d'amino-acide d'origine naturelle selon une séquence $-[-R_1—R_2—R_3-]-$, dans laquelle:

R_1 est un résidu d'un amino-acide basique relié éventuellement à un ou plusieurs restes d'un amino-acide neutre non hydrophobe et/ou à un ou plusieurs autres restes d'un amino-acide basique;

R_2 est formé par un ou plusieurs restes d'un amino-acide neutre non hydrophobe; et

R_3 est un reste d'un amino-acide hydrophobe relié éventuellement à un ou plusieurs restes d'un amino-acide neutre non hydrophobe et/ou à un ou plusieurs autres restes d'un amino-acide hydrophobe;

les restes d'amino-acide basique sont choisis parmi l'arginyle, le lysyle et l'ornithyle;

les restes d'amino-acide neutre non hydrophobe sont choisis parmi le glycyle, l'alanyle, le séryle et le thréonyle; et

les restes d'amino-acide hydrophobe sont choisis parmi le phénylalanyle, le valyle et le leucyle;

ce peptide répondant à la formule (I):

$$X—R-[-R_1—R_2R_3-]-Y \qquad (I)$$

dans laquelle la séquence $-[-R_1—R_2—R_3-]-$ est telle que définie, et X est de l'hydrogène ou un groupe de protection à l'azote; Y est un hydroxy ou un groupe de protection du carbone terminal, et

R est un groupe éventuellement présent capable de conférer à un peptide une résistance à une dégradation par les enzymes.

2. Peptide suivant la revendication 1, caractérisé en ce que R n'est pas présent, X est de l'hydrogene et Y est un hydroxy, un amino ou un méthoxy.

3. Peptide suivant la revendication 1 ou 2, caractérisé en ce que la séquence $-[-R_1—R_2—R_3-]-$ renferme de 8 à 10 restes d'amino-acide.

4. Peptide suivant la revendication 1, 2 ou 3, caractérisé en ce que R_3 contient simplement des restes d'amino-acide hydrophobe.

5. Peptide suivant la revendication 1, présentant la structure:

$$X—R—[b—c—d—e]—[f—g—h]—[i—j—k—l]—Y$$

dans laquelle X, Y et R ont la même signification que dans la revendication 1; c et e sont du lysyle, de l'arginyle ou de l'ornithyle; d est du thréonyle ou du séryle; b est de l'arginyle, du lysyle ou de l'ornithyle éventuellement présent; f et h sont du glycyle ou de l'alanyle; g est du séryle ou du thréonyle; i et j sont du phénylalanyle, du valyle ou du leucyle; et k et l sont du phénylalanyle, du valyle ou du leucyle éventuellement présent; ou sel de ce peptide.

6. Peptide suivant la revendication 5, caractérisé en ce que X est de l'hydrogène, Y est un hydroxy, un amino ou un méthoxy et R n'est pas présent.

7. Peptide suivant la revendication 1, choisi parmi:

Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Phe Phe Val Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1

où Y^1 est un hydroxy, —NH_2 ou méthoxy, ou sel de ce peptide.

8. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe OH, ou sel de celui-ci.

9. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe NH_2, ou sel de celui-ci.

10. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe OCH_3, ou sel de celui-ci.

11. Composition pharmaceutique destinée à être utilisée en thérapeutique de désensibilisation, caractérisée en ce qu'elle renferme un peptide suivant l'une quelconque des revendications précédentes ou un sel pharmaceutiquement acceptable de celui-ci, conjointement à un véhicule ou excipient pharmaceutiquement acceptable convenant à une administration parentérale, intra-nasale ou buccale.

12. Peptide ou sel de celui-ci suivant l'une quelconque des revendications 1 à 10, destiné à être utilisé en thérapeutique de désensibilisation contre les allergies.

13. Procédé pour la préparation d'un peptide ou d'un sel de celui-ci suivant l'une quelconque des revendications 1 à 10, caractérisé en ce qu'il consiste à effectuer la condensation d'un peptide plus court éventuellement protégé avec soit un amino-acide éventuellement protégé, soit un autre peptide plus court éventuellement protégé; puis si désiré à éliminir les groupes de protection présents et si désiré à former un sel du peptide.

Patentansprüche

1. Peptid oder dessen Salz, gekennzeichnet durch einen Gehalt an 6 bis 12 natürlich vorkommenden Aminosäuren in einer Folge $-[-R_1-R_2-R_3-]-$, in der

R_1 aus einem Rest einer basischen Aminosäure besteht, die gegebenenfalls an einen oder mehrere Reste einer neutralen nicht-hydrophoben Aminosäure und/oder an einen oder mehrere Reste einer basischen Aminosäure gebunden ist,

R_2 aus einem oder mehreren Resten einer neutralen nicht-hydrophoben Aminosäure besteht und

R_3 aus einem Rest einer hydrophoben Aminosäure besteht, die gegebenenfalls an einen oder mehrere Reste einer neutralen nicht-hydrophoben Aminosäure und/oder an einen oder mehrere weitere Reste einer hydrophoben Aminosäure gebunden ist,

wobei die basischen Aminosäurereste aus des Gruppe Arginyl, Lysyl und Ornithyl ausgewählt sind und die neutralen nicht-hydrophoben Aminosäurereste aus der Gruppe Glycyl, Alanyl, Seryl und Threonyl ausgewählt sind und die hydrophoben Aminosäurereste aus der Gruppe Phenylalanyl, Valyl und Leucyl ausgewählt sind und

wobei das Peptid die Formel (I) hat:

$$X-R-[-R_1-R_2-R_3-]-Y \qquad (I)$$

in der die Folge $-[-R_1-R_2-R_3-]-$ wie vorstehend definiert ist und

X ein Wasserstoffatom oder eine N-Schutzgruppe ist,

Y die Hydroxylgruppe oder eine Schutzgruppe für ein endständiges Kohlenstoffatom ist und

R eine gegebenenfalls vorliegende Gruppe ist, die einem Peptid Widerstandsfähigkeit gegen eine Enzymzersetzung verleihen kann.

2. Peptide nach Anspruch 1, wobei R nicht vorhanden ist, X ein Wasserstoffatom ist und Y die Hydroxyl-, Amino- oder Methoxygruppe ist.

3. Peptid nach Ansprüchen 1 oder 2, wobei die $-[-R_1-R_2-R_3-]-$-Folge 8 bis 10 Aminosäurereste hat.

4. Peptid nach den Ansprüchen 1, 2 oder 3, wobei R_3 nur hydrophobe Aminosäurereste enthält.

5. Peptid nach Anspruch 1 mit der Struktur

$$X-R-[b-c-d-e]-[f-g-h]-[i-j-k-l]-Y,$$

in der X, Y und R wie in Anspruch 1 definiert sind, c und e Lysyl, Arginyl oder Ornithyl sind, d Threonyl oder Seryl ist, b gegebenenfalls vorhandenes Arginyl, Lysyl oder Ornithyl ist, f und h Glycyl oder Alanyl sind, g Seryl oder Threonyl ist, i und j Phenylalanyl, Valyl oder Leucyl sind, k und l gegebenenfalls vorhandenes Phenylalanyl, Valyl oder Leucyl sind, oder dessen Salz.

6. Peptid nach Anspruch 5, wobei X ein Wasserstoffatom ist, Y die Hydroxyl-, Amino- oder Methoxygruppe ist und R nicht vorhanden ist.

7. Peptid nach Anspruch 1, ausgewählt aus der Gruppe

Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1
Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe—Y^1
Prog Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe—Y^1

in der Y^1 die Hydroxyl-, —NH_2— oder Methoxygruppe oder ein Salz davon ist.

8. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe OH oder dessen Salz.

9. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe NH_2 oder dessen Salz.

10. Lys Thr Lys Gly Ser Gly Phe Phe Val Phe OCH_3 oder dessen Salz.

11. Pharmakologische Zubereitung zur Verwendung in der Desensitivierungstherapie, enthaltend ein Peptid nach einem der vorstehenden Ansprüche oder dessen pharmakologisch verträgliches Salz zusammen mit einem pharmakologisch verträglichen Trägermaterial für eine parenterale, intranasala oder bukkale Verabreichung.

12. Peptid oder dessen Salz nach einem der Ansprüche 1 bis 10 zur Verwendung in der Desensitivierungstherapie von Allergien.

13. Verfahren zur Herstellung eines Peptids oder dessen Salz nach einem der Ansprüche 1 bis 10, die Maßnahme der Kupplung eines gegebenenfalls geschützten kürzerkettigen Peptids mit entweder einer gegebenenfalls geschützten Aminosäure oder mit einem anderen gegebenenfalls geschützten kürzerkettigen Peptid und anschließend gegebenenfalls der Abspaltung vorhandener Schutzgruppe und gegebenenfalls der Bildung eines Salzes des Peptids umfassend.