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(21) International Application Number: PCT/US93/07694 (22) International Filing Date: 13 August 1993 (13.08.93) (30) Priority data: 07/936,504 28 August 1992 (28.08.92) US (71) Applicant: MAGAININ PHARMACEUTICALS, INC. [US/US]; 5110 Campus Drive, Plymouth Meeting, PA 19462 (US). (72) Inventor: WILLIAMS, Jon, I. ; 11 Darvel Drive, Robbins- ville, NJ 08691 (US). (74) Agents: OLSTEIN, Elliot, M. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068-1739 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: PURIFICATION OF AMPHIPHILIC COMPOUNDS (57) Abstract A method for purifying at least one amphiphilic peptide or protein, wherein a material containing the at least one amphiphilic peptide or protein is contacted with a mixture of an aprotic organic solvent and an alcohol is added to form a single liquid phase, to which is subsequently added an aqueous material, such as a salt solution, to form a primarily aqueous phase containing the at least one amphiphilic peptide or protein, and an immiscible organic phase. The at least one amphiphilic peptide or protein is then recovered from the aqueous phase. In one embodiment, prior to contacting said material with a mixture of an aprotic organic solvent and an alcohol, a material containing at least one amphiphilic peptide or protein is contacted with a solution including an aprotic organic solvent, at an acidic pH, to provide an extract solution containing the at least one amphiphilic peptide or protein, and at least a portion of the aprotic organic solvent is removed from the extract solution to provide a remainder material containing the at least one amphiphilic peptide or protein. This method also may be used in multiple successive applications so as to purify further the at least one amphiphilic peptide or protein. These methods enable one to purify biologically active peptides or proteins without the use of costly purification agents and techniques.		

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PURIFICATION OF AMPHIPHILIC COMPOUNDS

This application is a continuation-in-part of application Serial No. 936,504, filed August 28, 1992.

This invention relates to the purification of amphiphilic compounds. More particularly, this invention relates to the purification of amphiphilic compounds, in particular amphiphilic peptides, from a starting material, such as, for example, biological tissues, cell lysates, solutions containing chemically synthesized peptides, or materials containing peptides made by recombinant techniques, without using chaotropic agents.

Amphiphilic peptides, in general, have distinct hydrophobic and hydrophilic regions due to the presence of hydrophobic and hydrophilic amino acids in the peptide chain, and many are capable of forming an alpha-helical structure. Such amphiphilic peptides may also possess important biological or therapeutic properties; i.e., such peptides may inhibit, prevent, or destroy the growth or proliferation of target cells or viruses. Such peptides consequently may be used as antibiotics, anti-microbial agents, anti-fungal agents, anti-parasitic agents, anti-tumor agents, anti-cancer agents, and/or anti-viral agents. The peptides may be administered to a human or non-human animal or to plants, in order to inhibit growth of a target cell or virus or virally-infected cell in a human

or non-human animal, or in a plant. In addition, such biologically active amphiphilic peptides may be used in vitro as preservatives or sterilants to protect materials from microbial or viral contamination.

Many amphiphilic peptides are membrane-active; i.e., the peptides are able to pass into and/or through cell membranes and disrupt cellular functions. Biologically active amphiphilic peptides in many instances are also ion-channel forming peptides, whereby the peptides enable ions having pharmacological properties, such as, for example, fluoride ions, peroxide ions, bicarbonate ions, silver ions, zinc ions, mercury ions, arsenic ions, copper ions, antimony ions, gold ions, thallium ions, nickel ions, selenium ions, bismuth ions, and cadmium ions, to pass through cell membranes. In such cases, the peptide may potentiate the activity of the ion having pharmacological properties, and the ion having pharmacological properties may concomitantly potentiate the peptide.

Examples of biologically active amphiphilic peptides include magainin peptides, CPF peptides, XPF peptides, PGLa peptides, melittin peptides, cecropins, sarcotoxins, and certain synthetic amphiphilic peptides. Many amphiphilic peptides are obtained naturally from various organisms. For example, magainin peptides may be obtained from the skin of the African clawed frog Xenopus laevis, and melittins may be obtained from the hemolymph of the honeybee Apis mellifera. Thus, in some instances, one obtains the peptides by purifying a biological material, such as animal tissue or cell lysate, which contains the peptides. Also, such peptides may be made by recombinant gene expression techniques, wherein DNA encoding the peptide is constructed from codons encoding the various amino acids, and such DNA is placed into an organism which expresses the peptide; or by solution or solid phase peptide synthesis techniques, and

a solution containing such peptides must then be processed to obtain purified amphiphilic peptide. These types of purifications are often carried out with the use of chaotropic agents such as urea, guanidine hydrochloride, guanidinium isothiocyanate, and the like, followed by one or more chromatographic steps involving an immobile solid phase material. Such a procedure, however, requires expensive materials and often requires a considerable amount of time. Individual purification steps which do not require expensive reagents and which provide high levels of peptide product recovery are very desirable for both experimental and commercial scale purification processes for pharmacologically or therapeutically valuable amphiphilic peptides.

Researchers in the field of amphiphilic peptides have used substantially standard protein biochemistry techniques for purifying such peptides. For example, U.S. Patent No. 4,355,104, issued to Hultmark, et al., discloses the purification of Cecropin A and Cecropin B by successive applications of ion exchange chromatography on CM-Sepharose, using a 0.4M to 1.0M linear gradient of ammonium acetate buffer, pH6.6, and then on a second CM-Sepharose column using a linear 0.1M to 0.5M or 0.2M to 0.6M gradient of ammonium formate, pH6.6. PCT Application No. W086/04356 and related U.S. Patent No. 5,206,154 disclose the purification of cecropins from genetically engineered bacteria following cyanogen bromide cleavage of a fusion protein containing a cecropin molecule using high pressure liquid chromatography (HPLC) on a C18 reverse phase column. Zasloff, PNAS, Vol. 84, pgs. 5449-5453 (1987), discloses the isolation of magainin peptides from the skin of the frog Xenopus laevis using batch isolation on CM52 (Whatman) ion exchange media in 0.2M ammonium acetate, pH5.1-5.2, followed by isolation from a Bio-Gel P-30 (Bio-Rad) size exclusion chromatography

column, and purification by HPLC on a Vydac C4 reverse phase column. Several defensin peptides, characterized by the presence of six cysteine residues and three disulfide bonds per molecule, have been isolated from insects and mammals using some combination of ion exchange chromatography, size exclusion chromatography, affinity chromatography (eg., dye ligand affinity chromatography), preparative polyacrylamide gel electrophoresis, and reverse phase HPLC. (Gleich, et al., PNAS, Vol. 83, 3146-3150 (1986); Wasmoen, et al., J. Biol. Chem., Vol. 263, pgs. 12559-12563 (1988), Lambert, et al., PNAS, Vol. 86, pgs. 262-266 (1989); Gabay, et al., PNAS, Vol. 86, pgs. 5610-5614 (1989); Yamashita, et al., Infect. and Immun., Vol. 57, pgs. 2405-2409 (1989); Bulet, et al., Eur. J. Biochem., Vol. 209, pgs. 977-984 (1992)). Also, the purification of synthetic antimicrobial peptides typically has involved some combination of ion exchange chromatography, size exclusion chromatography, and reverse phase HPLC. (Zasloff, et al., PNAS, Vol 85, pgs. 910-913 (1988); Cuervo, et al., Peptide Research, Vol. 1, pgs. 81-86 (1988); Wade, et al., PNAS, Vol. 87, pgs. 4761-4765 (1990); Wade, et al., Int. J. Peptide Protein Res., Vol. 40, pgs. 429-436 (1992); Rao, et al., Int. J. Peptide Protein Res.; Vol. 40, pgs. 507-514 (1992)). In the above references, all of the separation steps are multiple in number, time-consuming, costly, and poorly adapted to large scale isolation or purification of amphiphilic antimicrobial peptides.

U.S. Patent No. 3,989,819, issued to Brockman, discloses a process for separating proteins from central nervous system tissue wherein mammalian central nervous system tissue is contacted first with organic solvents, and then the tissue (not the organic solvent phase) is contacted with saline and aqueous acid. Brockman does not re-extract the organic solvent phase to obtain an amphiphilic protein.

U.S. Patent No. 5,086,002, issued to Hillyard, et al., and U.S. Patent No. 5,116,952, issued to Martin, et al., disclose acetonitrile extraction to obtain peptides from a chemically prepared peptide preparation, followed by extraction with high performance liquid chromatography (HPLC) purification.

U.S. Patent No. 4,647,445, issued to Lees, discloses the separation of the protein portion of low density lipoprotein away from a chloroform:methanol (2:1) phase and into an aqueous phase. Lees does not teach, however, the partitioning of a protein fraction into a lipophilic solvent or solvent mixture such as chloroform:methanol, nor does Lees teach the partitioning of lipophilic proteins into an organic solvent or any solvent which is capable of dissolving lipids in the presence of water.

Articles have been published which address the use of specific organic reagents for the removal of proteins and other water soluble materials from cellular lipids, and for the purification of specific membrane associated proteins. Folch, et al., J. Biol. Chem., Vol. 226, pgs. 497-509 (1957) discloses the purification of total lipids from animal tissues using chloroform: methanol mixtures. This reference, however, does not discuss the use of chloroform:methanol mixtures for purifying membrane-bound or membrane-associated or membrane active peptide or protein fractions. Altendorf, et al., J. Supramolecular Structure, Vol. 6, pgs. 229-238 (1977) reveal purification of bacterial membrane proteolipids and proteins such as lactose permease using organic solvents such as n-butanol, chloroform, hexamethyl-phosphoric triamide, N-methylpyrrolidone or dimethylsulfoxide. This reference does not disclose the utility of such two phase methods for further purification of any protein species partitioning into the organic phase. Fillingame, J. Biol. Chem., Vol. 251, pgs. 6630-6637 (1976) discloses purification of the proteolipid

carbodiimide-reactive protein component of the ATP energy-transducing system of E.coli using chloroform:methanol mixtures; recovery of the proteolipid fraction is accomplished by evaporation of the organic mixture, resuspension in chloroform:methanol (2:1), precipitation with diethyl ether and chromatography on DEAE-cellulose in chloroform:methanol (2:1). Criado, et al., Analytical Biochemistry, Vol. 103, pgs. 289-294 (1980) disclose the benefits of using p-toluene sulfonate as an additive in fractionating membrane proteins using chloroform:methanol mixtures; however, this reference does not discuss the benefits of subsequent aqueous extractions of proteinaceous material from the organic phases. Brett, et al., Biochem. J., Vol. 211, pgs. 661-670 (1983) disclose the isolation and fractionation of CNBr-produced peptides from ovine opsin using solubilization and chromatography on Sephadex LH-20 or LH-60 resins in organic solvents; however, this reference does not disclose further fractionation of the peptides in aqueous-organic two phase solvent systems.

Further, none of the above-mentioned publications describes methods or benefits relating to successive multiple use of organic extraction techniques to purify further or refine a mixture containing at least one amphiphilic peptide or protein.

In accordance with an aspect of the present invention, there is provided a method of purifying at least one amphiphilic peptide or protein. The method comprises contacting a material containing at least one amphiphilic peptide or protein with a mixture of an aprotic organic solvent and an alcohol in an amount effective to form a single miscible solution. The miscible solution is contacted with an aqueous solution. The aqueous solution is added in an amount effective to form a first phase, which includes an aqueous solution containing the amphiphilic

peptide or protein, and a second phase which includes the organic solvent. The at least one amphiphilic peptide or protein then is recovered from the first phase.

In one embodiment, the aprotic organic solvent is chloroform or diethyl ether. Preferably, the aprotic organic solvent is chloroform. The alcohol may be, in one embodiment, an alkanol, and in particular may be selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, isopropanol, isobutyl alcohol and butanol, including 1-butanol and 2-butanol, and pentanol (including N-pentanol). The mixture contains the aprotic organic solvent and alcohol in a volume ratio of from about 1:5 to about 5:1, preferably from about 1:2 to about 2:1. The mixture of aprotic organic solvent and alcohol is added to the material, which may be an aqueous solution containing the at least one amphiphilic peptide or protein or a dry material containing the amphiphilic peptide or protein, in an amount sufficient to form a single miscible solution.

The single miscible solution which is formed by the addition of the aprotic organic solvent and alcohol to the remainder material, is capable of being separated into a first aqueous phase and a second organic phase upon the addition of a separate aqueous solution.

The aqueous solution, which is added to the miscible solution in an amount effective to form a first aqueous phase containing the at least one amphiphilic peptide or protein, and a second organic phase, may, in one embodiment, be water or an aqueous salt solution. Preferably the aqueous material is an aqueous salt solution, and more preferably a lithium chloride solution, a potassium chloride solution, or a sodium chloride solution. The salt may be at a concentration of from about .04M to about 1M, preferably about 0.1M. Applicant has found, surprisingly, that when an aqueous salt solution is added to the single miscible

solution, the at least one amphiphilic peptide or protein which is soluble in the single miscible solution is subsequently found almost entirely in the first aqueous phase, while the second organic phase, which contains an organic solvent, such as, for example, chloroform, with some portion of an alcohol and some portion of water, contains hydrophobic materials and insoluble precipitates but virtually no amphiphilic peptides or proteins. Although amphiphilic molecules can dissolve in chloroform:alcohol mixtures, the aqueous solubility of such amphiphilic molecules in the presence of an immiscible aqueous phase such as a salt solution far outweighs their ability to remain dissolved in chloroform:alcohol mixtures.

The at least one amphiphilic peptide or protein may be recovered from the final aqueous phase by means known to those skilled in the art. Such recovery means can include, but are not limited to, application of one or more methods selected from the group consisting of liquid chromatography, high performance liquid chromatography, reextraction, diafiltration, ultrafiltration, crystallization, or other chemical processing to remove unwanted molecular species such as salt ions.

In one embodiment, prior to contacting the material with a mixture of an aprotic solvent and an alcohol, a material containing at least one amphiphilic peptide or protein is contacted with a solution including an aprotic organic solvent. The solution is preferably at an acidic pH. The contacting of the material with the solution at an acidic pH provides an extract solution containing the at least one amphiphilic peptide or protein. At least a portion of the aprotic organic solvent then is removed from the extract solution to provide a remainder material containing at least the at least one amphiphilic peptide or protein. The remainder material then is contacted with an

aprotic organic solvent and an alcohol such as hereinabove described, and processed as hereinabove described in order to obtain a purified amphiphilic peptide or protein.

In one embodiment, the solution at an acidic pH includes an aprotic organic solvent selected from the group consisting of acetonitrile and acetone. Preferably, the aprotic organic solvent in such a solution is acetonitrile. In a preferred embodiment, the solution at an acidic pH further includes an acid, which in ionic strength and chemical acid character provides a solution which preferably has a pH no greater than 4.0. Preferably, the acid is selected from the group consisting of trifluoroacetic acid and trichloroacetic acid. Most preferably, the acid is trifluoroacetic acid. Preferably, the trifluoroacetic acid is present in an amount of about 0.1% (vol./vol.).

Contacting a material including at least one amphiphilic peptide or protein with a solution including an aprotic organic solvent at an acidic pH enables the at least one amphiphilic peptide or protein to be dissolved in the solvent, thereby providing an extract solution containing the at least one amphiphilic peptide or protein. This extract solution then is separated from any insoluble materials if such are present. The insoluble materials may be re-extracted one or more additional times to increase the yield of the at least one amphiphilic peptide or protein. Then, at least the aprotic organic solvent (eg., acetonitrile) which contains hydrophobic materials, is removed from the solution. The aprotic organic solvent may be removed by a variety of means, such as, for example, by evaporation, distillation, lyophilization, or the like. Such removal of at least the aprotic organic solvent provides a remainder material enriched in the at least one amphiphilic peptide or protein. It is contemplated that, in addition to the aprotic organic solvent, all or a portion of

the water and/or acid may be removed from the extract solution as well. In one embodiment, the extract solution may be evaporated to dryness, thereby leaving a dry, solid remainder material containing the at least one amphiphilic peptide or protein.

The organic extraction and phase partitioning methods of the present invention have selective advantage for the purification of amphiphilic peptides, or proteins, which advantage can be further utilized by successive or multiple extractions and phase partitionings in solvent systems embodied herein. For example, in one embodiment of the present invention, a sample mixture containing at least one amphiphilic peptide, or protein, can be prepared as a material which is exposed to a chloroform:methanol mixture so as to form a single miscible solution containing the at least one amphiphilic peptide, or protein, and then mixed with a portion of an aqueous salt solution. Amphiphilic peptides, or proteins, would preferentially partition into the primarily aqueous phase. The primarily aqueous phase can subsequently be reduced in volume by vacuum evaporation, distillation, lyophilization or the like to reduce or eliminate any organic solvent components to the primarily aqueous phase and then re-exposed to a second chloroform:methanol mixture so as to form a new single miscible solution. This new single miscible solution can then be extracted with an aqueous salt solution to form a second primarily aqueous phase which will contain a more pure sample of amphiphilic peptides, or proteins. This recurring method can be used two or more times to produce a more pure sample of amphiphilic peptide, or protein, when compared to the original sample mixture. It is also possible to modify the selection of organic phase solvents or solvent mixture constituents or volume ratios for any or all of the multiple steps so as to favor the degree of

purification of amphiphilic peptides, or proteins, away from other non-desirable components of the original sample mixture such as lipids, proteolipids, or glycoproteins, or non-amphiphilic peptides, or proteins. For example, in one embodiment of the present invention the initial organic solvent mixture used during the initial organic extraction step of a multiple extraction procedure can be carried out with chloroform:methanol in a volume ratio of 1:2 and in subsequent organic extraction steps in a volume ratio of 2:1. In another exemplary embodiment, the initial organic extraction step can use chloroform:n-butanol in a volume ratio of 4:1, a second organic extraction step could use chloroform:methanol in a volume ratio of 1:2 and subsequent organic extraction steps could use chloroform:methanol in a volume ratio of 2:1.

The method of the present invention is particularly applicable to the purification of amphiphilic peptides, or proteins, many of which are biologically active.

In one embodiment, the amphiphilic peptide is a basic (positively charged) polypeptide having at least sixteen amino acids wherein the polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids. Still more particularly, the hydrophobic amino acids are in groups of two adjacent amino acids, and each group of two hydrophobic amino acids is spaced from another group of two hydrophobic amino acids by at least one amino acid other than a hydrophobic amino acid (preferably at least two amino acids) and generally by no greater than four amino acids, and the amino acids between pairs of hydrophobic amino acids may or may not be hydrophilic.

The hydrophilic amino acids are generally also in groups of two adjacent amino acids in which at least one of the two amino acids is a basic hydrophilic amino acid, with such groups of two hydrophilic amino acids being spaced from

each other by at least one amino acid other than a hydrophilic amino acid (preferably at least two amino acids) and generally no greater than four amino acids, and the amino acids between pairs of hydrophilic amino acids may or may not be hydrophobic.

In accordance with a particularly preferred embodiment, the polypeptide comprises a chain of at least four groups of amino acids, with each group consisting of four amino acids. Two of the four amino acids in each group are hydrophobic amino acids, and two of the four amino acids in each group are hydrophilic, with at least one of the hydrophilic amino acids in each group being a basic hydrophilic amino acid and the other being a basic or neutral hydrophilic amino acid.

The hydrophobic amino acids may be selected from the class consisting of Ala, Cys, Phe, Gly, Ile, Leu, Met, Pro, Val, Trp, Tyr, norleucine (Nle), norvaline (Nva), and cyclohexylalanine (Cha). The neutral hydrophilic amino acids may be selected from the class consisting of Asn, Gln, Ser, Thr, and homoserine (Hse). The basic hydrophilic amino acids may be selected from the class consisting of Lys, Arg, His, Orn, homoarginine (Har), 2, 4-diaminobutyric acid (Dbu), and p-aminophenylalanine.

Each of the groups of four amino acids may be of the sequence ABCD, BCDA, CDAB, or DABC, wherein A and B are each hydrophobic amino acids and may be the same or different, one of C or D is a basic hydrophilic amino acid, and the other of C or D is a basic or neutral hydrophilic amino acid and may be the same or different. In one embodiment, the polypeptide chain may comprise 5 or 6 groups of this sequence. In each group, each of A, B, C and D may be the same in some or all of the groups or may be different in some or all of the groups.

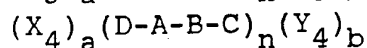
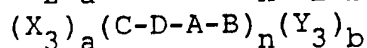
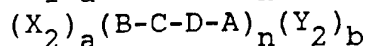
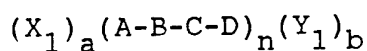
The polypeptide chain of the at least one amphiphilic peptide or protein preferably has at least 12 amino acids,

and no greater than 50 amino acids. It is to be understood, however, that the polypeptide does not have to consist entirely of the groups described above. The polypeptide may have amino acids extending from either or both ends of the noted groups forming the polypeptide chain and/or there may be amino acids between one or more of the at least four groups and still remain within the scope of the invention.

The groups of amino acids may be repeating groups of amino acids, or the amino acids in the various groups may vary provided that in each group of the at least four groups of amino acids there are two hydrophobic and two hydrophilic amino acids as hereinabove noted.

Thus the biologically active polypeptide may comprise a chain including at least four groups of amino acids, each containing four amino acids. Two of the four amino acids in each group are hydrophobic, at least one amino acid is basic hydrophilic, and the remaining one is basic or neutral hydrophilic, with the polypeptide chain preferably having at least 20 amino acids but no greater than 50 amino acids.

In one embodiment, each of the at least four groups of amino acids which are in the peptide chain is of the sequence A-B-C-D, B-C-D-A, C-D-A-B or D-A-B-C wherein A and B are hydrophobic amino acids, one of C or D is a basic hydrophilic amino acid, and the other of C or D is basic or neutral hydrophilic amino acid. The resulting polypeptide chain, therefore, may have one of the following sequences:



wherein X_1 is D; C-D- or B-C-D-, Y_1 is -A or -A-B or -A-B-C

X_2 is A-, D-A- or C-D-A-

Y_2 is -B, -B-C or B-C-D

X_3 is B-, A-B-, D-A-B-

Y_3 is -C, -C-D, -C-D-A
 X_4 is C-, B-C-, A-B-C-
 Y_4 is -D, -D-A, -D-A-B
a is 0 or 1; b is 0 or 1
and n is at least 4.

It is to be understood that the peptide chain may include amino acids between the hereinabove noted groups of four amino acids provided that the spacing between such groups and the charge on the amino acids does not change the characteristics of the peptide chain which provide amphiphilicity and a positive charge and do not adversely affect the folding characteristics of the chain to that which is significantly different from one in which the hereinabove noted group of four amino acids are not spaced from each other.

As representative examples of such peptides, there may be mentioned.

- I Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys
 (SEQ ID NO:1)
- II Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys
 (SEQ ID NO:2)
- III Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala
 (SEQ ID NO:3)
- IV Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe
 (SEQ ID NO:4)
- V Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser
 (SEQ ID NO:5)

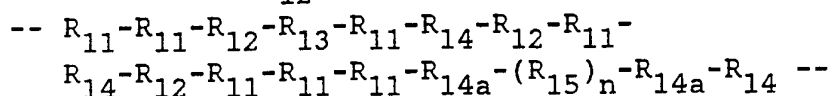
The peptide may have amino acids extending from either end of the chain. For example, the chains may have a Ser-Lys sequence before the "Ala" end, and/or an Ala-Phe

sequence after the "Lys" end. Other amino acid sequences may also be attached to the "Ala" and/or the "Lys" end.

Similarly, in any polypeptide chain having at least four groups of amino acids of the sequence as described above, the chain may have, for example, a C-D sequence before the first A-B-C-D group. Also other amino acid sequences may be attached to the "A" and/or the "D" end of one of these polypeptide chains. Also there may be amino acids in the chain which space one or more groups of the hereinabove noted four amino acids from each other.

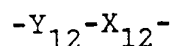
In accordance with another embodiment, the peptide may be a magainin peptide.

A magainin peptide is either a magainin such as magainin I, II or III or an analogue or derivative thereof. The magainin peptides preferably include the following basic peptide structure X_{12}



wherein R_{11} is a hydrophobic amino acid, R_{12} is a basic hydrophilic amino acid; R_{13} is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid; R_{14} and R_{14a} are hydrophobic or basic hydrophilic amino acids; R_{15} is glutamic acid or aspartic acid, or a hydrophobic or a basic hydrophilic amino acid, and n is 0 or 1. In a preferred embodiment, R_{13} is a hydrophobic or neutral hydrophilic amino acid, R_{14a} is a hydrophobic amino acid, and R_{15} is glutamic acid or aspartic acid.

Thus, for example, a magainin peptide may include the following structure:



where X_{12} is the hereinabove described basic peptide structure and Y_{12} is

- (i) R_{12}
- (ii) $R_{14a}\text{--}R_{12}$

(iii) $R_{11}-R_{14a}-R_{12}$

(iv) $R_{14}-R_{11}-R_{14a}-R_{12}$

where R_{11} , R_{12} , R_{14} and R_{14a} are as previously defined.

A magainin peptide may also have the following structure:

$-X_{12}-Z_{12}-$

wherein X_{12} is as previously defined and Z_{12} is:

(i) R_{16} where R_{16} is a basic hydrophilic amino acid or asparagine or glutamine.

(ii) $R_{16}-R_{17}$ where R_{17} is a neutral hydrophilic amino acid, a hydrophobic amino acid, or a basic hydrophilic amino acid. Preferably, R_{17} is a neutral hydrophilic amino acid.

A magainin peptide may also have the following structure:

$(Y_{12})_a-X_{12}-(Z_{12})_b$

where X_{12} , Y_{12} and Z_{12} are as previously defined and a is 0 or 1 and b is 0 or 1.

The magainin peptides may also include the following basic peptide structure X_{13} :

$--R_{14}-R_{11}-R_{14a}-R_{12}-R_{11}-R_{11}-R_{12}-R_{13}-$

$R_{11}-R_{14}-R_{12}-R_{11}-R_{11}-R_{12}-$, wherein R_{11} , R_{12} , R_{13} , R_{14} , and R_{14a} are amino acids as hereinabove described.

The magainin peptide may also include the following structure $X_{13}-Z_{13}$; wherein X_{13} is the hereinabove described basic peptide structure and Z_{13} is

$(R_{11})_n-(R_{11})_n-(R_{11})_n-(R_{14a})_n-(R_{15})_n-(R_{14a})_n-(R_{14})_n--$

$(R_{16})_n-(R_{17})_n$ wherein R_{11} , R_{14} , R_{14a} , R_{15} , R_{16} , and R_{17} are as hereinabove described, and n is 0 or 1, and each n may be the same or different.

The magainin peptides generally include at least fourteen amino acids and may include up to forty amino acids. A magainin peptide preferably has from 21 to 23

amino acids. Accordingly, the hereinabove described basic peptide structures of a magainin peptide may include additional amino acids at the amino end or at the carboxyl end, or at both ends.

As representative examples of such magainin peptides, there may be mentioned peptides having the following primary sequences as given in the accompanying sequence listing as well as appropriate analogues and derivatives thereof:

- (a) (SEQ ID NO:6) (OH) or (NH₂)
(Magainin I)
- (b) (SEQ ID NO:7) (OH) or (NH₂)
(Magainin II)
- (c) (SEQ ID NO:8) (OH) or (NH₂)
(Magainin III)

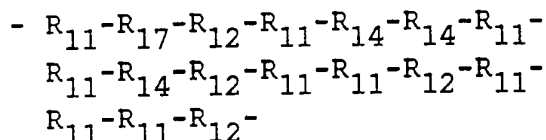
The following are examples of peptide derivatives or analogs of the basic structure:

- (d) (SEQ ID NO:9) (OH) or (NH₂)
- (e) (SEQ ID NO:10) (OH) or (NH₂)
- (f) (SEQ ID NO:11) (OH) or (NH₂)

Some natural magainin peptides are described in Proc. Natl. Acad. Sci. Vol. 84, pp. 5449-53 (August 1987). The term "magainin peptides" as used herein refers to the basic magainin structure as well as derivatives and analogs thereof, including but not limited to the representative derivatives or analogs.

In accordance with a further embodiment, the peptide may be a PGLa peptide or an XPF peptide.

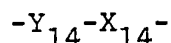
A PGLa peptide is either PGLa or an analogue or derivative thereof. The PGLa peptides preferably include the following basic peptide structure X₁₄:



where R₁₁, R₁₂, R₁₄, and R₁₇ are as previously defined.

The PGLa peptides generally include at least seventeen amino acids and may include as many as forty amino acids. Accordingly, the hereinabove described basic peptide structure for a PGLa peptide may include additional amino acids at the amino end or at the carboxyl end, or at both the amino and carboxyl end.

Thus, for example, a PGLa peptide may have the following structure:



where X_{14} is as previously defined and

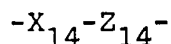
Y_{14} is

(i) R_{11} ; or

(ii) $R_{14}-R_{11}$

where R_{11} and R_{14} are as previously defined.

For example, a PGLa peptide may also have the following structure:



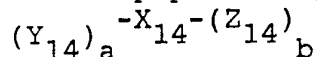
where X_{14} is as previously defined; and Z_{14} is:

(i) R_{11} ; or

(ii) $R_{11}-R_{11}$

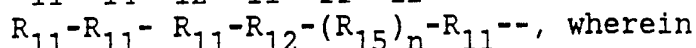
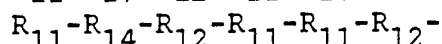
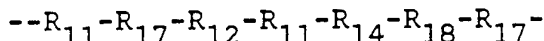
where R_{11} is as previously defined.

A PGLa peptide may also have the following structure:



where X_{14} , Y_{14} , and Z_{14} are as previously defined, a is 0 or 1 and b is 0 or 1.

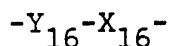
An XPF peptide is either XPF or an analogue or derivative thereof. The XPF peptides preferably include the following basic peptide structure X_{16} :



R_{11} , R_{12} , R_{14} , R_{15} and R_{17} are as previously defined and R_{18} is glutamine or asparagine or a basic hydrophilic or hydrophobic amino acid, and n is 0 or 1.

The XPF peptides generally include at least nineteen amino acids and may include up to forty amino acids. Accordingly, the hereinabove described basic peptide structure of XPF may include additional amino acids at the amino end or at the carboxyl end, or at both the amino and carboxyl ends.

Thus, for example, an XPF peptide may include the following structure:



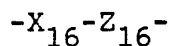
where X_{16} is as previously defined and Y_{16} is

(i) R_{11} ; or

(ii) $R_{14}-R_{11}$

where R_{11} and R_{14} are as previously defined.

An XPF peptide may include the following structure:



where X_{16} is as previously defined and Z_{16} is

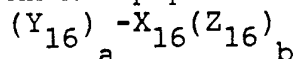
(i) R_{11} ; or

(ii) $R_{11}-R_{18}$; or

(iii) $R_{11}-R_{18}$ -Proline; or

(iv) $R_{11}-R_{18}$ -Proline- R_{12}

An XPF peptide may also have the following structure:



where X_{16} , Y_{16} and Z_{16} are as previously defined, a is 0 or 1 and b is 0 or 1.

Preferred are XPF or PGLa peptides, which are characterized by the following primary amino acid sequences as given in the accompanying sequence listing:

PGLa : (SEQ ID NO:12) (NH_2)

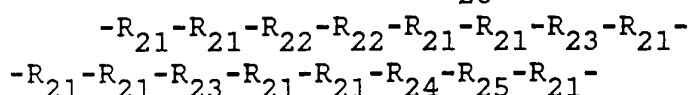
XPF : (SEQ ID NO:13)

A review of XPF and PGLa can be found in Hoffman et al, EMBO J. 2:711-714 (1983); Andreu, et al, J. Biochem. 149:531-535 (1985); Gibson, et al J. Biol. Chem. 261:5341-5349 (1986); and Giovannini, et al, Biochem J. 243:113-120 (1987).

In accordance with yet another embodiment, the peptide may be a CPF peptide or appropriate analogue or derivative thereof.

CPF peptides as well as analogues and derivatives thereof are herein sometimes referred to collectively as CPF peptides.

The CPF peptide may be one which includes the following basic peptide structure X_{20} :



wherein R_{21} is a hydrophobic amino acid;

R_{22} is a hydrophobic amino acid or a basic hydrophilic amino acid;

R_{23} is a basic hydrophilic amino acid;

R_{24} is a hydrophobic or neutral hydrophilic amino acid;

and

R_{25} is a basic or neutral hydrophilic amino acid.

The hereinabove basic structure is hereinafter symbolically indicated as X_{20} .

The hydrophobic amino acids are Ala, Cys, Phe, Gly, Ile, Leu, Met, Val, Trp, Tyr, norleucine (Nle), norvaline (Nva), and cyclohexylalanine (Cha).

The neutral hydrophilic amino acids are Asn, Gln, Ser, and Thr.

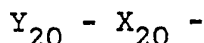
The basic hydrophilic amino acids are Lys, Arg, His, Orn, homoarginine (Har), 2,4-diaminobutyric acid (Dbu), and p-aminophenylalanine.

The CPF peptide may include only the hereinabove noted amino acids or may include additional amino acids at the

amino and/or carboxyl ends or both the amino and carboxyl end. In general, the peptide does not include more than 40 amino acids.

The CPF peptides including the above basic structure preferably have from 1 to 4 additional amino acids at the amino end.

Accordingly, such preferred peptides may be represented by the structural formula:



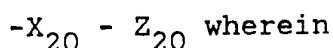
wherein X_{20} is the hereinabove described basic peptide structure and Y_{20} is

- (i) $R_{25}-$; or
- (ii) $R_{22}-R_{25}-$; or
- (iii) $R_{21}-R_{22}-R_{25}$; or
- (iv) $R_{22}-R_{21}-R_{22}-R_{25}$; preferably
Glycine - $R_{21}-R_{22}-R_{25}$.

wherein R_{21} , R_{22} and R_{25} are as previously defined.

The carboxyl end of the basic peptide structure may also have additional amino acids which may range from 1 to 13 additional amino acids.

In a preferred embodiment, the basic structure may have from 1 to 7 additional amino acids at the carboxyl end, which may be represented as follows:



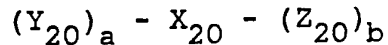
X is the hereinabove defined basic peptide structure

and Z_{20} is

- (i) $R_{21}-$, or
- (ii) $R_{21}-R_{21}-$; or
- (iii) $R_{21}-R_{21}-R_{24}$; or
- (iv) $R_{21}-R_{21}-R_{24}-R_{24}$; or
- (v) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}$; or
- (vi) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}-\text{Gln}$; or

(vii) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}-\text{Gln}-\text{Gln}$, wherein R_{21} and R_{24} are as previously defined, and R_{26} is proline or a hydrophobic amino acid.

Preferred peptides may be represented by the following structural formula



wherein X_{20} , Y_{20} and Z_{20} are as previously defined and a is 0 or 1 and b is 0 or 1.

Representative examples of CPF peptides which may be employed, some of which have been described in the literature, include the following sequences as given in the accompanying sequence listing:

(SEQ ID NO:14)

(SEQ ID NO:15)

(SEQ ID NO:16)

(SEQ ID NO:17)

(SEQ ID NO:18)

(SEQ ID NO:19)

(SEQ ID NO:20)

(SEQ ID NO:21)

(SEQ ID NO:22)

(SEQ ID NO:23)

(SEQ ID NO:24)

(SEQ ID NO:25)

(SEQ ID NO:26)

A review of the natural CPF peptides can be found in Richter, K., Egger, R., and Kreil J. Biol. Chem 261, 3676-3680 (1986); Wakabayashi, T., Kato, H., and Tachibana, S. Nucleic Acids Research 13, 1817-1828 (1985); Gibson, B.W., Poulter, L., Williams, D.H., and Maggio, J.E. J. Biol. Chem 261, 5341-5349 (1986).

In accordance with yet another embodiment, the peptide being purified in accordance with the present invention may

include one of the following basic structures X_{31} through X_{37} wherein:

X_{31} is $-[R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}]_n$;
 X_{32} is $-[R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}]_n$;
 X_{33} is $-[R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}]_n$;
 X_{34} is $-[R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}]_n$;
 X_{35} is $-[R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}]_n$;
 X_{36} is $-[R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}]_n$; and
 X_{37} is $-[R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}]_n$;

wherein R_{31} is a basic hydrophilic amino acid, R_{32} is a hydrophobic amino acid, R_{33} is a neutral hydrophilic, basic hydrophilic, or hydrophobic amino acid, and n is from 2 to 5.

The basic hydrophilic amino acids may be selected from the class consisting of Lys, Arg, His, Orn, homoarginine (Har), 2,4-diaminobutyric acid (Dbu), and p-aminophenylalanine.

The hydrophobic amino acids may be selected from the class consisting of Ala, Cys, Phe, Gly, Ile, Leu, Met, Pro, Val, Trp and Tyr, norleucine (Nle), norvaline (Nva), and cyclohexylalanine (Cha).

The neutral hydrophilic amino acids may be selected from the class consisting of Asn, Gln, Ser and Thr.

In accordance with one embodiment, when the peptide includes the structure X_{31} , the peptide may include the following structure:

$Y_{31}-X_{31}$, wherein X_{31} is as hereinabove described, and Y_{31} is:

- (i) R_{32} ;
- (ii) $R_{32}-R_{32}$;
- (iii) $R_{31}-R_{32}-R_{32}$;
- (iv) $R_{33}-R_{31}-R_{32}-R_{32}$;
- (v) $R_{32}-R_{33}-R_{31}-R_{32}-R_{32}$; or

(vi) $R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}$, wherein R_{31} , R_{32} , and R_{33} are as hereinabove described

In accordance with another embodiment, when the peptide includes the structure X_{31} , the peptide may include the following structure:

$X_{31}-Z_{31}$, wherein X_{31} is as hereinabove described, and Z_{31} is:

- (i) R_{31} ;
- (ii) $R_{31}-R_{32}$;
- (iii) $R_{31}-R_{32}-R_{32}$;
- (iv) $R_{31}-R_{32}-R_{32}-R_{33}$;
- (v) $R_{31}-R_{32}-R_{32}-R_{33}-R_{31}$; or
- (vi) $R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{31})_a-X_{31}-(Z_{31})_b$, wherein Y_{31} and Z_{31} are as previously defined, a is 0 or 1, and b is 0 or 1.

When the peptide includes the structure X_{32} , the peptide may include the following structure:

$Y_{32}-X_{32}$, wherein X_{32} is as hereinabove described, and Y_{32} is:

- (i) R_{31} ;
- (ii) $R_{32}-R_{31}$;
- (iii) $R_{32}-R_{32}-R_{31}$;
- (iv) $R_{31}-R_{32}-R_{32}-R_{31}$;
- (v) $R_{33}-R_{31}-R_{32}-R_{32}-R_{31}$; or
- (vi) $R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}$.

In another embodiment, when the peptide includes the structure X_{32} , the peptide may include the following structure:

$X_{32}-Z_{32}$, wherein X_{32} is as hereinabove described, and Z_{32} is:

- (i) R_{32} ;
- (ii) $R_{32}-R_{32}$;

- (iii) $R_{32}-R_{32}-R_{33}$;
- (iv) $R_{32}-R_{32}-R_{33}-R_{31}$;
- (v) $R_{32}-R_{32}-R_{33}-R_{31}-R_{32}$; or
- (vi) $R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{32})_a - X_{32} - (Z_{32})_b$, wherein Y_{32} and Z_{32} are as previously defined, a is 0 or 1, and b is 0 or 1.

In accordance with another embodiment, when the peptide includes the structure X_{33} , the peptide may include the following structure:

$Y_{33} - X_{33}$ wherein X_{33} is as hereinabove described, and Y_{33} is:

- (i) R_{32} ;
- (ii) $R_{31}-R_{32}$;
- (iii) $R_{32}-R_{31}-R_{32}$;
- (iv) $R_{32}-R_{32}-R_{31}-R_{32}$;
- (v) $R_{31}-R_{32}-R_{32}-R_{31}-R_{32}$; or
- (vi) $R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}$, wherein R_{31} , R_{32} , and R_{33} are as hereinabove described.

In accordance with another embodiment, when the peptide includes the structure X_{33} , the peptide may include the following structure:

$X_{33} - Z_{33}$ wherein X_{33} is as hereinabove described, and Z_{33} is:

- (i) R_{32} ;
- (ii) $R_{32}-R_{33}$;
- (iii) $R_{32}-R_{33}-R_{31}$;
- (iv) $R_{32}-R_{33}-R_{31}-R_{32}$;
- (v) $R_{32}-R_{33}-R_{31}-R_{32}-R_{32}$; or
- (vi) $R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{33})_a - X_{33} - (Z_{33})_b$, wherein Y_{33} and Z_{33} are as previously defined, a is 0 or 1, and b is 0 or 1.

In accordance with yet another embodiment, when the peptide includes the structure X_{34} , the peptide may include the following structure:

$Y_{34} - X_{34}$, wherein X_{34} is as hereinabove described, and Y_{34} is:

- (i) R_{32} ;
- (ii) $R_{32}-R_{32}$;
- (iii) $R_{31}-R_{32}-R_{32}$;
- (iv) $R_{32}-R_{31}-R_{32}-R_{32}$;
- (v) $R_{32}-R_{32}-R_{31}-R_{32}-R_{32}$; or
- (vi) $R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}$, wherein R_{31} , R_{32} and R_{33} are as hereinabove described.

In accordance with another embodiment, when the peptide includes the structure X_{34} , the peptide may include the following structure:

$X_{34}-Z_{34}$, wherein X_{34} is as hereinabove described, and Z_{34} is:

- (i) R_{33} ;
- (ii) $R_{33}-R_{31}$;
- (iii) $R_{33}-R_{31}-R_{32}$;
- (iv) $R_{33}-R_{31}-R_{32}-R_{32}$;
- (v) $R_{33}-R_{31}-R_{32}-R_{32}-R_{31}$; or
- (vi) $R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{34})_a - X_{34} - (Z_{34})_b$, wherein X_{34} and Z_{34} are as previously defined, a is 0 or 1, and b is 0 or 1.

In accordance with a further embodiment, when the peptide includes the structure X_{35} , the peptide may include the following structure:

$Y_{35}-X_{35}$, wherein X_{35} is as hereinabove described, and Y_{35} is:

- (i) R_{33} ;
- (ii) $R_{32}-R_{33}$;
- (iii) $R_{32}-R_{32}-R_{33}$;
- (iv) $R_{31}-R_{32}-R_{32}-R_{33}$;
- (v) $R_{32}-R_{31}-R_{32}-R_{32}-R_{33}$; or
- (vi) $R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}$, wherein R_{31} , R_{32} , and R_{33} are as hereinabove described.

In accordance with another embodiment, when the peptide includes the structure X_{35} , the peptide may include the following structure:

$X_{35} - Z_{35}$ wherein X_{35} is as hereinabove described, and Z_{35} is:

- (i) R_{31} ;
- (ii) $R_{31}-R_{32}$;
- (iii) $R_{31}-R_{32}-R_{32}$;
- (iv) $R_{31}-R_{32}-R_{32}-R_{31}$;
- (v) $R_{31}-R_{32}-R_{32}-R_{31}-R_{32}$; or
- (vi) $R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{35})_a - X_{35} - (Z_{35})_b$, wherein X_{35} and Z_{35} are as previously defined, a is 0 or 1, and b is 0 or 1.

In accordance with a further embodiment, when the peptide includes the structure X_{36} , the peptide may include the following structure:

$Y_{36} - X_{36}$ wherein X_{36} is as hereinabove described, and Y_{36} is:

- (i) R_{31} ;
- (ii) $R_{33}-R_{31}$;
- (iii) $R_{32}-R_{33}-R_{31}$;
- (iv) $R_{32}-R_{32}-R_{33}-R_{31}$;
- (v) $R_{31}-R_{32}-R_{32}-R_{33}-R_{31}$; or
- (vi) $R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}$, wherein R_{31} , R_{32} , and R_{33} are as hereinabove described.

In accordance with another embodiment, when the peptide includes the structure X_{36} , the peptide may include the following structure:

X_{36} - Z_{36} , wherein X_{36} is as hereinabove described, and Z_{36} is:

- (i) R_{32} ;
- (ii) R_{32} - R_{32} ;
- (iii) R_{32} - R_{32} - R_{31} ;
- (iv) R_{32} - R_{32} - R_{31} - R_{32} ;
- (v) R_{32} - R_{32} - R_{31} - R_{32} - R_{32} ; or
- (vi) R_{32} - R_{32} - R_{31} - R_{32} - R_{32} - R_{33} .

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{36})_a$ - X_{36} -(Z_{36}) $_b$, wherein Y_{36} and Z_{36} are as previously defined, a is 0 or 1, and b is 0 or 1.

In accordance with one embodiment, when the peptide includes the structure X_{37} , the peptide may include the structure Y_{37} - X_{37} , wherein X_{37} is as hereinabove described, and Y_{37} is:

- (i) R_{32} ;
- (ii) R_{31} - R_{32} ;
- (iii) R_{33} - R_{31} - R_{32} ;
- (iv) R_{32} - R_{33} - R_{31} - R_{32} ;
- (v) R_{32} - R_{32} - R_{33} - R_{31} - R_{32} ; or
- (vi) R_{31} - R_{32} - R_{32} - R_{33} - R_{31} - R_{32} , wherein R_{31} , R_{32} , and R_{33} are as hereinabove described.

In accordance with a further embodiment, when the peptide includes the structure X_{37} , the peptide may include the following structure:

X_{37} - Z_{37} wherein X_{37} is as hereinabove described, and Z_{37} is:

- (i) R_{32} ;
- (ii) R_{32} - R_{31} ;
- (iii) R_{32} - R_{31} - R_{32} ;

- (iv) $R_{32}-R_{31}-R_{32}-R_{32}$;
- (v) $R_{32}-R_{31}-R_{32}-R_{32}-R_{33}$; or
- (vi) $R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}$.

In accordance with yet another embodiment, the peptide may include the following structure:

$(Y_{37})_a - X_{37} - (Z_{37})_b$, wherein Y_{37} and Z_{37} are as previously defined, a is 0 or 1, and b is 0 or 1.

In a preferred embodiment, n is 3, and most preferably the peptide has one of the following structures as given in the accompanying sequence listing:

- (Lys Ile Ala Gly Lys Ile Ala)₃ (SEQ ID NO:27).
- (Lys Ile Ala Lys Ile Ala Gly)₃ (SEQ ID NO:28).
- (Lys Ile Ala Gly Lys Ile Gly)₃ (SEQ ID NO:29).
- (Lys Leu Ala Gly Lys Leu Ala)₃ (SEQ ID NO:30).
- (Lys Phe Ala Gly Lys Phe Ala)₃ (SEQ ID NO:31).
- (Lys Ala Leu Ser Lys Ala Leu)₃ (SEQ ID NO:32).
- (Lys Leu Leu Lys Ala Leu Gly)₃ (SEQ ID NO:33).
- (Lys Ala Ile Gly Lys Ala Ile)₃ (SEQ ID NO:34).
- (Gly Ile Ala Lys Ile Ala Lys)₃ (SEQ ID NO:35).
- (Lys Ile Ala Lys Ile Phe Gly)₃ (SEQ ID NO:36).
- (Gly Ile Ala Arg Ile Ala Lys)₃ (SEQ ID NO:37).
- (Lys Phe Ala Arg Ile Ala Gly)₃ (SEQ ID NO:38).
- (Gly Phe Ala Lys Ile Ala Lys)₃ (SEQ ID NO:39).
- (Lys Ile Ala Gly Orn Ile Ala)₃ (SEQ ID NO:40).
- (Lys Ile Ala Arg Ile Ala Gly)₃ (SEQ ID NO:41).
- (Orn Ile Ala Gly Lys Ile Ala)₃ (SEQ ID NO:42).
- (Gly Ile Ala Arg Ile Phe Lys)₃ (SEQ ID NO:43).
- (Lys Nle Ala Gly Lys Nle Ala)₃ (SEQ ID NO:44).
- (Lys Nle Ala Gly Lys Ile Ala)₃ (SEQ ID NO:45).
- (Lys Ile Ala Gly Lys Nle Ala)₃ (SEQ ID NO:46).
- (Lys Nva Ala Gly Lys Nva Ala)₃ (SEQ ID NO:47).
- (Lys Nva Ala Gly Lys Ile Ala)₃ (SEQ ID NO:48).
- (Lys Leu Leu Ser Lys Leu Gly)₃ (SEQ ID NO:49).
- (Lys Leu Leu Ser Lys Phe Gly)₃ (SEQ ID NO:50).

(Lys Ile Ala Gly Lys Nva Ala)₃ (SEQ ID NO:51).
 (His Ile Ala Gly His Ile Ala)₃ (SEQ ID NO:52).
 (Ala Gly Lys Ile Ala Lys Ile)₃ (SEQ ID NO:53).
 (Ile Ala Lys Ile Ala Gly Lys)₃ (SEQ ID NO:54).
 (Lys Ile Ala Gly Arg Ile Ala)₃ (SEQ ID NO:55).
 (Arg Ile Ala Gly Arg Ile Ala)₃ (SEQ ID NO:56).
 (Lys Val Ala Gly Lys Ile Ala)₃ (SEQ ID NO:57).
 (Lys Ile Ala Gly Lys Val Ala)₃ (SEQ ID NO:58).
 (Ala Lys Ile Ala Gly Lys Ile)₃ (SEQ ID NO:59).
 (Orn Ile Ala Gly Orn Ile Ala)₃ (SEQ ID NO:60).
 (Lys Phe Ala Gly Lys Ile Ala)₃ (SEQ ID NO:61).
 (Lys Ile Ala Gly Lys Phe Ala)₃ (SEQ ID NO:62).
 (Lys Cha Ala Gly Lys Ile Ala)₃ (SEQ ID NO:63).
 (Lys Nle Ala Lys Ile Ala Gly)₃ (SEQ ID NO:64).
 (Arg Ile Ala Gly Lys Ile Ala)₃ (SEQ ID NO:65).
 (Har Ile Ala Gly Har Ile Ala)₃ (SEQ ID NO:66).
 (Xaa Ile Ala Gly Lys Ile Ala)₃ (SEQ ID NO:67).
 (Lys Ile Ala Gly Xaa Ile Ala)₃ (SEQ ID NO:68).
 Lys Ile Ala (Lys Ile Ala Gly Lys Ile Ala)₃ (SEQ ID NO:69)

In (SEQ ID NO:67) and (SEQ ID NO:68), Xaa is p-aminophenylalanine.

In accordance with another embodiment, the amphiphilic peptide includes the following basic structure X₄₀:

$R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{32}-R_{34}-R_{32}-R_{32}$,
 wherein R₃₁, R₃₂, and R₃₃ are as hereinabove described,
 and R₃₄ is a basic hydrophilic or hydrophobic amino acid.

In accordance with one embodiment, the peptide may include the following structure:

Y₄₀-X₄₀, wherein X₄₀ is as hereinabove described, and Y₄₀ is:

- (i) R₃₂;
- (ii) R₃₂-R₃₂;

- (iii) $R_{34}-R_{32}-R_{32}$;
- (iv) $R_{33}-R_{34}-R_{32}-R_{32}$;
- (v) $R_{32}-R_{33}-R_{34}-R_{32}-R_{32}$;
- (v) $R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}$, or
- (vii) $R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}$, wherein R_{31} , R_{32} , R_{33} and R_{34} are as hereinabove described.

In accordance with another embodiment, the peptide may include the following structure:

$X_{40}-Z_{40}$, wherein X_{40} is as hereinabove described and Z_{40} is:

- (i) R_{31} ;
- (ii) $R_{31}-R_{32}$;
- (iii) $R_{31}-R_{32}-R_{32}$;
- (iv) $R_{31}-R_{32}-R_{32}-R_{33}$;
- (v) $R_{31}-R_{32}-R_{32}-R_{33}-R_{34}$;
- (vi) $R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}$; or
- (vii) $R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}$, wherein R_{31} , R_{32} , R_{33} , and R_{34} are as hereinabove described.

In accordance with yet another embodiment the peptide may include the following structure:

$(Y_{40})_a-X_{40}-(Z_{40})_b$, wherein Y_{40} and Z_{40} are as previously defined, a is 0 or 1, and b is 0 or 1. In a preferred embodiment, the peptide has the following structural formula as given in the accompanying sequence listing:

(SEQ ID NO:70)

In another preferred embodiment, the peptide has the following structural formula as given in the accompanying sequence listing:

(SEQ ID NO:71)

In accordance with a further embodiment, the peptide has one of the one of the following structural formulae as given in the accompanying sequence listing:

(SEQ ID NO:72)

(SEQ ID NO:73)
(SEQ ID NO:74)
(SEQ ID NO:75)
(SEQ ID NO:76)
(SEQ ID NO:77)
(SEQ ID NO:78)
(SEQ ID NO:79)
(SEQ ID NO:80)
(SEQ ID NO:81)
(SEQ ID NO:82)
(SEQ ID NO:83)
(SEQ ID NO:84)
(SEQ ID NO:85)
(SEQ ID NO:86)
(SEQ ID NO:87)

In accordance with another embodiment, the peptide purified in accordance with the present invention may include the following structural formula:

- (Lys Ile Ala Lys Lys Ile Ala)-_n, wherein n is from 2 to 5. Preferably, n is 3, and the peptide has the following structural formula:

(Lys Ile Ala Lys Lys Ile Ala)₃
(SEQ ID NO:88)

In accordance with another embodiment, the peptide may include the following structural formula:

-(Lys Phe Ala Lys Lys Phe Ala)-_n
wherein n is from 2 to 5.

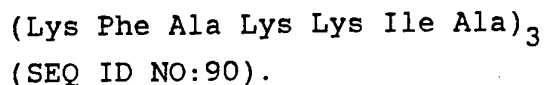
Preferably, n is 3, and the peptide has the following structural formula:

(Lys Phe Ala Lys Lys Phe Ala)₃
(SEQ ID NO:89)

In accordance with another embodiment, the peptide may include the following structural formula:

-(Lys Phe Ala Lys Lys Ile Ala)-_n

wherein n is from 2 to 5. Preferably n is 3, and the peptide has the following structural formula:



In accordance with another embodiment, the peptide may be selected from the group consisting of the following structural formulae as given in the accompanying sequence listing:

(SEQ ID NO:91)

(SEQ ID NO:92)

(SEQ ID NO:93)

(SEQ ID NO:94)

In accordance with yet another embodiment, the peptide may be a cecropin or sarcotoxin, or analogue or derivative thereof.

The term cecropins includes any of the natural structures as well as analogues and derivatives thereof. The cecropins and analogues and derivatives thereof are described in U.S. Patent No. 4,355,104, Ann. Rev. Microbiol., Vol. 41, pages 103-26 (1987), in particular page 108, and in Christensen, et al., Proc. Nat. Acad. Sci., Vol. 85, pgs. 5072-76 (1988), which are hereby incorporated by reference.

The term sarcotoxins includes the basic materials as well as analogues and derivatives thereof. The sarcotoxins and analogues and derivatives thereof are described in Molecular Entomology, pages 369-78, in particular page 375, Alan R. Liss, Inc. (1987), which is hereby incorporated by reference.

In accordance with another embodiment, the amphiphilic peptide may be melittin or an analogue or derivative thereof.

Melittin is an amphipathic peptide consisting of 26 amino acid residues, and is isolated from honeybee (*Apis*

mellifera) venom. The peptide is known to be cytolytic. See Habermann, et al., Hoppe-Seyler's Zeitschrift Physiol. Chem., Vol. 348, pgs. 37-50 (1987). Melittin has the following structural formula as represented by the three-letter amino acid code:

Gly Ile Gly Ala Val Leu Lys Val Leu

5

Thr Thr Gly Leu Pro Ala Leu Ile Ser

10

15

Trp Ile Lys Arg Lys Arg Gln Gln

20

25

(SEQ ID NO:95)

In another embodiment, the peptide purified in accordance with the present invention is an apidaecin. The term apidaecin as used herein includes the basic structure as well as analogues and derivatives thereof. Apidaecins are further described in European Patent Application No. 299,828.

In another embodiment, the amphiphilic peptide purified in accordance with the present invention includes the following basic structure X_{50} :

$R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}$.

R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid.

In one further embodiment, the peptide includes the basic structure $Y_{50}-X_{50}$ wherein X_{50} is as hereinabove described and Y_{50} is:

(i) R_{41} ;

(ii) $R_{42}-R_{41}$; or

(iii) $R_{42}-R_{42}-R_{41}$, wherein R_{41} and R_{42} are as hereinabove described.

In one embodiment, R_{41} is leucine. In another embodiment, R_{42} is lysine. Representative examples of

peptides in accordance with this aspect of the present invention include those having the following structures:

(SEQ ID NO: 96)

(SEQ ID NO: 97)

(SEQ ID NO: 98)

(SEQ ID NO: 99)

In accordance with another embodiment, the amphiphilic peptide includes the following basic structure X_{52} :

$R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}$, wherein R_{41} is a hydrophobic amino acid and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid.

In one embodiment R_{41} is leucine. In another embodiment, R_{42} is lysine.

In one embodiment, the peptide includes the basic structure $Y_{52}-X_{52}$, wherein X_{52} is as hereinabove described, and Y_{52} is:

- (i) R_{42} ;
- (ii) $R_{41}-R_{42}$;
- (iii) $R_{41}-R_{41}-R_{42}$;
- (iv) $R_{42}-R_{41}-R_{41}-R_{42}$; or
- (v) $R_{42}-R_{42}-R_{41}-R_{41}-R_{42}$.

In one embodiment, the peptide may have the following structure;

Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu
5 10

Leu Lys Lys Leu Arg Arg
15

(SEQ ID NO.:100)

In another embodiment, the peptide includes the basic structure $X_{52} - Z_{52}$, wherein X_{52} is as hereinabove described, and Z_{52} is:

- (i) R_{41} ;

- (ii) $R_{41}-R_{41};$
- (iii) $R_{41}-R_{41}-R_{42};$
- (iv) $R_{41}-R_{41}-R_{42}-R_{42};$ or
- (v) $R_{41}-R_{41}-R_{42}-R_{42}-R_{41};$

In one embodiment, the peptide may have the following structure:

Lys Leu Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu Leu Lys Lys
 Leu 5 10
 15

(SEQ ID NO: 101)

In another embodiment, the peptide may include the structure:

$(Y_{52})_a - X_{52} - (Z_{52})_b$, wherein X_{52} , Y_{52} and Z_{52} are as hereinabove described, and a is 0 or 1, and b is 0 or 1.

In accordance with another embodiment, the peptide includes the following basic structure X_{54} :

$-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{43}-$, wherein R_{41} and R_{42} are as hereinabove described, and R_{43} is a natural hydrophilic amino acid.

In one embodiment, the peptide may have the following structure:

(SEQ ID NO:102)

In another embodiment, the peptide may have the following structure:

(SEQ ID NO:103)

In accordance with yet another embodiment, the peptide includes the following basic structure X_{56} :

$R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{44}$, wherein R_{41} and R_{42} are as hereinabove described, and R_{44} is a neutral hydrophilic amino acid or proline.

In one embodiment, the peptide may include the following structure $Y_{56}-X_{56}$, wherein X_{56} is the basic peptide structure hereinabove described, and Y_{56} is:

- (i) $-R_{41}$
- (ii) $-R_{41}-R_{41};$
- (iii) $-R_{42}-R_{41}-R_{41};$
- (iv) $-R_{41}-R_{42}-R_{41}-R_{41};$
- (v) $-R_{41}-R_{41}-R_{42}-R_{41}-R_{41};$
- (vi) $-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41};$ or
- (vii) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41};$

wherein R_{41} and R_{42} are as hereinabove described.

In one embodiment, the peptide may include the structure:

$X_{56}-Z_{56}$, wherein X_{56} is as hereinabove described, and Z_{56} is:

- (i) $-R_{42};$
- (ii) $-R_{42}-R_{42};$
- (iii) $-R_{42}-R_{42}-R_{41};$
- (iv) $-R_{42}-R_{42}-R_{41}-R_{41};$
- (v) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42};$
- (vi) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42};$ or
- (vii) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41};$

In a preferred embodiment, the peptide may have one of the following structures:

(SEQ ID NO:104); or

(SEQ ID NO:105).

In another embodiment, the peptide may have the structure $(Y_{56})_a-X_{56}-(Z_{56})_b$, wherein X_{56} , Y_{56} , and Z_{56} are as hereinabove described, a is 0 or 1, and b is 0 or 1.

In accordance with another embodiment, the peptide includes the following basic structure X_{58} :

$R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{43}$,
wherein R_{41} , R_{42} and R_{43} are as hereinabove described.

In accordance with another embodiment, the peptide may include the structure $Y_{58}-X_{58}$, wherein X_{58} is as hereinabove described, and Y_{58} is:

- (i) $-R_{41};$

- (ii) $-R_{42}-R_{41};$
- (iii) $-R_{42}-R_{42}-R_{41};$
- (iv) $-R_{41}-R_{42}-R_{42}-R_{41};$
- (v) $-R_{41}-R_{41}-R_{42}-R_{42}-R_{41};$
- (vi) $-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41};$ or
- (vii) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41},$ wherein R_{41} and R_{42} are as hereinabove described.

In another embodiment, the peptide includes the structure $X_{58}-Z_{58}$, wherein X_{58} is as hereinabove described, and Z_{58} is:

- (i) $-R_{41};$
- (ii) $-R_{41}-R_{45};$
- (iii) $-R_{41}-R_{45}-R_{45};$
- (iv) $-R_{41}-R_{45}-R_{45}-R_{43};$
- (v) $-R_{41}-R_{45}-R_{45}-R_{43}-R_{41};$
- (vi) $-R_{41}-R_{45}-R_{45}-R_{43}-R_{41}-R_{43};$
- (vii) $-R_{41}-R_{45}-R_{45}-R_{43}-R_{41}-R_{43}-R_{43};$
- (viii) $-R_{41}-R_{45}-R_{45}-R_{43}-R_{41}-R_{43}-R_{43}-R_{45};$ or
- (ix) $-R_{41}-R_{45}-R_{45}-R_{43}-R_{41}-R_{43}-R_{43}-R_{45}-R_{43},$ wherein R_{41} and R_{43} are as hereinabove described, and R_{45} is proline.

In one embodiment, the peptide has the following structure:

(SEQ ID NO:106).

In one embodiment, the peptide may have the structure $(Y_{58})_a-X_{58}-(Z_{58})_b$, wherein X_{58} , Y_{58} , and Z_{58} are as hereinabove described, a is 0 or 1, and b is 0 or 1.

In accordance with another embodiment, the peptide includes the following basic structure X_{60} ;

$R_{41}-R_{41}-R_{43}-R_{42}-R_{41}-R_{41}-R_{41}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41},$ wherein R_{41} , R_{42} , and R_{43} are as hereinabove described. In one embodiment, the peptide may have the following structure:

(SEQ ID NO:107).

In another embodiment, the peptide may include the structure $X_{60}-Z_{60}$, wherein X_{60} is as hereinabove described, and Z_{60} is:

- (i) $-R_{42}$;
- (ii) $-R_{42}-R_{42}$;
- (iii) $-R_{42}-R_{42}-R_{41}$;
- (iv) $-R_{42}-R_{42}-R_{41}-R_{41}$;
- (v) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}$;
- (vi) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}$; or
- (vii) $-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}$.

In accordance with yet another embodiment, the peptide has a structure selected from the group consisting of:

- (a) $R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (b) $R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (c) $R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (d) $R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$; and
- (e) $R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$,

wherein R_{41} and R_{42} are as hereinabove described.

In one embodiment, the peptide has the structure (a), and a representative example of such a structure is (SEQ ID NO:108), which is given in the accompanying sequence listing.

In another embodiment, the peptide has the structure (b), and a representative example of such a structure is (SEQ ID NO:109), which is given in the accompanying sequence listing.

In another embodiment, the peptide has the structure (c), and a representative example of such a structure is (SEQ ID NO:110) as given the accompanying sequence listing.

In yet another embodiment, the peptide has the structure (d), and a representative example of such a structure is (SEQ ID NO:111) as given in the accompanying sequence listing.

In a further embodiment, the peptide has the structure (e), and representative examples of such a structure are (SEQ ID NO:112) and (SEQ ID NO:113) as given in the accompanying sequence listing.

In accordance with another embodiment, the peptide has the following structural formula:

(SEQ ID NO:114).

In accordance with yet another embodiment, the amphiphilic peptide or protein may be an ion channel-forming peptide or protein.

Ion channel-forming proteins or peptides which may be employed include defensins, also known as human neutrophil antimicrobial peptides (HNP), major basic protein (MBP) of eosinophils, bactericidal permeability-increasing protein (BPI), or a pore-forming cytotoxin called variously perforin, cytolysin, or pore-forming protein. Defensins are described in Selsted, et al., J. Clin. Invest., Vol. 76, pgs. 1436-1439 (1985). MBP proteins are described in Wasmoen, et al., J. Biol. Chem., Vol. 263, pgs 12559-12563. (1988). BPI proteins are described in Ooi, et al, J. Biol. Chem., Vol. 262, pgs. 14891-14894 (1987). Perforin is described in Henkart, et al., J. Exp. Med., 160: 75 (1984), and in Podack, et al., J. Exp. Med., 160:695 (1984). The above articles are hereby incorporated by reference.

The term ion channel-forming proteins includes the basic structures of the ion channel-forming proteins as well as analogues and derivatives.

The method of the present invention may be used to obtain amphiphilic peptides or proteins from any starting material containing amphiphilic peptides or proteins. Examples of starting materials include, but are not limited to, solutions of amphiphilic peptides or proteins which were chemically synthesized, were made by recombinant techniques, were isolated from or found in biological tissues, or were

isolated from or found in cell lysates. Such starting materials include the at least one amphiphilic peptide or protein and, depending upon the starting material, may also include lipids, glycolipids, polysaccharides, membrane proteins, soluble or cytosolic proteins, RNA and DNA.

In a preferred embodiment, a starting material such as any of those hereinabove described, which contains at least one amphiphilic peptide or protein, is placed into an aqueous solution containing acetonitrile and trifluoroacetic acid. The at least one amphiphilic peptide or protein is dissolved in the solution to form an extract solution containing the at least one amphiphilic peptide or protein. The extract solution is then separated from any insoluble materials, if present, and evaporated to dryness, leaving a dry composition containing the at least one amphiphilic peptide or protein. Hydrophobic materials, which are extracted by the acetonitrile, are thereby removed from the starting material. A mixture of chloroform and methanol in a chloroform-to-methanol volume ratio of about 2:1 is added to the dry composition in an amount sufficient to form a single liquid phase, to which is then added 0.1M potassium chloride in an amount sufficient to form an upper aqueous phase containing the at least one amphiphilic peptide or protein, and a lower organic phase containing primarily hydrophobic peptides and proteins, lipids, and other molecules. Other non-desirable materials may be found as insoluble precipitates in the organic phase or in a boundary layer between the aqueous or organic phases. The aqueous phase is then separated from the boundary layer and the primarily organic phase, and the at least one amphiphilic peptide or protein is recovered from the primarily aqueous phase by means such as those hereinabove described.

In another preferred embodiment, a starting material such as any of those hereinabove described, which contains

at least one amphiphilic peptide or protein, is present as an aqueous solution or suspension from which any insoluble materials, if present, are separated. A mixture of chloroform and methanol in a volume ratio of 2:1 is added to the aqueous solution in an amount sufficient to form a single miscible phase, to which is added a solution of sodium chloride in water in a concentration of at least 40 mM and in an amount sufficient to form an upper aqueous phase containing the at least one amphiphilic peptide or protein, and a lower organic phase containing primarily hydrophobic peptides and proteins, lipids, and other possible molecular contaminants. The aqueous phase is separated from any boundary layer and the primarily organic phase, and the at least one amphiphilic peptide or protein is recovered from the primarily aqueous phase by means such as those hereinabove described.

The invention will now be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Approximately 1.0 mL of a thawed E. coli strain HB101 cell lysate (sonicated cell mixture) was placed in each of two tubes. 100 microliters (uL) of (SEQ ID NO:7) - NH₂ was added to one tube (at a concentration of 1.0 mg/mL) and 100 uL of (SEQ ID NO:115) was added to the other tube (at a concentration of 1.0 mg/mL), after first adding 10.0 mL 60% acetonitrile, 1% trifluoroacetic acid (TFA) to each HB101 cell sonicate. The samples were placed on ice and then shaken gently in a cold room at 4°C overnight.

The two samples then were spun in a Beckman GPR centrifuge for 15 minutes at 4°C and at 3,000 rpm to form extract supernatants. The (SEQ ID NO:7)-NH₂ and (SEQ ID NO:115) extracts each were placed in approximate 6.0 mL portions into glass 13x100 mm test tubes. The tubes'

contents were lyophilized to dryness under vacuum over a 6-hour period. Each precipitate then was reconstituted in 400 μ L of 0.1% TFA by vortexing. 50 μ L of each of the HB101 cell lysate extracts were set aside in plastic Eppendorf tubes and placed on ice. Fifteen volumes of chloroform:methanol (2:1, vol:vol) were added to each of the HB101 cell lysates, each sample then was mixed and was spun 15 minutes at 1,500 rpm and at 4°C to break any emulsion. In each sample, a top insoluble thin phase was formed which was removed. One-fifth volume (1.2mL) of 0.1M potassium chloride (KCl) was added to the remaining portion of each sample and the samples were mixed. The tubes were spun 15 minutes at 1,500 rpm and at 4°C and the top (aqueous) phase (about 2.1 mL in each case) was transferred to a fresh glass tube. Each aqueous layer then was reduced to 0.5 mL under vacuum. The bottom organic phases were evaporated to dryness under vacuum and 0.50 mL of 0.1% TFA was added to each dried organic phase sample. The bottom (organic) phase samples were vortexed vigorously and then were centrifuged for 15 minutes at 1,500 rpm and at 4°C.

The two 50 μ L portions of each 0.1% TFA samples set aside earlier, 125 μ L of each of the top (aqueous) layer samples, 100 μ L of a (SEQ ID NO:7) - NH₂ (1.0 mg/mL) standard (tube 1S_f), and 100 μ L of a (SEQ ID NO:115) (1.0 mg/mL) standard (tube 2S_f) were filtered through 0.5 μ m PTFE filters (Millipore Corporation, Redford, MA) using 1.0 mL syringes. The samples were filtered into plastic conical bottom HPLC ampoules. 100 μ L of a (SEQ ID NO:7)-NH₂ (1.0 mg/mL) standard (tube 1S), and 100 μ L of a (SEQ ID NO:115) (1.0 mg/mL) standard (tube 2S) were placed directly into HPLC ampoules without filtration. Then, 125 μ L of each of the bottom (organic) phase sample extracts were filtered into HPLC ampoules.

Two samples from each tube, each of which was 2 uL in volume, were subjected to HPLC analysis on a 1 x 50 mm applied Biosystems C8 Aquapore reverse phase column (300 A particle diameter, 20 micron pore size) operated on a Hewlett-Packard (Series II) model 1090 liquid chromatograph using a 20% to 30% acetonitrile in water gradient in the presence of 0.1% TFA; elution was over a 10 minute interval. Eluents were continuously monitored at both 200.4 and 214.4 nm.

Sample results are given in Table I below. Single peptide peak areas were quantified for samples originating from peptide standards added to HB101 cell lysates based upon retention times for (SEQ ID NO:7) - NH₂ and (SEQ ID NO:115) peptide standards. Absorbance values are the mean for the two independent aliquots taken from each HPLC sample:

Table I

<u>Sample</u>	Mean Peak Area Absorbance at 200.4 nm (<u>Arbitrary Units</u>)
1S (SEQ ID NO:7)-NH ₂	18784
1S _f	15504
2S _f (SEQ ID NO:115)	15949
2S _f	10840
Sample 1, (SEQ ID NO:7)-NH ₂ in HB101 lysate:	
Total 60% acetonitrile, 1%	
TFA extract	560*
Top (aqueous) phase	538
Bottom (organic) phase extract	134
Sample 2, (SEQ ID NO:7)-NH ₂ in HB101 lysate:	
Total 60% acetonitrile, 1%	
TFA extract	560*
Top (aqueous) phase	561
Bottom (organic) phase extract	89

Sample 1, (SEQ ID NO:115) in

HB101 lysate:

Total 60% acetonitrile, 1%	
TFA extract	304*
Top (aqueous) phase	366
Bottom (organic)	
phase extract	0

Sample 2, (SEQ ID NO:115) in

HB101 lysate:

Total 60% acetonitrile, 1%	
TFA extract	304*
Top (aqueous) phase	399
Bottom (organic)	
phase extract	46

The (*) sample values are the same for each pair of samples in the data sets with (SEQ ID NO:7)-NH₂ and (SEQ ID NO:115) since only one 50 uL sample of total acetonitrile/TFA extract was set aside for each extract type.

Several conclusions can be drawn from this table and from measurements of total cell protein in the primary HB101 cell lysate. The amount of peptide added to the primary extracts corresponds to about 3% of total bacterial cell protein. Integration of the peak areas on the HPLC profiles for peptide and protein recovered from the acetonitrile/TFA extractions and resuspended in 0.1% TFA suggests that the acetonitrile/TFA extraction concentrates the added peptides at least ten-fold from the crude cell lysates. The single extraction of (SEQ ID NO:7) - NH₂ or (SEQ ID NO:115) into 0.1 M potassium chloride from chloroform:methanol (2:1, vol:vol) further increases the peptide purity by greater than two-fold and is an efficient extraction step. Compensating for sample volumes, the 0.1 M potassium

chloride extraction step has an average efficiency of about 83% for (SEQ ID NO:7) - NH₂ and an average efficiency of about 95% for (SEQ ID NO:115). Efficiency is determined by dividing the arbitrary area units of the aqueous phase by the total sum of the arbitrary area units of the aqueous and organic phases. Net yield of peptides (SEQ ID NO:7) - NH₂ and (SEQ ID NO:115) into the total aqueous and organic phases is greater than that in the parent 0.1% TFA sample, presumably because peptide-containing material has precipitated or is aggregated that is filtered away upon preparation of the total extract HPLC sample. This undetected peptide fraction is probably solubilized upon mixing of the 0.2% TFA sample with 15 volumes of chloroform:methanol (2:1).

Example 2

A cyanogen bromide-treated sample of protein isolated from inclusion bodies produced upon 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) induction of 500 mL of a recombinant E.coli strain produced an E.coli bacteriophage T7 gene 10 gene product--(SEQ ID NO:116) fusion protein. The fusion protein was evaporated to dryness under vacuum and resuspended in 5.0 mL 0.1 M Tris-HCl, pH 8.0, 6 M guanidine hydrochloride (GuHCl). Cyanogen bromide treatment of solubilized inclusion body protein from this culture releases the amphiphilic peptide

(SEQ ID NO:116) from the fusion protein. This sample was incubated 15 minutes at 55°C before it was spun at 2000 rpm for 5 minutes, 4°C, in a Beckman GPR centrifuge and 50 microliters (μL) of the 0.1 M Tris-HCl/6 M GuHCl extract supernatant was placed in each of two glass 17 x 100 mm test tubes. Control experiments studying the effect of repeated extractions of the pellet fraction from the 0.1 M Tris-HCl/6 M GuHCl extraction step revealed that greater than 90% of the extracted amphiphilic peptide (SEQ ID NO:116) was solubilized by a single extraction into this buffer. Fifteen volumes (0.75 mL) of chloroform:methanol (2:1) then were added to the tubes, the tubes were mixed, then 100 μL 0.1 M potassium chloride (KCl) was added to each solution and the tubes were spun at 2000 rpm for 5 minutes, 4°C, in a Beckman GPR centrifuge. The aqueous top phases were transferred to new glass test tubes. The top phases were evaporated to dryness under vacuum, resuspended in 100 μL deionized water and one sample was set aside. The other sample extract volume was increased to 160 μL with deionized water, 2.4 mL chloroform:methanol (2:1) was added to the tube, the sample was mixed, then 512 μL 0.1 M potassium chloride (KCl) was added to the solution and the sample was spun 2000 rpm for 5 minutes, 4°C before the aqueous top phase was transferred to a new glass tube. Again the aqueous top phase was evaporated to dryness under vacuum and resuspended in 250 μL deionized water before adding 3.75 mL

chloroform:methanol (2:1). The sample was mixed, and 800 μ L 40 mM KCl was added. The sample was again mixed, spun 2000 rpm for 5 minutes, 4°C, and the aqueous top phase was again transferred to a new glass test tube. The aqueous top phase was evaporated to dryness again, and the precipitate was resuspended in 520 μ L deionized water before adding 7.8 mL chloroform:methanol (2:1) and mixing the sample. About 1.65 mL of 40 mM KCl then was added, the sample was mixed, and it then was spun 2000 rpm for 5 minutes, 4°C, before the top aqueous phase was transferred to another glass test tube. The volume of this sample was reduced under vacuum from about 3.0 mL to about 1.2 mL before 100 μ L of this sample and the 100 μ L sample set aside earlier were each filtered into an HPLC vial through an 0.45 micron cellulose acetate filter (Millipore). The samples were analyzed by HPLC analysis on a Rainin Dynamax instrument using an Applied Biosystems 2.5 mm x 50 mm C8 Aquapore reverse phase column (300 Angstrom particle size, 20 micron pore size) with a 2%-60% acetonitrile in 0.1% TFA mobile phase gradient of 30 minute duration. The A₂₂₀ peak material corresponding to (SEQ ID NO:116) was identified using a chemically synthesized (SEQ ID NO:116) standard chromatographed under the same conditions. The percent total A₂₂₀ peak material present as (SEQ ID NO:116) was calculated by the Rainin instrument software and gave values of 6.8% for one process purification cycle and 8.8% (mean, two readings) for four

process purification cycles. However, it was noted that the (SEQ ID NO:116) chromatographic peak was superimposed on a complex background of A_{220} absorbing material following one process purification cycle, suggesting that the 6.8% value is a substantial overestimate of (SEQ ID NO:116) purity. By contrast, the (SEQ ID NO:116) peak and other chromatographic peaks seen following four process purification cycles approached or achieved chromatographic detector base line values, indicating that the 8.8% estimated purity value for four cycles is correct. Subsequent collection of the (SEQ ID NO:116) peak material following one or four process cycle applications and analysis for amino acid content on an Applied Biosystems amino acid analyzer suggested that the (SEQ ID NO:116) peak material is only about 30% pure following one process cycle application, but about 80% pure following four process cycle applications.

Example 3

Twelve 50 μ L portions of the 0.1 M Tris-HCl guanidine hydrochloride (GuHCl) extract described in Example 2 were placed in glass 17 x 100 mM test tubes and were mixed with 750 μ L chloroform:methanol (2:1) before adding 0-100 μ L of either 0.1 M NaCl or 0.1 M KCl to each tube. The aqueous phase additions were all standardized at 100 μ L by adding additional deionized water where appropriate. These samples were thoroughly mixed and were spun 2000 rpm for 5 minutes,

4°C, before the top aqueous phases were removed to new test tubes and evaporated to dryness under vacuum. Each dried sample was resuspended in 100 µL deionized water and filtered through 0.45 micron cellulose acetate filters (Millipore) before being subjected to HPLC analysis as described in Example 2. The results are shown in Table 2. They provide evidence that NaCl works as well or better than KCl as an aqueous extraction phase additive at all concentrations tested and that deionized water alone is about 80% as efficient as 100 mM salt solutions in re-extraction of amphiphilic peptide (SEQ ID NO:116) from the organic phase.

Table 2

<u>Salt Additive</u>	<u>Concentration of Salt Additive (mM)</u>	<u>A₂₂₀ Peak Area Counts (x 10⁻³) (Arbitrary Units)</u>
NaCl	0	24.5
	20	30.1
	40	30.6
	60	29.1
	80	28.6
	100	31.2
KCl	0	25.8
	20	28.1
	40	28.3
	60	27.7
	80	31.0
	100	30.2

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments or teachings described above. The invention may

be practiced other than as particularly described and still
be within the scope of the accompanying claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Williams, Jon

(ii) TITLE OF INVENTION: Purification of Amphiphilic
Compounds

(iii) NUMBER OF SEQUENCES: 116

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Carella, Byrne, Bain, Gilfillan,
Cecchi & Stewart

(B) STREET: 6 Becker Farm Road

(C) CITY: Roseland

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch diskette

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: DW4.V2

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/936,504

(B) FILING DATE: 28-AUG-1992

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Olstein, Elliot M.
- (B) REGISTRATION NUMBER: 24,025
- (C) REFERENCE/DOCKET NUMBER: 421250-184

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 201-994-1700
- (B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: W089/11290
- (I) FILING DATE: 19-MAY-1989
- (J) PUBLICATION DATE: 30-NOV-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala	Phe	Ser	Lys	Ala	Phe	Ser	Lys	Ala	Phe
				5					10
Ser	Lys	Ala	Phe	Ser	Lys	Ala	Phe	Ser	Lys
				15					20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: W089/11290

(I) FILING DATE: 19-MAY-1989

(J) PUBLICATION DATE: 30-NOV-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Phe Ser Lys Ala Phe Ser Lys Ala Phe

5

10

Ser Lys Ala Phe Ser Lys Ala Phe Ser Lys

15

20

Ala Phe Ser Lys

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: W089/11290

(I) FILING DATE: 19-MAY-1989

(J) PUBLICATION DATE: 30-NOV-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Phe Ser Lys Ala Phe Ser Lys Ala Phe Ser
 5 10
Lys Ala Phe Ser Lys Ala
 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: W089/11290
- (I) FILING DATE: 19-MAY-1989
- (J) PUBLICATION DATE: 30-NOV-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Lys Ala Phe Ser Lys Ala Phe Ser Lys
 5 10
Ala Phe Ser Lys Ala Phe Ser Lys Ala Phe
 15 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: W089/11290
- (I) FILING DATE: 19-MAY-1989
- (J) PUBLICATION DATE: 30-NOV-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Ala Phe Ser Lys Ala Phe Ser Lys Ala
 5 10
Phe Ser Lys Ala Phe Ser
 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Magainin I peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
Gly Ile Gly Lys Phe Leu His Ser Ala Gly
 5 10
Lys Phe Gly Lys Ala Phe Val Gly Glu Ile
 15 20
Met Lys Ser

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Magainin II peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Ile Gly Lys Phe Leu His Ser Ala Lys
 5 10
Lys Phe Gly Lys Ala Phe Val Gly Glu Ile
 15 20
Met Asn Ser

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Magainin III peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Ile Gly Lys Phe Leu His Ser Ala Lys
 5 10
Lys Phe Gly Lys Ala Phe Val Gly Glu Ile
 15 20
Met Asn

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: magainin peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
Ile Gly Lys Phe Leu His Ser Ala Lys Lys
 5 10
Phe Gly Lys Ala Phe Val Gly Glu Ile Met
 15 20
Asn Ser

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: magainin peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
Gly Lys Phe Leu His Ser Ala Lys Lys Phe
 5 10
Gly Lys Ala Phe Val Gly Glu Ile Met Asn
 15 20
Ser

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: magainin peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Zasloff, Michael
- (C) JOURNAL: Proc. Nat. Acad. Sci.
- (D) VOLUME: 84
- (F) PAGES: 5449-5453
- (G) DATE: AUG - 1987
- (H) DOCUMENT NUMBER: US 4810777
- (I) FILING DATE: 04-MAR-1987
- (J) PUBLICATION DATE: 07-MAR-1989

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
Lys Phe Leu His Ser Ala Lys Lys Phe Gly
 5 10
Lys Ala Phe Val Gly Glu Ile Met Asn Ser
 15 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: PGLa peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Hoffman, et al.
- (C) JOURNAL: EMBO J.
- (D) VOLUME: 2
- (F) PAGES: 711-714
- (G) DATE: 1983
- (A) AUTHOR: Andreu, et al.
- (C) JOURNAL: Journal of Biochemistry
- (D) VOLUME: 149
- (F) PAGES: 531-535
- (G) DATE: 1985
- (A) AUTHOR: Gibson, et al.
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 5341-5349
- (G) DATE: 1986
- (A) AUTHOR: Giovannini, et al.
- (C) JOURNAL: Biochem J.
- (D) VOLUME: 243
- (F) PAGES: 113-120
- (G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
Gly Met Ala Ser Lys Ala Gly Ala Ile Ala
 5 10
Gly Lys Ile Ala Lys Val Ala Leu Lys Ala
 15 20
Leu

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: XPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Hoffman, et al.1
- (C) JOURNAL: EMBO J.
- (D) VOLUME: 2
- (F) PAGES: 711-714
- (G) DATE: 1983
- (A) AUTHOR: Andreu, et al.
- (C) JOURNAL: Journal of Biochemistry
- (D) VOLUME: 149
- (F) PAGES: 531-535
- (G) DATE: 1985
- (A) AUTHOR: Gibson, et al.
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 5341-5349

(G) DATE: 1986
(A) AUTHOR: Giovannini, et al.
(C) JOURNAL: Biochem J.
(D) VOLUME: 243
(F) PAGES: 113-120
(G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
Gly Trp Ala Ser Lys Ile Gly Gln Thr Leu
 5 10
Gly Lys Ile Ala Lys Val Gly Leu Lys Glu
 15 20
Leu Ile Gln Pro Lys
 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K.
 Egger, R.
 Kreil
(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 3676-3680

(G) DATE: 1986
(A) AUTHOR: Wakabayashi, T.
Kato, H.
Tachibana, S.
(C) JOURNAL: Nucleic Acids Research
(D) VOLUME: 13
(F) PAGES: 1817-1828
(G) DATE: 1985
(A) AUTHOR: Gibson, B.W.
Poulter, L.
Williams, D.H.
Maggio, J.E.
(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 5341-5349
(G) DATE: 1986
(H) DOCUMENT NUMBER: W090/04407
(I) FILING DATE: 16-OCT-1989
(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
Gly Phe Gly Ser Phe Leu Gly Leu Ala Leu
5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala
15 20
Leu Gly Gly Ala Pro Gln Gln
25

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K

Egger, R.

Kreil

(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 3676-3680

(G) DATE: 1986

(A) AUTHOR: Wakabayashi, T.

Kato, H.

Tachibaba, S.

(C) JOURNAL: Nucleic Acids Research

(D) VOLUME: 13

(F) PAGES: 1817-1828

(G) DATE: 1985

(A) AUTHOR: Gibson, B.W.

Poulter, L.

Williams, D.H.

Maggio, J.E.

(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 5341-5349

(G) DATE: 1986

(H) DOCUMENT NUMBER: W090/04407

(I) FILING DATE: 16-OCT-1989

(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
Gly Leu Ala Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Gly Leu Lys Ile Gly Ala His Leu
 15 20
Leu Gly Gly Ala Pro Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
 Egger, R.
 Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.
 Kato, H.
 Tachibana, S.
- (C) JOURNAL: Nucleic Acids Research
- (D) VOLUME: 13
- (F) PAGES: 1817-1828

(G) DATE: 1985
(A) AUTHOR: Gibson, B.W.
Poulter, L.
Williams, D.H.
Maggio, J.E.
(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 5341-5349
(G) DATE: 1986
(H) DOCUMENT NUMBER: W090/04407
(I) FILING DATE: 16-OCT-1989
(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
Gly Leu Ala Ser Leu Leu Gly Lys Ala Leu
 5 10
Lys Ala Gly Leu Lys Ile Gly Thr His Phe
 15 20
Leu Gly Gly Ala Pro Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
Egger, R.
Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.
Kato, H.
Tachibana, S.
- (C) JOURNAL: Nucleic Acids Research
- (D) VOLUME: 13
- (F) PAGES: 1817-1828
- (G) DATE: 1985
- (A) AUTHOR: Gibson, B.W.
Poulter, L.
Williams, D.H.
Maggio, J.E.
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 5341-5349
- (G) DATE: 1986
- (H) DOCUMENT NUMBER: W090/04407
- (I) FILING DATE: 16-OCT-1989
- (J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly	Leu	Ala	Ser	Leu	Leu	Gly	Lys	Ala	Leu
				5					10
Lys	Ala	Thr	Leu	Lys	Ile	Gly	Thr	His	Phe
				15					20
Leu	Gly	Gly	Ala	Pro	Gln	Gln			
				25					

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
Egger, R.
Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.
Kato, H.
Tachibana, S.
- (C) JOURNAL: Nucleic Acids Research
- (D) VOLUME: 13
- (F) PAGES: 1817-1828
- (G) DATE: 1985
- (A) AUTHOR: Gibson, B.W.
Poulter, L.
Williams, D.H.
Maggio, J.E.
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 5341-5349

- (G) DATE: 1986
- (H) DOCUMENT NUMBER: WO90/04407
- (I) FILING DATE: 16-OCT-1989
- (J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gly	Phe	Ala	Ser	Phe	Leu	Gly	Lys	Ala	Leu
					5				10
Lys	Ala	Ala	Leu	Lys	Ile	Gly	Ala	Asn	Met
					15				20
Leu	Gly	Gly	Thr	Pro	Gln	Gln			
					25				

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
Egger, R.
Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.

Kato, H.

Tachibana, S.

(C) JOURNAL: Nucleic Acids Research

(D) VOLUME: 13

(F) PAGES: 1817-1828

(G) DATE: 1985

(A) AUTHOR: Gibson, B.W.

Poulter, L.

Williams, D.H.

Maggio, J.E.

(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 5341-5349

(G) DATE: 1986

(H) DOCUMENT NUMBER: W090/04407

(I) FILING DATE: 16-OCT-1989

(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Phe Gly Ser Phe Leu Gly Lys Ala Leu

5

10

Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala

15

20

Leu Gly Gly Ala Pro Gln Gln

25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K.

Egger, R.

Kreil

(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 3676-3680

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(F) PAGES: 5341-5349

(G) DATE: 1986

(H) DOCUMENT NUMBER: W090/04407

(I) FILING DATE: 16-OCT-1989

(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
Gly Phe Gly Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala
 15 20
Leu Gly Gly Ser Pro Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
 Egger, R.
 Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
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- (G) DATE: 1986
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(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 5341-5349
(G) DATE: 1986
(H) DOCUMENT NUMBER: W090/04407
(I) FILING DATE: 16-OCT-1989
(J) PUBLICATION DATE: 03-MAY-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
Gly Phe Ala Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Leu
 15 20
Leu Gly Gly Thr Pro Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
Egger, R.
Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.
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- (D) VOLUME: 13
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- (A) AUTHOR: Gibson, B.W.
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- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 5341-5349
- (G) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Phe Ala Ser Phe Leu Gly Lys Ala Leu
5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala
15 20
Leu Gly Gly Ala Pro Gln Gln
25

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K.
Egger, R.
Kreil

(C) JOURNAL: J. Biol. Chem.

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(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 5341-5349

(G) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
Gly Phe Ala Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Met
 15 20
Leu Gly Gly Ala Pro Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

- (A) AUTHOR: Richter, K.
 Egger, R.
 Kreil
- (C) JOURNAL: J. Biol. Chem.
- (D) VOLUME: 261
- (F) PAGES: 3676-3680
- (G) DATE: 1986
- (A) AUTHOR: Wakabayashi, T.
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- (C) JOURNAL: Nucleic Acids Research
- (D) VOLUME: 13
- (F) PAGES: 1817-1828

(G) DATE: 1985
(A) AUTHOR: Gibson, B.W.
Poulter, L.
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Maggio, J.E.
(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 5341-5349
(G) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
Gly Phe Gly Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala
 15 20
Leu Gly Gly Ser Leu Gln Gln
 25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K.
Egger, R.
Kreil

- (C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 3676-3680
(G) DATE: 1986
(A) AUTHOR: Wakabayashi, T.
Kato, H.
Tachibana, S.
(C) JOURNAL: Nucleic Acids Research
(D) VOLUME: 13
(F) PAGES: 1817-1828
(G) DATE: 1985
(A) AUTHOR: Gibson, B.W.
Poulter, L.
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Maggio, J.E.
(C) JOURNAL: J. Biol. Chem.
(D) VOLUME: 261
(F) PAGES: 5341-5349
(G) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
Gly Phe Gly Ser Phe Leu Gly Lys Ala Leu
 5 10
Lys Ala Gly Leu Lys Ile Gly Thr Asn Phe
 15 20
Leu Gly Gly Ala Pro Gln Gln
 25

- (2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CPF peptide.

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Richter, K
Egger, R.
Kreil

(C) JOURNAL: J. Biol. Chem.

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(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 261

(F) PAGES: 5341-5349

(G) DATE: 1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Leu Ala Ser Leu Leu Gly Lys Ala Leu

Lys Ala Ala Leu Lys Ile Gly Ala Asn Ala
15 20
Leu Gly Gly Ser Pro Gln Gln
25

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
5 10
Gly Lys Ile Ala Lys Ile Ala Gly Lys Ile
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Lys Ile Ala Lys Ile Ala Gly Lys Ile Ala
5 10

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
15 20
Gly

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
Lys Ile Ala Gly Lys Ile Gly Lys Ile Ala
5 10
Gly Lys Ile Gly Lys Ile Ala Gly Lys Ile
15 20
Gly

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
Lys Leu Ala Gly Lys Leu Ala Lys Leu Ala
5 10

Gly Lys Leu Ala Lys Leu Ala Gly Lys Leu
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Lys Phe Ala Gly Lys Phe Ala Lys Phe Ala
5 10
Gly Lys Phe Ala Lys Phe Ala Gly Lys Phe
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Lys Ala Leu Ser Lys Ala Leu Lys Ala Leu
5 10

Ser Lys Ala Leu Lys Ala Leu Ser Lys Ala
15 20
Leu

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Lys Leu Leu Lys Ala Leu Gly Lys Leu Leu
5 10
Lys Ala Leu Gly Lys Leu Leu Lys Ala Leu
15 20
Gly

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Lys Ala Ile Gly Lys Ala Ile Lys Ala Ile
5 10

Gly Lys Ala Ile Lys Ala Ile Gly Lys Ala
15 20
Ile

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Ile Ala Lys Ile Ala Lys Gly Ile Ala
5 10
Lys Ile Ala Lys Gly Ile Ala Lys Ile Ala
15 20
Lys

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Lys Ile Ala Lys Ile Phe Gly Lys Ile Ala
5 10

Lys Ile Phe Gly Lys Ile Ala Lys Ile Phe
 15 20
Gly

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Gly Ile Ala Arg Ile Ala Lys Gly Ile Ala
 5 10
Arg Ile Ala Lys Gly Ile Ala Arg Ile Ala
 15 20
Lys

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Lys Phe Ala Arg Ile Ala Gly Lys Phe Ala
 5 10

Arg Ile Ala Gly Lys Phe Ala Arg Ile Ala
 15 20
Gly

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Phe Ala Lys Ile Ala Lys Gly Phe Ala
 5 10
Lys Ile Ala Lys Gly Phe Ala Lys Ile Ala
 15 20
Lys

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is ornithine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Lys Ile Ala Gly Xaa Ile Ala Lys Ile Ala
 5 10

Gly Xaa Ile Ala Lys Ile Ala Gly Xaa Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Ile Ala Arg Ile Ala Gly Lys Ile Ala
 5 10
Arg Ile Ala Gly Lys Ile Ala Arg Ile Ala
 15 20
Gly

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is ornithine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Xaa Ile Ala Gly Lys Ile Ala Xaa Ile Ala
5 10
Gly Lys Ile Ala Xaa Ile Ala Gly Lys Ile
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Ile Ala Arg Ile Phe Lys Gly Ile Ala
5 10
Arg Ile Phe Lys Gly Ile Ala Arg Ile Phe
15 20
Lys

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Lys Xaa Ala Gly Lys Xaa Ala Lys Xaa Ala
 5 10
Gly Lys Xaa Ala Lys Xaa Ala Gly Lys Xaa
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Lys Xaa Ala Gly Lys Ile Ala Lys Xaa Ala
 5 10
Gly Lys Ile Ala Lys Xaa Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Ile Ala Gly Lys Xaa Ala Lys Ile Ala
 5 10
Gly Lys Xaa Ala Lys Ile Ala Gly Lys Xaa
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norvaline.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Lys Xaa Ala Gly Lys Xaa Ala Lys Xaa Ala
 5 10
Gly Lys Xaa Ala Lys Xaa Ala Gly Lys Xaa
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norvaline.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Lys	Xaa	Ala	Gly	Lys	Ile	Ala	Lys	Xaa	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Xaa	Ala	Gly	Lys	Xaa
				15				20	
Ala									

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Lys	Leu	Leu	Ser	Lys	Leu	Gly	Lys	Leu	Leu
				5				10	
Ser	Lys	Leu	Gly	Lys	Leu	Leu	Ser	Lys	Leu
				15				20	
Gly									

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Lys Leu Leu Ser Lys Phe Gly Lys Leu Leu
 5 10
Ser Lys Phe Gly Lys Leu Leu Ser Lys Phe
 15 20
Gly

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norvaline.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Lys Ile Ala Gly Lys Xaa Ala Lys Ile Ala
 5 10
Gly Lys Xaa Ala Lys Ile Ala Gly Lys Xaa
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
His Ile Ala Gly His Ile Ala His Ile Ala
 5 10
Gly His Ile Ala His Ile Ala Gly His Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
Ala Gly Lys Ile Ala Lys Ile Ala Gly Lys
 5 10
Ile Ala Lys Ile Ala Gly Lys Ile Ala Lys
 15 20
Ile

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ile	Ala	Lys	Ile	Ala	Gly	Lys	Ile	Ala	Lys
			5					10	
Ile	Ala	Gly	Lys	Ile	Ala	Lys	Ile	Ala	Gly
			15					20	
Lys									

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Lys	Ile	Ala	Gly	Arg	Ile	Ala	Lys	Ile	Ala
			5					10	
Gly	Arg	Ile	Ala	Lys	Ile	Ala	Gly	Arg	Ile
			15					20	
Ala									

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Arg Ile Ala Gly Arg Ile Ala Arg Ile Ala
 5 10
Gly Arg Ile Ala Arg Ile Ala Gly Arg Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Lys Val Ala Gly Lys Ile Ala Lys Val Ala
 5 10
Gly Lys Ile Ala Lys Val Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Lys Ile Ala Gly Lys Val Ala Lys Ile Ala
 5 10
Gly Lys Val Ala Lys Ile Ala Gly Lys Val
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Ala Lys Ile Ala Gly Lys Ile Ala Lys Ile
 5 10
Ala Gly Lys Ile Ala Lys Ile Ala Gly Lys
 15 20
Ile

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is ornithine.

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Xaa Ile Ala Gly Xaa Ile Ala Xaa Ile Ala
 5 10
Gly Xaa Ile Ala Xaa Ile Ala Gly Xaa Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Lys Phe Ala Gly Lys Ile Ala Lys Phe Ala
 5 10
Gly Lys Ile Ala Lys Phe Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Ile Ala Gly Lys Phe Ala Lys Ile Ala
 5 10
Gly Lys Phe Ala Lys Ile Ala Gly Lys Phe
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is cyclohexylalanine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Lys Xaa Ala Gly Lys Ile Ala Lys Xaa Ala
 5 10
Gly Lys Ile Ala Lys Xaa Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Lys Xaa Ala Lys Ile Ala Gly Lys Xaa Ala

5 10

Lys Ile Ala Gly Lys Xaa Ala Lys Ile Ala

15 20

Gly

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 AMINO ACIDS

(B) TYPE: amino acids

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Arg Ile Ala Gly Lys Ile Ala Arg Ile Ala

5 10

Gly Lys Ile Ala Arg Ile Ala Gly Lys Ile

15 20

Ala

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is homoarginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Xaa	Ile	Ala	Gly	Xaa	Ile	Ala	Xaa	Ile	Ala
			5					10	
Gly	Xaa	Ile	Ala	Xaa	Ile	Ala	Gly	Xaa	Ile
			15					20	
Ala									

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE: Xaa is p-aminophenylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Xaa	Ile	Ala	Gly	Lys	Ile	Ala	Xaa	Ile	Ala
			5					10	
Gly	Lys	Ile	Ala	Xaa	Ile	Ala	Gly	Lys	Ile
			15					20	
Ala									

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE: Xaa is p-aminophenylalanine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Lys Ile Ala Gly Xaa Ile Ala Lys Ile Ala
 5 10
Gly Xaa Ile Ala Lys Ile Ala Gly Xaa Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Lys Ile Ala Lys Ile Ala Gly Lys Ile Ala
 5 10
Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
 15 20
Gly Lys Ile Ala

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Lys Leu Ala Ser Lys Ala Gly Lys Ile Ala Gly
 5 10
Lys Ile Ala Lys Val Ala Leu Lys Ala Leu
 15 20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is ornithine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala Gly
 5 10
Xaa Ile Ala Lys Ile Ala Gly Lys Ile Ala
 15 20

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
 5 10
Gly Arg Ile Ala Lys Ile Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
 5 10
Gly Xaa Ile Ala Lys Ile Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norvaline.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
 5 10
Gly Xaa Ile Ala Lys Ile Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is ornithine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Lys Phe Ala Gly Lys Phe Ala Lys Phe Ala Gly
 5 10
Xaa Phe Ala Lys Phe Ala Gly Lys Phe Ala
 15 20

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is ornithine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Lys Ile Ala Gly Lys Phe Ala Lys Ile Ala

5

10

Gly Xaa Phe Ala Lys Ile Ala Gly Lys Phe

15

20

Ala

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa at residues 6, 13, and 20
is norleucine; Xaa at residue
12 is ornithine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Lys Ile Ala Gly Lys Xaa Ala Lys Ile Ala

5

10

Gly Xaa Xaa Ala Lys Ile Ala Gly Lys Xaa

15

20

Ala

(2) INFORMATION FOR SEQ ID NO:78:

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(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Lys	Met	Ala	Ser	Lys	Ala	Gly	Lys	Ile	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Val	Ala	Leu	Lys	Ala
				15				20	
Leu									

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79

Lys	Ile	Ala	Ser	Lys	Ala	Gly	Lys	Ile	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Val	Ala	Leu	Lys	Ala
				15				20	
Leu									

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Lys	Ile	Ala	Ser	Lys	Ala	Gly	Lys	Xaa	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Val	Ala	Leu	Lys	Ala
				15				20	

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Lys	Leu	Ala	Ser	Lys	Ala	Gly	Lys	Xaa	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Val	Ala	Leu	Lys	Ala
				15				20	

Leu

(2) INFORMATION FOR SEQ ID NO:82:

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(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is norleucine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Lys	Xaa	Ala	Ser	Lys	Ala	Gly	Lys	Xaa	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Val	Ala	Leu	Lys	Ala
				15				20	Leu

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa is p-aminophenylalanine.

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
 5 10
Gly Xaa Ile Ala Lys Ile Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Lys Ile Ala Gly Ala Ile Ala Lys Ile Ala
 5 10
Gly Lys Ile Ala Lys Ile Ala Gly Lys Ile
 15 20
Ala

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
5 10
Gly Ala Ile Ala Lys Ile Ala Gly Lys Ile
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala
5 10
Gly Lys Ile Ala Lys Ile Ala Gly Ala Ile
15 20
Ala

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Lys Leu Ala Ser Lys Ala Ala Lys Ile Ala
5 10

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Ala Lys Ile Ala Lys Val Ala Leu Lys Ala

10

20

Leu

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Lys Ile Ala Lys Lys Ile Ala Lys Ile Ala

5

10

Lys Lys Ile Ala Lys Ile Ala Lys Lys Ile

15

20

Ala

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Lys Phe Ala Lys Lys Phe Ala Lys Phe Ala

5

10

Lys Lys Phe Ala Lys Phe Ala Lys Lys Phe

15

20

Ala

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(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Lys	Phe	Ala	Lys	Lys	Ile	Ala	Lys	Phe	Ala
				5				10	
Lys	Lys	Ile	Ala	Lys	Phe	Ala	Lys	Lys	Ile
				15				20	
Ala									

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ala	Ile	Ala	Gly	Lys	Ile	Ala	Lys	Ile	Ala
				5				10	
Gly	Lys	Ile	Ala	Lys	Ile	Ala	Gly	Lys	Ile
				15				20	
Ala									

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 21 amino acids

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(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Lys Ile Ala Gly Lys Ile Ala Ala Ile Ala

5 10

Gly Lys Ile Ala Lys Ile Ala Gly Lys Ile

15 20

Ala

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Lys Ile Ala Gly Lys Ile Ala Lys Ile Ala

5 10

Gly Lys Ile Ala Ala Ile Ala Gly Lys Ile

15 20

Ala

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Gly Met Ala Ser Lys Ala Gly Lys Ile Ala
 5 10
Gly Lys Ile Ala Lys Val Ala Leu Lys Ala
 15 20
Leu

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE

- (A) ORGANISM: *Apis mellifera*

(vii) FEATURE

- (A) NAME/KEY: melittin peptide

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Habermann, E.
 Jentsch, J.
- (B) TITLE: Sequenzanalyse des Melittins aus
 den tryptischen and peptischen
 Spaltstucken
- (C) JOURNAL: Hoppe-Seyler's Zeitschrift
 Physiol. Chem
- (D) VOLUME: 348
- (F) PAGES: 37-50
- (G) DATE: 1987

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Gly Ile Gly Ala Val Leu Lys Val Leu

5

Thr Thr Gly Leu Pro Ala Leu Ile Ser Trp

10

15

Ile Lys Arg Lys Arg Gln Gln

20

25

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:96:

Leu Lys Lys Leu Lys Lys Leu Leu Lys Leu

5

10

Leu

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION:SEQ ID NO:97:

Leu Leu Lys Lys Leu Lys Lys Leu Leu Lys

5

10

Leu Leu

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:98:

Lys Leu Leu Lys Lys Leu Lys Lys Leu Leu

5

10

Lys Leu Leu

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:99:

Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu

5

10

Leu Lys Leu Leu

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(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu Leu Lys Lys Leu Arg Arg
5 10 15

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:101:

Lys Leu Lys Lys Leu Leu Lys Lys Leu Lys
5 10
Lys Leu Leu Lys Leu Leu
15

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Leu Lys Lys Leu Leu Lys Lys Leu Lys Lys

5

10

Leu Leu Lys Lys Asn

15

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: xaa is homoserine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Leu Lys Lys Leu Leu Lys Lys Leu Lys Lys

5

10

Leu Leu Lys Lys Xaa

15

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Leu Lys Leu Leu Lys Lys Leu Leu Lys Lys
5 10
Asn Lys Lys Leu Leu Lys Lys Leu
15

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Leu Lys Leu Leu Lys Lys Leu Leu Lys Lys
5 10
Pro Lys Lys Leu Leu Lys Lys Leu
15

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Leu Leu Lys Lys Leu Lys Lys Leu Leu Lys

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	5	10
Lys Leu Gln Gly Pro Pro Gln Gly Gln Ser		
	15	20
Pro Gln		

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Leu Ala Ser Lys Ala Gly Ala Ile Ala Gly	
	5 10
Lys Ile Ala Lys Lys Leu Leu Lys Lys Leu	
	15 20

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Leu Lys Lys Leu Lys Lys Leu
5

(2) INFORMATION FOR SEQ ID NO:109:

-123-

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Leu Leu Lys Lys Leu Lys Lys Leu

5

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Lys Leu Leu Lys Lys Leu Lys Lys Leu

5

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

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Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu
5 10

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Leu Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu
5 10

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Ala Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu
5 10

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Leu Lys Lys Leu Leu Lys Lys Leu Lys Lys Leu
5 10

Leu Lys Arg

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Gly Ile Gly Lys Phe Leu Lys Lys Ala Lys
5 10
Lys Phe Gly Lys Ala Phe Val Lys Ile Leu
15 20
Lys Lys

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:116:

Gly	Ile	Gly	Lys	Phe	Leu	Lys	Lys	Ala	Lys
			5					10	
Lys	Phe	Gly	Lys	Ala	Phe	Val	Lys	Ile	Leu
			15					20	
Lys	Lys	Gly							

WHAT IS CLAIMED IS:

1. A method of purifying at least one amphiphilic peptide or protein comprising:

(a) contacting a material containing at least one amphiphilic peptide or protein with a mixture of an aprotic organic solvent and an alcohol in an amount effective to form a single miscible solution;

(b) contacting said miscible solution with an aqueous solution, said aqueous solution being added in an amount effective to form a first phase, said first phase including an aqueous solution containing said at least one amphiphilic peptide or protein, and a second phase, said second phase including an organic solvent; and

(c) recovering said at least one amphiphilic peptide or protein from said first phase.

2. The method of Claim 1 wherein prior to contacting said material with a mixture of an aprotic organic solvent and an alcohol, a material containing at least one amphiphilic peptide or protein is contacted with a solution including an aprotic organic solvent, at an acidic pH, to provide an extract solution containing said at least one amphiphilic peptide or protein, and at least a portion of the aprotic organic solvent is removed from the extract solution to provide a remainder material containing at least said at least one amphiphilic peptide or protein.

3. The method of Claim 1 wherein said aprotic organic solvent is chloroform.

4. The method of Claim 1 wherein said alcohol is selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, isopropanol, isobutyl alcohol, 1-butanol, 2-butanol, and pentanol.

5. The method of Claim 1 wherein said aqueous solution is an aqueous salt solution.

6. The method of Claim 5 wherein said salt solution is a potassium chloride solution.

7. The method of Claim 5 wherein said salt solution is a sodium chloride solution.

8. The method of Claim 2 wherein said solution including an aprotic organic solvent at an acidic pH further includes an acid such that the solution has a pH no greater than about 4.0.

9. The method of Claim 2 wherein said aprotic organic solvent in said solution at an acidic pH is acetonitrile.

10. The method of Claim 8 wherein said acid is selected from the group consisting of trifluoroacetic acid and trichloroacetic acid.

11. The method of Claim 10 wherein said acid is trifluoroacetic acid.

12. The method of Claim 1 wherein said amphiphilic peptide is selected from the group consisting of:

- (i) a magainin peptide;
- (ii) a cecropin;
- (iii) a sarcotoxin;
- (iv) an XPF peptide;
- (v) a PGLa peptide;
- (vi) a CPF peptide;
- (vii) a melittin;
- (viii) an apidaecin;
- (ix) a peptide including one of the following basic

structures X_{31} through X_{37} wherein:

X_{31} is $-(R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32})-n$;

X_{32} is $-(R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31})-n$;

X_{33} is $-(R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32})-n$;

X_{34} is $-(R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32})-n$;

X_{35} is $-(R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33})-n$;

X_{36} is $-(R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31})-n$; and

X_{37} is $-(R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32})-n$;

wherein R_{31} is a basic hydrophilic amino acid, R_{32} is a hydrophobic amino acid, R_{33} is a neutral hydrophilic, basic hydrophilic, or hydrophobic amino acid, and n is from 2 to 5;

(x) a peptide including the following basic structure X_{40} :
 $R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{32}-R_{34}-R_{32}-R_{32}-$,
 wherein R_{31} is a basic hydrophilic amino acid, R_{32} is a hydrophobic amino acid, R_{33} is a neutral hydrophilic, basic hydrophilic, or hydrophobic amino acid, and R_{34} is a basic hydrophilic or hydrophobic amino acid;

(xi) a peptide including the following basic structure X_{50} :
 $R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}-$,
 wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid;

(xii) a peptide including the following basic structure X_{52} :
 $R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-$,
 wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid;

(xiii) a peptide including the following basic structure X_{54} :
 $-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-$
 $-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{43}$; wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid;

(xiv) a peptide including the following basic structure X_{56} :
 $-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{44}-$
 wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{44} is a neutral hydrophilic amino acid or proline;

(xv) a peptide including the following basic structure X_{58} :
 $-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{42}-R_{41}-R_{43}-$,
 wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid;

(xvi) a peptide including the following basic structure X_{60} :
 $-R_{41}-R_{41}-R_{43}-R_{42}-R_{41}-R_{41}-R_{41}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-$
 $-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}$, wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid;

(xvii) a peptide having a structure selected from the group consisting of:

- (a) $R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
 - (b) $R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
 - (c) $R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
 - (d) $R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$; and
 - (e) $R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$,
- wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid; and

(xviii) a basic polypeptide having at least sixteen amino acids, wherein said basic polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids.

13. The method of Claim 12 wherein said peptide is a magainin peptide.

14. The method of Claim 12 wherein said peptide is a cecropin.

15. The method of Claim 12 wherein said peptide is a sarcotoxin.

16. The method of Claim 12 wherein said peptide is an XPF peptide.

17. The method of Claim 12 wherein said peptide is a PGLa peptide.

18. The method of Claim 12 wherein said peptide is a CPF peptide.

19. The method of Claim 12 wherein said peptide is a melittin.

20. The method of Claim 12 wherein said peptide is an apidaecin.

21. The method of Claim 12 wherein said peptide includes one of the following basic structures X_{31} through X_{37} , wherein:

- X_{31} is $-[R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}]_n$;
- X_{32} is $-[R_{32}-R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}]_n$;
- X_{33} is $-[R_{32}-R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}]_n$;

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X_{34} is $-[R_{33}-R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}]_n-$;
 X_{35} is $-[R_{31}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}]_n-$;
 X_{36} is $-[R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}]_n-$; and
 X_{37} is $-[R_{32}-R_{31}-R_{32}-R_{32}-R_{33}-R_{31}-R_{32}]_n-$;

wherein R_{31} is a basic hydrophilic amino acid, R_{32} is a hydrophobic amino acid, R_{33} is a neutral hydrophilic, basic hydrophilic, or hydrophobic amino acid, and n is from 2 to 5.

22. The method of Claim 12 wherein said peptide includes the following basic structure X_{40} :

$R_{31}-R_{32}-R_{32}-R_{33}-R_{34}-R_{32}-R_{32}-R_{31}-R_{32}-R_{32}-R_{32}-R_{34}-R_{32}-R_{32}$,
 wherein R_{31} is a basic hydrophilic amino acid, R_{32} is a hydrophobic amino acid, R_{33} is a neutral hydrophilic or hydrophobic amino acid, and R_{34} is a basic hydrophilic or hydrophobic amino acid.

23. The method of Claim 12 wherein said peptide includes the following basic structure X_{50} :

$R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}$,
 wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid.

24. The method of Claim 12 wherein said peptide includes the following basic structure X_{52} :

$R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}$,
 wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid.

25. The process of Claim 12 wherein said peptide includes the following structure X_{54} :

$-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{43}$,
 wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid.

26. The process of Claim 12 wherein said peptide includes the following structure X_{56} :

$-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{44}$, wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral

hydrophilic amino acid, and R_{44} is a neutral hydrophilic amino acid or proline.

27. The process of Claim 12 wherein said peptide includes the following structure X_{58} :

$-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{42}-R_{41}-R_{43}$, wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid.

28. The process of Claim 12 wherein said peptide includes the following structure X_{60} :

$-R_{41}-R_{41}-R_{43}-R_{42}-R_{41}-R_{41}-R_{41}-R_{41}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-$, wherein R_{41} is a hydrophobic amino acid, R_{42} is a basic hydrophilic or neutral hydrophilic amino acid, and R_{43} is a neutral hydrophilic amino acid.

29. The process of Claim 12 wherein said peptide has a structure selected from the group consisting of:

- (a) $R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (b) $R_{42}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (c) $R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$;
- (d) $R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$; and
- (e) $R_{41}-R_{42}-R_{42}-R_{41}-R_{41}-R_{42}-R_{42}-R_{41}-R_{42}-R_{42}-R_{41}$, wherein R_{41} is a hydrophobic amino acid, and R_{42} is a basic hydrophilic or neutral hydrophilic amino acid.

30. The method of Claim 12 wherein said peptide is a basic polypeptide having at least sixteen amino acids, wherein said basic polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids.

31. The method of Claim 1 wherein said peptide is a peptide produced by recombinant techniques.

32. The method of Claim 1 wherein said peptide is an ion channel-forming peptide.

33. The method of Claim 1 wherein steps (a) through (c) are repeated at least once.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/07694

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00
US CL :530/324

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 530/422, 530/424, 530/426

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: extraction, solvent, acetonitrile, chloroform, ethanol, peptides or proteins

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<u>Meth. Enzymol. Vol 22</u> , issued 1971, Penefsky et al., "Extraction of Water Soluble Enzymes and Proteins from Membranes", pp. 204-219, see entire document.	1-33
Y	<u>Meth. Enzymol. Vol 1</u> , issued 1955, R.K. Morton, "Methods of Extraction of Enzymes from Animal Tissues", pp. 25-50, see entire document.	1-33
Y	US, A, 3,989,819 (Brockman) 02 November 1976, col. 1, line 53 to col 3, line 10; col. 4, line 30 to col 5, line 50.	1-33
Y	US, A, 4,647,445 (Lees) 03 March 1987, col 8, lines 23-40.	1-33

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

05 OCTOBER 1993

Date of mailing of the international search report

NOV 24 1993

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/07694

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,116,952 (Martin et al.) 26 May 1992, col 9, lines 65-66.	1-33