

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
26 August 2004 (26.08.2004)

PCT

(10) International Publication Number
WO 2004/072095 A2

- (51) International Patent Classification⁷: **C07K**
- (21) International Application Number:
PCT/IL2004/000141
- (22) International Filing Date: 12 February 2004 (12.02.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/446,519 12 February 2003 (12.02.2003) US
- (71) Applicant (for all designated States except US): **YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM** [IL/IL]; Hi Tech Park, Edmond J Safra Campus, Givat Ram, P.O. Box 39135, 91390 Jerusalem (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **GILON, Haim** [IL/IL]; 18 Gelber Street, 96755 Jerusalem (IL). **ARAD, Gila** [IL/IL]; 1 Hachoshen Street, 90805 Mevaseret Zion (IL). **KAEMPFER, Raymond** [IL/IL]; 18 Neve Sha'anani Street, 93707 Jerusalem (IL). **GLUCHOV, Ivgenia** [IL/IL]; 18 Neve Sha'anani Street, 93707 Jerusalem (IL).
- (74) Agents: **LUZZATTO, Kfir** et al.; Luzzatto & Luzzatto, P.O. Box 5352, 84152 Beer Sheva (IL).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: CYCLIZED PEPTIDES AS EXOTOXIN ANTAGONISTS

(57) Abstract: The invention relates to peptides comprising eight to twelve amino acids, cyclized by backbone-to-backbone or backbone-to-side chain bridge, and is further directed to the use of the peptides for treating and preventing severe effects of bacterial pyrogenic exotoxins, including incapacitation induced by exposure to the exotoxins. The peptides antagonize exotoxin-mediated activation of T-lymphocytes, and elicit protective immunity against toxic shock induced by one or more pyrogenic exotoxins.

WO 2004/072095 A2

CYCLIZED PEPTIDES AS EXOTOXIN ANTAGONISTS

Field of the Invention

The present invention relates to peptides comprising eight to twelve amino acids cyclized by backbone to backbone or backbone to side chain bridges, enabling the treatment and prevention of severe effects of bacterial pyrogenic exotoxins.

Background of the Invention

Pyrogenic exotoxins, known also as superantigenic toxins, produced by *Staphylococcus aureus* and *Streptococcus pyogenes*, cause severe human poisoning. These toxins bind directly to the MHC class II molecule and the T-cell receptor, at different sites than conventional antigens, thus activating T-cells in amounts larger by two orders than conventional antigens. Among the major serological types within the family of *Staphylococcus aureus* exotoxins, SEB is the most prominent, having been recognized as a leading cause of toxic shock syndrome accompanying surgical or injurious wound infections, as well as viral infections of the respiratory tract of influenza patients. Toxic shock syndrome, in its most severe form, causes shock and death [Murray et al.: ASM News 61 (1995) 29]. More generally, members of the staphylococcal exotoxin family, including SEA to SEE and toxic shock (TS) syndrome toxin 1 (TSST-1), have been implicated in food poisoning, toxic shock syndrome, in atopic dermatitis [Schlievert: Infect. Dis. 167 (1993) 997] and in Kawasaki's syndrome [Bohach et al., Crit. Rev. Microbiol. 17 (1990) 251]. However, life threatening TS-like syndrome may result from exposure to these toxins through a nonenteric route, such as inhalation. The incapacitating consequences of an exposure to the agents, such as SEB, during a war or a terrorist attack, are of great concern in the context of biological warfare. The dose estimated to be lethal for 50% of the human population is 0.02 µg/kg, and the dose estimated to incapacitate 50% of the exposed human population is 0.0004 µg/kg [Ulrich et al. in Medical Aspects of Chemical and Biological

- 2 -

Warfare, Published by the Office of The Surgeon General, Department of the Army, USA, Washington (1997) pp 621-30].

5 The toxicity of SEB and related exotoxins is thought to be related to the capacity of these molecules to stimulate the rapid and excessive production of cytokines, especially of IL-2, IFN- γ and tumor necrosis factors (TNFs) [Schad et al., EMBO J. 14 (1995) 3292] leading to the toxic shock.

10 The incapacitating and potentially lethal effects of the mentioned superantigens in humans, whether exerted on civilians or on military personnel, create a need for prophylaxis against the toxins, for treatment of toxin-exposed individuals, and for a safe vaccine against the toxin. Despite the urgency of this need, methods of protection or treatment have been lacking. The developments of efficient protective means, using an animal
15 model, is further complicated by the fact that the sensitivity to staphylococcal toxins in humans is usually higher than in animals, e.g. the human sensitivity exceeds that of mice by a factor of 100. Attempts to use fragments of SEB protein, about 30 amino acids long, for generating antisera in rabbits were not successful, whether comprising amino acids 113-144, 130-
20 160, 151-180, or 171-200 of the SEB protein molecule [Jett et al., Infect. Immun. 62 (1994) 3408].

As mentioned above, both *S. aureus* and *S. pyogenes* produce a variety of toxin families. In staphylococcal food poisonings and, more seriously, in
25 biological warfare or in toxic shock caused by *S. pyogenes*, mixtures of toxins can be involved whose composition cannot be anticipated. An attack by crude natural mixtures of toxins, which are readily attainable in cultures of *S. aureus*, would be frightening. Clearly, this complexity demands the development of broad-spectrum antagonists of pyrogenic exotoxins as well as
30 broad-spectrum vaccines.

- 3 -

There exists, therefore, a long-felt need to design a SEB vaccine that is free of sensitizing potential, yet is capable of protecting test animals or humans against lethal doses of toxin. Still more precious would be a vaccine with a wider protective spectrum in the SE toxin family, including, for example,
 5 SEA, SEC, TSST-1, SPEA, etc.

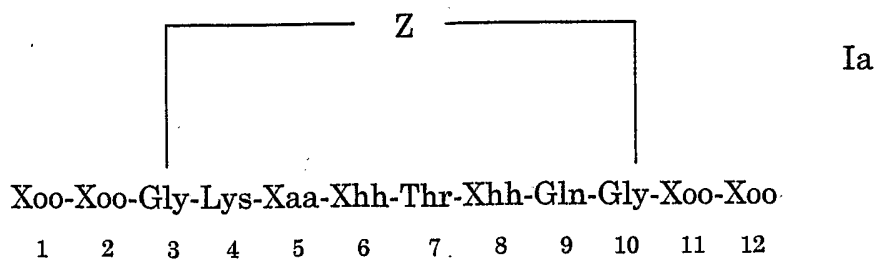
WO 98/29444 discloses a peptide, homologous to SEB fragment 150-161, that elicits protective immunity against TS induced by a pyrogenic exotoxin. However, relatively high amounts of said peptide are necessary to make up
 10 for the limited halflife in vivo expected for a linear peptide.

It is therefore an object of this invention to provide a more stable antagonist against pyrogenic exotoxins, for use in immediate treatment, or short term prevention and rapid prophylaxis, of acute toxic shock and of the harmful
 15 effects of such toxins which may be due to, for example, accidental food poisoning, and for a vaccine for immunization against intoxication by pyrogenic exotoxins for long term protection thereagainst.

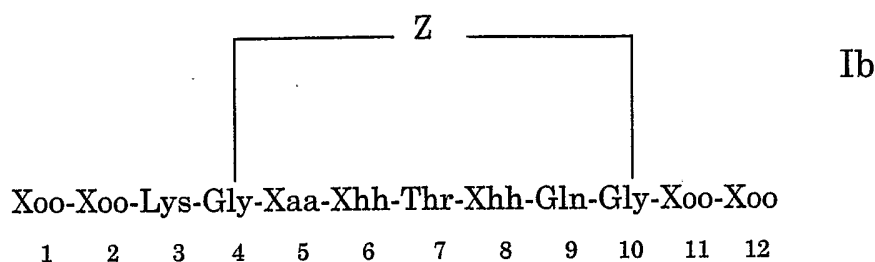
The inventors have designed a series of SEB antagonists based on cyclized
 20 oligopeptides comprising a homolog of SEB 153-158 hexapeptide which blocks the action of pyrogenic exotoxins on the human immune response in vitro, severely inhibiting toxin-mediated induction of IL-2 and IFN- γ mRNA.

Summary of the Invention

25 This invention provides a cyclized peptide having formula Ia or Ib:

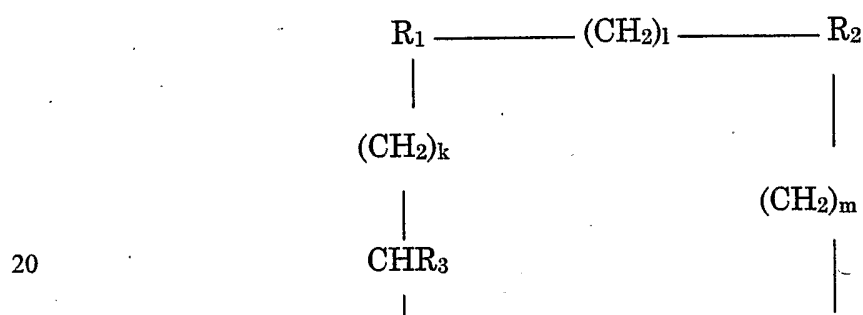


- 4 -



wherein Xoo is any standard amino acid (AA) or a deletion, Xaa is any standard AA, Xhh is a nonpolar AA selected from Ala, Val, Leu, Ile, and Phe, and wherein Z is a linker connecting two Gly residues through side chains or N-atoms of said Gly residues, which linker has a length from 5 to 21 atoms and is optionally substituted with one alkylamino group. A part of the invention is an analog of said cyclized peptide obtained by one AA replacement with another standard AA or with a modified AA, as well as derivatives of said peptide or its analog obtained by their esterification, amidation, acylation, and alkylation, preferably at free amino or free carboxy groups. Also a part of the invention is a salt of said peptide or its analog or derivative.

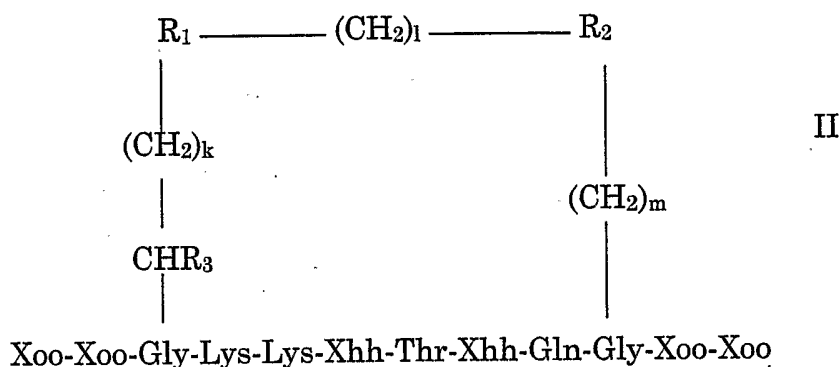
In a preferred cyclized peptide according to this invention, said linker Z has the following structure:



wherein R_1 is a group selected from $-NH-CO-$ and $-CO-NH-$, R_2 is either the same as R_1 , or represents bond when l is zero, R_3 is selected from hydrogen, $-NH_2$, and $-(CH_2)_j-NH_2$, k is an integer from 0 to 6, l is an integer from 0 to 3, m is an integer from 2 to 7, and j is an integer from 0 to 5.

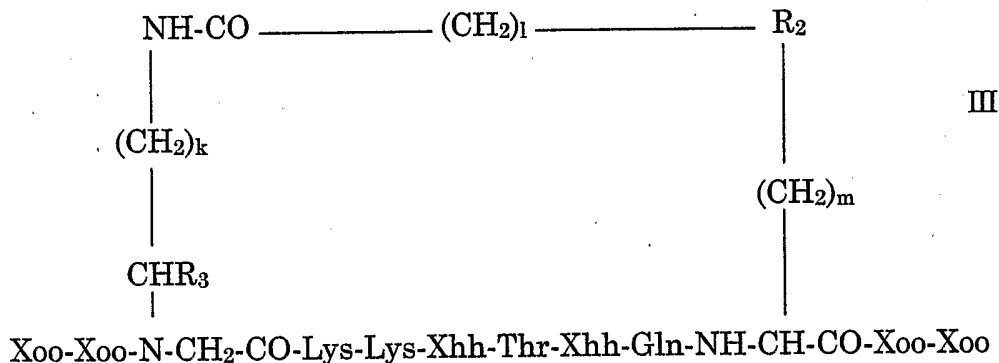
- 5 -

In a preferred embodiment of this invention, the cyclized peptide has formula II:



5 wherein Xoo, Xhh, R_1 , R_2 , R_3 , k , l , m , and j have the same meaning as above.

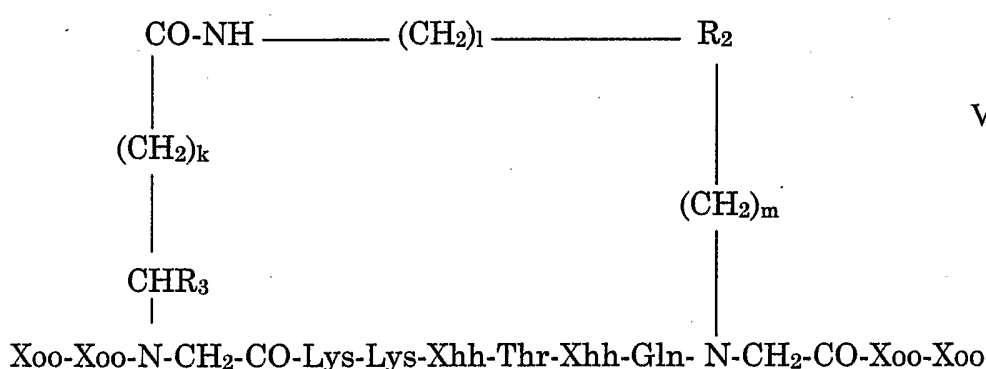
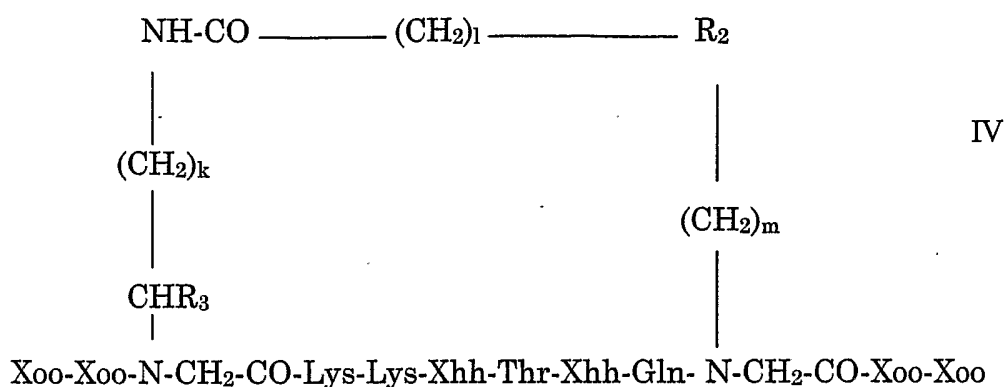
In another preferred embodiment of this invention, the cyclized peptide, comprising backbone to side chain cyclization, has formula III:



10 wherein Xoo, Xhh, R_3 , k , l , and m have the meaning as defined above, and R_2 is either the same as R_1 , or represents bond when l is zero.

In another preferred embodiment of the invention, the cyclized peptide, comprising backbone to backbone cyclization, has formula IV, and in still another preferred embodiment of the invention, the cyclized peptide, comprising backbone to backbone cyclization, has formula V, wherein the
 15 symbols have the same meaning as described above.

- 6 -



Derivatives and salts of the peptides having the above formulae are a part of
 5 the invention.

The peptide of this invention has preferably more positive charges, under usual physiological conditions, within group R_3 and first five amino acids, preferably in the form of two or three free amino groups. In a preferred
 10 embodiment, R_3 is $-(\text{CH}_2)_j\text{-NH}_2$ when only one or two Lys residues are present in the first five amino acids in the sequence. If Lys in a peptide analog of the invention, at position 4 of formula Ia or position 3 of formula Ib, is replaced, the preferred AA at position 5 is Lys. In a preferred embodiment of this invention, the cyclic peptide has Tyr at position 1, or they have Tyr-Asn at
 15 positions 1-2. In other preferred embodiments, AA at positions 1-2 are deleted. In a further preferred embodiment of this invention, the cyclic peptide has Asp at position 12, or it has Leu-Asp at positions 11-12. In a still further preferred embodiment AA at positions 11-12 are deleted. In a

- 7 -

preferred embodiment of the invention, AA at positions 6 and 8 are independently selected from Val and Ala.

Said linker connecting said two Gly residues in the cyclic peptide of this invention has preferably a length from 8 to 16 atoms. Still more preferably, said linker has a length from 10 to 14 atoms. Preferred derivatives of the peptide of this invention comprise an acetylated amino group, and an amidated carboxyl group. Preferred analogs of the peptides of this invention comprise modified amino acids, including D-amino acids, racemates, isomers, etc.

The invention also relates to the use of cyclized peptides of formulae I to V, as described above, in antagonizing toxin-mediated activation of T-lymphocytes, wherein the toxin is a pyrogenic bacterial exotoxin, and in eliciting protective immunity against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins derived preferably from *Staphylococcus aureus* or *Streptococcus pyogenes*. The invention further relates to the use of said cyclized peptides, analogs, derivatives, or salts thereof, in the preparation of a medicament, preferably a medicament for reducing harmful effects of bacterial pyrogenic exotoxins or their analogs. The use of this invention comprises inhibiting pyrogenic exotoxin-mediated activation of T-lymphocytes, and eliciting protective immunity against a toxic shock induced by a pyrogenic exotoxin. In a preferred embodiment of this invention, a cyclized peptide of a formula selected from I to V, or an analog, derivative, or salt thereof is used in the preparation of a medicament for the treatment of incapacitation induced by one or more pyrogenic exotoxins. The invention further relates to the use of said cyclized peptides, derivatives and salts in the preparation of a vaccine for protecting against toxic effects of a pyrogenic exotoxin.

- 8 -

In another aspect, this invention is directed to a cyclized peptide comprising a hexapeptide having sequence Lys-Xaa₁-Xaa₂-Thr-Xaa₃-Gln, SEQ ID NO. 1, wherein Xaa₁ is any standard AA, and Xaa₂ and Xaa₃ are nonpolar AAs independently selected from Ala, Val, Leu, Ile, and Phe, or to a homolog thereof obtained by replacing one AA in said sequence by another standard AA, which replacement does not result in any loss of activity, or to their pharmaceutically acceptable salts, for use in antagonizing exotoxin-mediated activation of T-lymphocytes, and in eliciting protective immunity against toxic shock induced by one or more pyrogenic exotoxins.

10

Also provided by this invention is a composition comprising at least one cyclized peptide of formulae I to V, homologs, derivatives or salts thereof, for reducing the toxic effect of a bacterial exotoxin or a mixture of bacterial exotoxins. The composition inhibits pyrogenic exotoxin-mediated activation of T-lymphocytes and protects against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins. The composition may further comprise pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient. The immunogenic composition of the invention elicits protective immunity against a toxic shock induced by a pyrogenic exotoxin. The invention also provides a composition for treating the incapacitation induced by one or more pyrogenic exotoxins, wherein said composition comprises a cyclized peptide, derivatives and salts thereof, of one of formulae I to V. In a preferred embodiment of this invention, a composition for reducing the harmful effects of pyrogenic exotoxins comprises a mixture of more different cyclized peptides that have structures I to V.

25

The invention is further directed to a method for preventing, treating, or reducing the harmful effects of a bacterial exotoxin or a mixture of bacterial exotoxins in a mammal, comprising administering to said mammal an effective amount of at least one cyclized peptide of formulae I to V, or a derivative or salt thereof. This method enables inhibiting the pyrogenic

30

- 9 -

exotoxin-mediated activation of T-lymphocytes and protecting against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins in a patient, and comprises administering to the patient an inhibitory effective amount of at least one cyclized peptide, having a formula selected from I to V, or its derivative or salt. The method enables the elicitation of protective immunity against a toxic shock induced by a pyrogenic exotoxin in a patient in need of such treatment.

In a very important aspect of this invention, a method is provided for preventing or treating incapacitation induced by at least one pyrogenic exotoxin comprising administering to a patient in need of such treatment a therapeutically effective dose of at least one cyclized peptide of formula selected from formulae I to V, a derivative or salt thereof, or of a composition comprising the same. In a preferred embodiment of the invention, said dose may contain a mixture of at least two cyclic peptides having structures as described by any one of formulae I to V. The effective dose may be administered repeatedly, at predetermined periods of time. Said incapacitation may be a result of an accidental exposure to a bacterial toxin at various situations, such as food poisoning, work, combat, or a terrorist attack.

The invention provides also a vaccine for protecting against toxic effects of a pyrogenic exotoxin or a mixture of pyrogenic exotoxins comprising as active ingredient an immunologically effective amount of at least one peptide of a formula selected from formulae I to V, or derivative or salt thereof. The vaccine confers immunity against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins, and comprises as active ingredient an immunologically effective amount of said at least one peptide or derivative or salt, and optionally an immunization adjuvant. The vaccine of the invention enhances the production of antibodies that block T-cell activation.

- 10 -

The invention also relates to antibodies directed against a cyclized peptide of a formula selected from formulae I to V, or its analog or derivative, which blocks T-cell activation. An antiserum containing antibodies directed against said cyclized peptide or analog is also related to by this invention. The antiserum may be obtained from a domestic animal. The antiserum is capable of alleviating harmful effects and toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins.

The invention provides a method for assessing the efficacy of a vaccine, which vaccine comprises at least one peptide of a formula selected from I to V, for conferring immunity against one or more pyrogenic bacterial toxins comprising determining the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells. This method enables to determine the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells by measuring the inhibition of expression of pyrogenic toxin-induced mRNA encoded by the IL-2 or IFN- γ genes. A part of the invention is a kit for assessing the efficacy of a vaccine for conferring immunity against one or more pyrogenic toxins comprising determining the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells by said method.

Brief Description of the Drawings

The above and other characteristics and advantages of the invention will be more readily apparent through the following examples, and with reference to the appended drawings, wherein:

- Fig. 1. shows schemes of synthesis of Fmoc-Gly(Nn)Boc-OH and Fmoc-Gly(Nn)Alloc-OH building units;
- Fig. 2. shows major synthetic steps in the synthesis of library SEB-1;
- Fig. 3. is a schematic structure of library SEB-1. "FG" are groups NH or CO;
- Fig. 4. demonstrates the activities of SEB-1 library peptides as compared with the activity of p12A. Scores were calculated from multiple

- 11 -

determinations of the inhibition power of the peptides, based on measurements of mRNA levels for IL-2 and IFN- γ cytokines induced by SEB exotoxin. Library peptides were measured at 70 μ M, p12 control at 700 μ M;

- 5 Fig. 5. shows the structure of peptides of library SEB-2, 5A being schematic structure of the library, "FG" are alternating groups CO or NH, dotted lines represent various cyclization possibilities, 5B, 5C, and 5D being detailed structures of the peptides;

Fig. 6. shows major synthetic steps in the synthesis of library SEB-2;

- 10 Fig. 7. demonstrates the activities of SEB-2 library peptides as compared with the activity of p12A. Scores were calculated from multiple determinations of the inhibition power of the peptides, based on measurements of mRNA levels for IL-2 and IFN- γ cytokines induced by SEB exotoxin. All peptides were measured at 700 μ M; and

- 15 Fig. 8. illustrates three-dimensional structure of SEB toxin as determined by X-ray, 8A showing the site comprising residues SEB(150-161), and 8B showing the whole structure of SEB.

Detailed Description of the Invention

- 20 It was shown, in WO 98/29444, coauthored by two of the present inventors, that a peptide comprising amino acid sequence substantially homologous to a SEB fragment, particularly fragment pSEB(150-161) having the sequence Thr-Asn-Lys-Lys-Lys-Val-Thr-Ala-Gln-Glu-Leu-Asp (SEQ ID NO 2, the amino acids of which are also numbered 1-12), is capable of blocking the
- 25 activation of IL-2 and IFN- γ gene expression by a pyrogenic exotoxin and of eliciting protective immunity against toxic shock induced by a pyrogenic exotoxin, and several homologs, including peptide p12, having Tyr instead Thr at position 1, are provided as good candidates for use in the blocking of the activation of IL-2 and IFN- γ gene expression by a pyrogenic exotoxin and
- 30 in the preparation of vaccines. However the homologs are linear peptides, and it is known that linear peptides, especially shorter ones, are not too

- 12 -

stable in vivo, mainly due to the action of exopeptidases. When trying to solve this problem, more possibilities may be considered, including oligomerization of the peptide, modification of some of amino acids involved in the sequence, cyclizations, etc. In WO 98/29444, two D-Ala amino acids
5 were attached at both ends of the linear peptide, forming peptide p12A. In this invention, cyclization of peptide p12 was employed. A random cyclization may be expected to increase the stability of a peptide, but it can also be expected to introduce a structural constraint preventing the peptide molecule from adjusting its tertiary structure to its molecular partners during
10 interactions important for the desired biological activity, resulting in a loss of activity.

Two libraries of peptides comprising side chain to side chain, backbone to side chain, and backbone to backbone cyclizations of peptide p12 were
15 prepared by the inventors. Since p12 and its homologs, as mentioned above, were good candidates for use as active antagonists of exotoxins, preserving a substantial part of their activity during the cyclization process was a desired objective. Surprisingly, some of cyclized peptides not only reached the activity of the linear form, but even surpassed that activity by tenfold.

20 When considering which two amino acids (AA) in the peptide should be selected as the points for linking, the inventors also referred to their studies of the crystallographic structure of p12. The study suggested that Lys 152 and Glu 159 of p12 participated in intramolecular interactions. Therefore the inventors presumed that the two residues might be less important for
25 intermolecular interactions. However, the inventors do not wish to be limited by any particular theory, since the above rationale may explain the preservation of the original activity, but the explanation of the observed sharp activity increase would probably require to include additional factors.

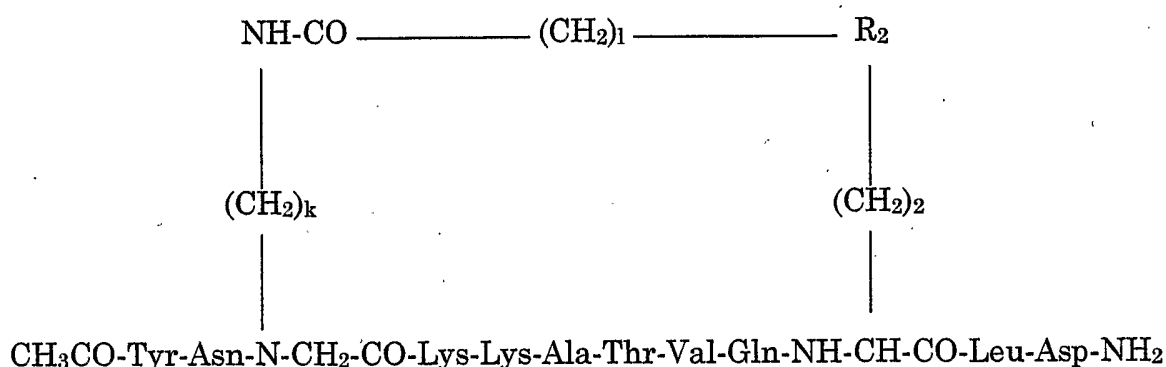
30 Two peptide libraries were synthesized comprising a side chain to side chain, backbone to backbone or a backbone to side chain bridge. In some cases, the

- 13 -

amino acids (AAs) at positions 3, 4, or 10 were replaced by N-(ω -aminoalkyl)glycine (denoted GlyN) or N-(ω -carboxyalkyl)glycine (denoted GlyC), and the ring comprising either AAs 3 and 10, or AAs 4 and 10, was closed by creating one or two peptide bonds; in the case of two peptide bonds, a linker was incorporated in said ring, comprising an ω -amino-alkanoic acid.

In some cases, the first two or the last two AA of the p12 sequence were deleted. The biological activity of the peptides was characterized by measuring their power to inhibit the superantigenic activity of SEB toxin. Capabilities of the cyclized peptides and p12A linear control to lower the formation of mRNA for IL-2 and IFN- γ , which mRNA formation is induced by SEB in peripheral blood mononuclear cells, were determined *in vitro*.

In the first series of experiments, a peptide library was synthesized comprising a bridge between positions 3 and 10, either through side chain (Lys at position 3, denoted Lys3 hereinafter) to side chain (Glu10) or through backbone (GlyN3) to side chain (Glu10). Peptides of the following structures were obtained:



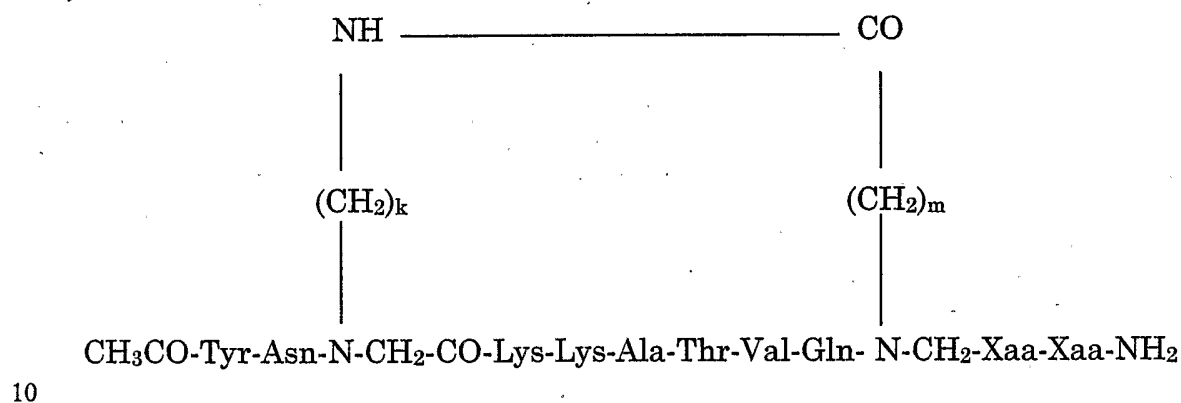
wherein l is either of 0, 1, 2, and 3, R_2 is either NH-CO when l is 1 to 3, or R_2 is a bond when l is zero, and k is either of 2, 3, 4, and 6.

Of 20 different peptides in the first library, denoted SEB1 to SEB20, fourteen either reached or surpassed the activity of the linear p12A protein. As

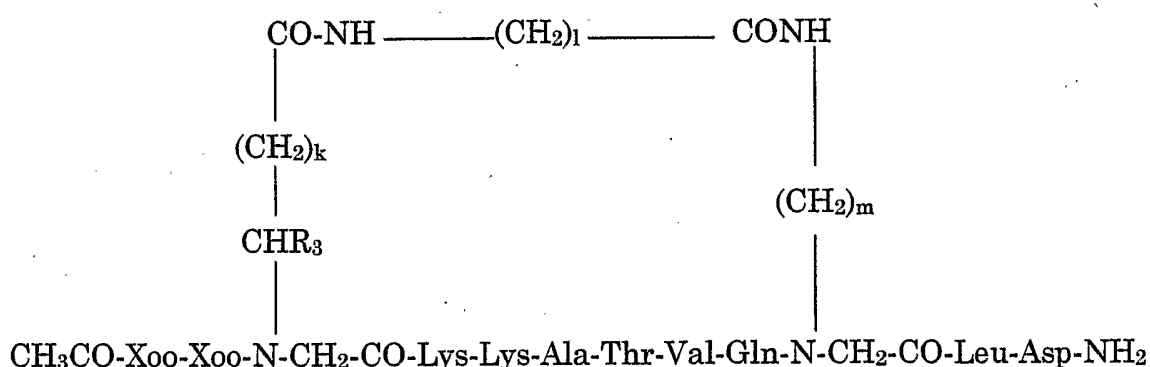
- 14 -

illustrated in Figure 4, several cyclized peptides, including SEB11, SEB13, SEB18, and SEB19, have much higher activity than peptide p12, even if used in 10 times lower concentration.

- 5 In the second series of experiments, a peptide library was synthesized comprising a bridge between positions 3 and 10 or between positions 4 and 10. The bridge connected backbone (GlyN3) to side chain (Glu10), or the backbone (GlyN3 or GlyC3) to backbone (GlyC10 or GlyN10). Peptides of the following three structures were obtained:

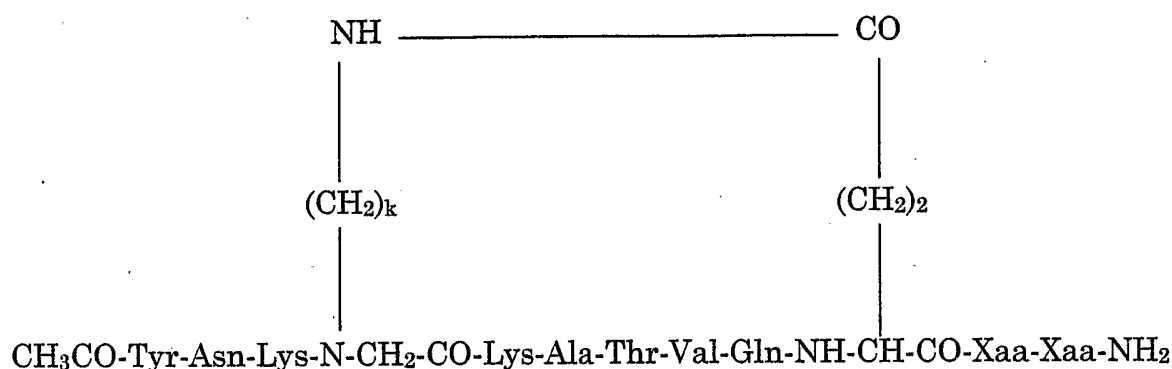


wherein k is 6, m is 2 or 3, and Xaa is a standard amino acid;



wherein R₃ is selected from hydrogen and -(CH₂)_j-NH₂, k is 2 or 3, m is either of 2, 3, 4, and 6, and l is 1; and

- 15 -



wherein k is 4 or 6.

Of 14 different peptides in the second library, denoted SEB21 to SEB34, ten reached or surpassed the activity of the linear p12 protein. As illustrated in Figure 7, several cyclized peptides, including SEB32, and SEB33, have much higher activity than peptide p12A.

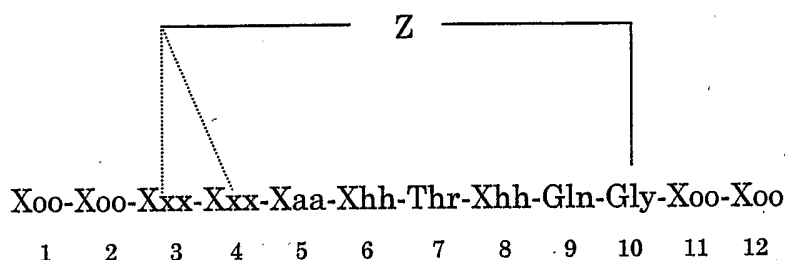
The cyclic peptidomimetics of this invention were synthesized mostly by techniques known in the art, comprising SPPS and SMPS, and closing the ring by the formation of intramolecular peptidic bond(s). The sequence of AA was designed to be homologous to pSEB(150-161). The results show that cyclic peptides based on pSEB(150-161) with a bridge between position 159 and 152 or 153 act as strong SEB antagonists, even superior to the linear analogs. The bridge preferably connects positions 152 and 159. The positive charge near positions 152-154 seems to be important for the antagonist activity of the peptide, and introducing an additional positive charge next to Lys153 and Lys154 enhances the antagonist activity. Two terminal amino acids are not essential for the activity, even if keeping them seems to be preferred in many cases. Of course, the peptides may be synthesized otherwise than in the shown examples.

When considering the ability of superantigen antagonists to protect against exotoxin shock [e.g., Arad et al.: Nature Medicine 6, (2000) 414], and when taking into consideration homologies among pyrogenic exotoxins at positions corresponding to SEB(150-161), as can be observed in nearly thirty published

- 16 -

sequences of the toxins of *S. pyogenes* and *S. aureus*, and when further bearing in mind the above observed preferred properties of the cyclized peptide, it can be noted that, after employing Asp159 and Lys152 or Lys153 in the bridge formation, a hexapeptide Lys-Xaa₁-Xaa₂-Thr-Xaa₃-Gln (SEQ ID NO. 1, wherein Xaa₁ is any standard AA, and Xaa₂ and Xaa₃ are nonpolar AAs independently selected from Ala, Val, Leu, Ile, and Phe) or its homolog not containing more than one replacement, turns out to be essential for the ability of a cyclized peptide of this invention to inhibit exotoxin-triggered cytokine expression in blood T-cells, the cycle in said cyclized peptide having preferably a length from 30 to 39 atoms, with preferably 8 to 16 atoms being a part of the linker.

The general structure of a cyclized antagonistic peptide of this invention can thus be described as the following structure:



15

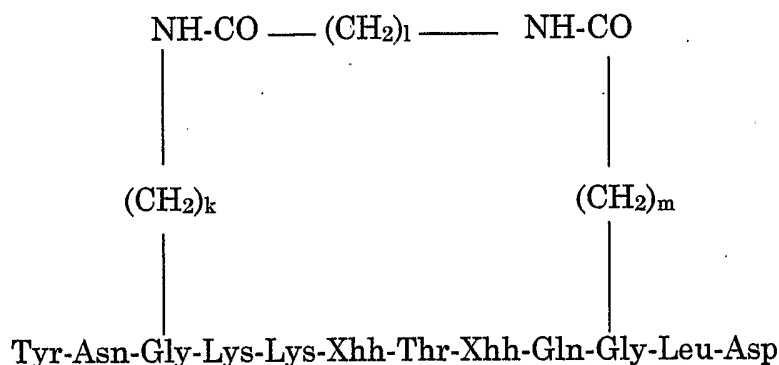
or analog thereof obtained by one AA replacement with another standard AA or with a modified AA; wherein Xoo is any standard AA or a deletion, Xaa is any standard AA, Xhh is a nonpolar AA selected from Ala, Val, Leu, Ile, and Phe, and Z is a linker connecting Gly10 with one of residues Xxx at position 3 or 4, wherein the other – nonlinked – residue Xxx is Lys. Linker Z comprises side chains or N-atoms of said linked Xxx residue and said Gly residue, and has a length from 5 to 21 atoms and is optionally substituted with an alkylamino group. A cyclized peptide of this invention or its analog may comprise carboxyl groups that are esterified or amidated, and amino groups

20

- 17 -

that are acylated or alkylated, and can be otherwise modified, wherein such derivatives and their salts are active exotoxin antagonists.

Preferred antagonists of pyrogenic exotoxins according to this invention have the following structure:



- 5 wherein two Xhh AAs are independently selected from Ala and Val, k is an integer from 2 to 6, l is an integer from 0 to 3, m is 2.

Examples

Chemicals and instruments

Protected amino acids, coupling reagents and resins for peptide synthesis
 10 were purchased from NovaBiochem, Bachem and Aldrich, except for
 Allyl/Alloc protected amino acids, which were purchased from Neosystem.
 TFA, HF, TIS, DIEA and organic synthesis reagents were purchased from
 Sigma-Aldrich and Merck. Protease inhibitors and phosphatases, bovine
 serum albumin (BSA), FCS and DMEM mediums were purchased from Beit
 15 Ha'emek Biological Industries Inc. SEB protein was supplied by the
 Toxicology Department of the United States Army Medical Research
 Institute of Infectious Diseases, Frederick, MD. NMP, DCM, DMF and HPLC
 grade acetonitrile were purchased from J.T. Baker or BioLab Inc. Other
 solvents for organic synthesis were purchased from Frutarom, and other
 20 chemicals from Sigma-Aldrich. Acetic aldehyde was distilled under

- 18 -

atmospheric pressure before use. Some building units carrying an Allyl (Fmoc-Gly(Cm)Allyl-OH m=2,3) or Alloc (Fmoc-Gly(Nn)Alloc-OH n=2,3) were supplied by Peptor Inc.

5 Separately prepared sequences were synthesized in an SPPS specific vessel. Most peptides were prepared by SMPS in polypropylene bags, which were shaken in polyethylene boxes. Peptide cleavage by HF was performed when MBHA resin was used with a KEL-F special system designed for the simultaneous cleavage of 10 peptides. Analytical and preparative RP-HPLC
10 chromatography was performed on two Merck-Hitachi systems at 215 nm, on Model 665A and on Model LaChrom. Analytical separation columns were LiChroCart RP-18 by Merck and Vydac, 25 cm long and 5 mm inner diameter. Preparative and semi-preparative separations columns were Vydac Protein & Peptide C18 25 cm long and 25 or 10 mm inner diameter
15 respectively. Mobile phase solvents were triple distilled water (TDW) and acetonitrile with 0.1% TFA. Mobile phase rate was 1 ml/min for analytical runs, 4.5 ml/min for semi-preparative runs and 9 ml/min for preparative runs. Most mass spectrometry (MS) measurements were conducted at the inter-division instruments lab at the medicine faculty of the Hebrew
20 university, on a ThermoQuest LC-Q^{DUO} Ion Trap MS by Finnigan. Mobile phase was a TDW:MeOH:AcOH (49:49:2) mixture. HNMR assays were conducted on an AMX-300 Bruker NMR at 295°K at the Margaret Thatcher NMR lab of the organic chemistry department of the Hebrew University. An 8452A diode array spectrophotometer by Hewlett Packard was used for
25 Fmoc-piperidine complex absorption determination. TLC analyses were performed on F₂₅₄ silica plates supplied by Merck.

Amino acid analysis was performed at the Life Science Institute of the Hebrew University. Peptides were hydrolyzed for 24 hours in HCl on a
30 Knauer protein hydrolyzer. Hydrolysis products were reacted with Fmoc-chloride. Determination of the amino acids was done on RP-8 column equipped HPLC with a fluorescence detector (λ_{ex} =263 nm, λ_{em} =313 nm).

- 19 -

Determination of resin loading was performed in the same manner, from a known dry peptide-resin amount compared with known standard volume and concentration.

5 **Inhibition of SEB-induced mRNA (IFN γ and IL-2)**
 formation by the peptides

In these assays human PBMC cells from healthy donors were used [Arad (2000) Ibid]. The cells were separated from Buffy-coat, purified, divided into 4x10⁶ cells/ml aliquots and incubated in PRMI. Buffer used: 2% fetal calf
10 serum, 2 mM Gln, non-specific amino acids, 10 mM HEPES, 10 mM sodium pyruvate, 5x10⁻⁵M 2-mercaptoethanol, pH=7.2, 100 units/ml penicillin, 100 μ g/ml streptomycin, 5 μ g/ml nystatin. 100 ng/ml SEB was introduced with or without the assayed peptides. Total RNA was extracted and serially diluted. All concentrations were blotted onto a nitrocellulose filter. IL-2 and IFN γ
15 specific RNA amounts were determined by blot hybridization with IL-2 and IFN γ RNA antisense probes detected by ³²P. The IL-2 probe, 600 nucleotides long, was transcribed of the T3 promotor complementary to the third exon and part of the third intron giving a 117 nucleotides sequence protected by mRNA. The IFN γ probe, 274 nucleotides long, was transcribed of the T7
20 promotor complementary to the third exon and part of the third intron giving a 183 nucleotides sequence protected by mRNA. These RNA antisense probes were detected by a UV/Vis detector at 630 nm.

Preparation of building blocks and
25 **intermediates for the peptide synthesis**

Mono-Boc-alkylenediamine (1)

This was prepared as described [Muller et al.: J.Org.Chem 62 (1997) 411].

1-Boc-6-Alloc-hexylenediamine (4)

30 To an ice cooled mixture of (1) (17 g, 1 eq.) in 4N KOH (pH=14) was added allyl chloroformate (5.8 ml, 1.1 eq.). The obtained white solid was filtered and

- 20 -

dissolved in 10 ml DCM. This organic phase was washed with 4N KOH (4 x 20 ml) in order to get rid of starting material, TDW (1 x 20 ml), dried over Na₂SO₄ and evaporated to dryness.

5 N α -Fmoc-N α -[ω -(Boc-amino)alkyl]Gly (Fmoc-Gly(Nn)Boc-OH) (2)

This was synthesized using reductive alkylation and in situ Fmocylation. To a solution of (1) (0.05 mole, 1 eq.) in 80 ml MeOH was added NaCNBH₃ (0.015 mole, 0.3 eq.) and the reaction mixture was cooled. Then glyoxylic acid (0.055 mole, 1.1 eq.) was added portion-wise. After 2.5 hours the solvent was
10 evaporated to dryness and to the obtained foam was added a solution of Et₃N (0.1-0.2 mole, 2-4 eq.) in 125 ml TDW. To this suspension was added a solution of Fmoc-Osu (0.05 mole, 1 eq.) in acetonitrile and the reaction mixture was left to stir for another 4 hours in room temperature. Quenching was done by addition of 120 ml TDW. The aqueous reaction mixture was
15 washed with petroleum ether (3 x 100 ml), petroleum ether:ether (7:3) (3 x 100 ml) and acidified under cooling with 1N HCl (about 70 ml) to pH=2. The product was extracted to ethyl acetate (3 x 100 ml) and the organic phase was washed with TDW (3 x 50 ml), dried over MgSO₄ and evaporated to dryness. Products were obtained as white solids. Material (2) was prepared
20 for ω being 2, 3, 4, and 6.

Fmoc-Gly(Nn)Alloc-OH (3)

This was achieved by replacing Boc with Alloc. To product (2) (3.38 g, 0.0072 mole for n=4; 3.06 g, 0.0062 mole for n=6) was added 10 ml DCM:TFA (1:1)
25 mixture. The resulting yellow reaction mixture was stirred for 90 min. Then solvents were evaporated *in vacuo* and the product was triturated with diethyl ether (2 x 100 ml). The obtained oil was dissolved in 30 ml TDW:acetonitrile (2:1) and the mixture was basified to pH=9 with powdered Na₂CO₃. To this solution was added a solution of allyl chloroformate (1.1 eq.)
30 in DCM and the reaction mixture was stirred for 1 hour while keeping the pH basic. Product was then acidified to pH=1 with saturated KHSO₄ and

- 21 -

extracted to ethyl acetate (3 x 40 ml), dried over MgSO_4 and evaporated to dryness.

Fmoc-Gly(N6)Alloc-OH (3)

5 This was synthesized using reductive alkylation and in situ Fmocylation. To product (4), 1-Boc-6-Alloc-hexylenediamine, (15 g) was added 20 ml DCM:TFA (1:1) mixture. The reaction mixture was stirred for 60 min. Then solvents were evaporated in vacuo and the obtained oil was triturated with diethyl ether (3 x 50 ml) and basified to pH=9 with KOH. This crude product
10 was used for preparation of the desired building unit according to the procedure used for the preparation of material (2).

Alloc-NH-(CH₂)_n-OH

Amino acid (0.1 mole) ($n=1,2,3$) was dissolved in 25 ml 4M NaOH while
15 stirring. The solution was cooled in an ice bath and to it was added a mixture of allyl chloroformate (13.8 ml, 0.13 mole, 1.3 eq.), 5 ml isopropanol and 20 ml 4M NaOH. The reaction mixture was stirred in room temperature for 1 hour. TLC system: chloroform:MeOH (4:1). Quenching was performed by the addition of 40 ml water followed by washing of the reaction mixture with
20 petroleum ether (3 x 30 ml), cooling and acidifying with concentrated HCl to pH=1. Product was extracted to ethyl acetate (3 x 45 ml), dried over MgSO_4 and evaporated to dryness.

Boc-NH-CH₂-CO-Allyl (5)

25 To a mixture of Boc-Gly-OH (8.75 g, 0.05 mole, 1 eq.) and allyl bromide (100 ml, 1.1 mole, 24 eq.) was added 0.034 mole DIEA until a clear solution was obtained. This solution was refluxed in a silicon oil bath for three hours. Then it was diluted with 200 ml ethyl acetate, washed from excess base and allyl bromide with 0.1N HCl (3 x 100 ml), saturated NaHCO_3 (4 x 100 ml)
30 and NaCl (3 x 100 ml). Product was dried over MgSO_4 and evaporated to dryness to obtain a yellow oil.

- 22 -

NH₂-CH₂-CO-Allyl (6)

Product (5) (8.44 g, 0.039 mole) was dissolved in 120 ml mixture of DCM:TFA (1:1) and was left for 1 hour while shaking occasionally. Then it was evaporated, triturated twice with diethyl ether (2 x 100 ml) and dissolved in 100 ml chloroform. The solution was basified by Et₃N to pH=8 and then washed with water (5 x 100 ml), dried over Na₂SO₄ and evaporated to dryness.

Gly(C1)Allyl-OH (7)

This was prepared by reductive alkylation. To a solution of (6) (0.004 mole, 1 eq.) in 50 ml MeOH glyoxylic acid (0.373 g, 1.1 eq.) was added portion-wise. After 5 minutes NaCNBH₃ (0.082 g, 0.013 mole, 0.3 eq.) was added and the reaction mixture was stirred for 16 hours, then it was evaporated to dryness.

Peptide synthesisGeneral SPPS techniques

Peptides were synthesized by SMPS or SMPS methods on a divinylbenzene cross-linked polypropylene resin of two types, both giving a carboxamide end. SMPS "tea bags" were prepared of a polypropylene sheet, 4.5x5 cm for 200 mg dry resin or 5x5 cm for larger resin quantities. Bags were marked and three sides of them were sealed. Then resin was introduced to the bags, they were weighed, sealed at the fourth side and cut with scissors to avoid rough edges. Bags were shaken in polypropylene boxes of various sizes according to bags size and number. Solvent volume of each reaction was about 10-15 ml/bag. Peptides of library SEB-1 were synthesized on MBHA resin (loading: 0.55-0.59 mmole/g). Other sequences were constructed on Rink-amide MBHA resin (loading: 0.55-0.60 mmole/g). Prior to synthesis bags resin was swollen by a two hours shaking in NMP. Synthesis direction was from the carboxyl end to the amine end using Fmoc or Boc/Fmoc chemistry. All reactions were conducted in room temperature in DCM, NMP, NMP:MeOH 1:1 or DMF. At the end of each synthetic step resin was washed followed by free amines

- 23 -

assaying, in order to qualitatively determine the success of that step. These assays are detailed herein. A positive answer (showing free amines presence) is expected following cleavage of amine protecting groups and reductive alkylation. A negative answer, showing absence of free amines, is expected following couplings, acylations and cyclizations. Neutralization of the MBHA resin was done by shaking it twice in 5% DIEA in NMP, 5 minutes each time.

Coupling of amino acids to each other or to the solid phase

The following techniques were employed.

- 10 i) An amino acid or N α blocked building unit (3 eq.) and coupling reagent (TBTU, PyBOP or PyBrOP, 3 eq.) were dissolved in DMF or NMP (10 ml minimum) in a 50 ml plastic tube. Then DIEA (7 eq.) was added and the tube was shaken for 10 minutes for pre-activation. Then this was added to the synthesis vessel and all was shaken for 2 hours. Whenever chloranil or
15 Kaiser tests were positive the coupling was repeated [Gazal et al.: J. Pept. Res. 58 (2001) 527].
- ii) An amino acid or N α blocked building unit (5 eq.) and coupling reagent (BTC, 1.65 eq.) were added to dry DCM (10 ml minimum) in a 50 ml plastic tube. Then 2,4,6-collidine (14 eq.) was added and a clear solution was
20 obtained. After a few seconds pH was tested and when lower than 8 more base was added. Then this was added to the synthesis vessel and all was shaken for 1 hour. Whenever chloranil or Kaiser tests were positive the coupling was repeated.
- iii) For the coupling of Fmoc-Asn(Trt)-OH to building units, amino acid (5
25 eq.) and HOBt (5 eq.) were dissolved in dry DMF (10 ml minimum) in a 50 ml plastic tube for 5 minutes until complete dissolution. Then DIC (8 eq.) was added under inert atmosphere and the solution was shaken for 30 minutes for pre-activation. After that this solution was added to the synthesis vessel under inert atmosphere and all was shaken for 1 hour.

- 24 -

Washings

Most synthetic steps were followed by washings (2 minutes each) of this order: 5 x NMP followed by 2 x DCM. BTC couplings were followed by washings of a different order: 2 x DCM, 3 x NMP, 2 x DCM. Removal of allyl/alloc (method H) groups was followed by these washings: 4 x DCM, 2 x DMF, 2 x NMP, 2 x 5% DIEA in NMP and 2 x DCM. SiCl₃I Boc removal was followed by this: 2 x DCM:acetonitrile (1:1), 2 x NMP, 2 x 5% DIEA in NMP and 2 x NMP. At the end of the whole synthetic procedure, the last washings were followed by 3 x DCM and 1 x MeOH.

Fmoc Removal

Reaction was performed in a fresh solution of 20% piperidine in DMF or NMP 2 x 30 minutes. Chloranil and Kaiser tests were almost negative after Fmoc removal from Asp and Glu.

Free active sites capping and amine acetylation

Shaking in 0.5M acetic anhydride and 0.125 M DIEA in NMP for 30 minutes was used [Gazal et al. (2001), Ibid].

Boc removal and neutralization

This was done in two different methods: i) Shaking in TFA:DCM (1:1) twice (1 x 5 min, 1 x 30 min) followed by neutralization with 5% DIEA in NMP. ii) 10 minutes shaking in room temperature in freshly prepared SiCl₃I solution transferred to reaction vessel via an argon washed needle. The solution was prepared by mixing NaI (10 eq.) and SiCl₄ (10 eq.) in DCM:acetonitrile (1:1) in room temperature.

Allyl/Alloc removal

Bags containing peptide on resin or MCH resin were pre-dried in dessicator overnight and then introduced into a hydrogenation vessel. The vessel was flushed with argon. N,N'-dimethyl barbituric acid (7.5 eq./g resin) was

- 25 -

dissolved in DCM. Ar was bubbled through the solution and then it was transferred to the hydrogenation vessel under Ar, followed by the addition of dry catalyst complex Pd(PPh₃)₄ (0.1 eq.) again under Ar. Reaction vessel was sealed with a rubber cap and parafilm, wrapped with aluminum foil and shaken for 3 hours [Unverzagt et al.: Bioorg. Med. Chem. 2 (1994) 1189-1201].

Cyclic anhydride coupling to the amine end

Cyclic anhydride (10 eq.) and DMAP (1 eq.) were dissolved in NMP (10 ml minimum) in a 50 ml plastic tube. Then DIEA (10 eq.) was added and solution was shaken for 30 minutes for pre-activation, added to synthesis vessel and all was shaken for 1 hour.

Dicarboxylic acids coupling to the amine end

Dicarboxylic acid (10 eq.) and DMAP (1 eq.) were dissolved in NMP (10 ml minimum) in a 50 ml plastic tube. Then DIC (10 eq.) was added and solution was shaken for 30 minutes for pre-activation, added to synthesis vessel and all was shaken for 1 hour.

Cyclization via lactam bond

First cyclization: PyBOP (10 eq.) was dissolved in dry NMP in a 50 ml plastic tube. Then added to synthesis vessel followed by addition of DIEA (10 eq.). Peptides were shaken in this solution for 2 hours. For the second and third cyclizations, reagents amounts were halved.

Amine end reductive alkylation

Distilled acetaldehyde (4 eq.) or m-anisaldehyde were dissolved in NMP:MeOH (1:1) with 1% acetic acid in the reaction vessel. 5 minutes later NaCNBH₃ (4 eq.) was added. Peptides were left to shake in this reaction mixture for 3 hours.

On-resin preparation of Gly building units

- 26 -

This was conducted according to Friedler et al. [Biochemistry 37 (1998) 5416].

Cleavage of peptides off the solid phase

5 When using dry HF, dried resin bound peptides and anisole (total concentration of 5%) were introduced into an HF specific teflon system by Japan Peptide Institute. To this was added liquid HF (10 ml/300-400 mg resin) at -2 to 0°C. After 2 hours HF was evaporated. Peptides were dissolved in 4 ml TFA. Workup was as described herein. When using TFA, a
10 cooled mixture of TFA:TDW:triisopropylsilane (95:2.5:2.5) was added to 50 ml plastic tubes with resin bound peptide (5-10 ml/tube). Tubes were shaken usually for 30 minutes at 0°C followed by 2 hours at room temperature. Crude peptides after cleavage were filtered through cotton or Altech plastic filtered columns. Most TFA was evaporated by strong bubbling of N₂ to a
15 final volume of 2-3 ml. Then ice cooled ether was added. The obtained precipitates were vortexed and then centrifuged for 5 minutes. Etheric phases were decanted. This washing was repeated 2-4 times. The peptides were then dried and dissolved in TDW:acetonitrile (1:1) with 0.1% TFA, filtered through HPLC filters, frozen in liquid nitrogen and lyophilized.

20

On-resin allylation of free carboxylic acid

Allyl alcohol (10 eq.) and DMAP (1 eq.) were dissolved in DCM (10 ml minimum) in a 50 ml plastic tube. Then DIC (10 eq.) was added and solution was shaken for 30 minutes for pre-activation, added to synthesis vessel and
25 all was shaken for 1 hour.

Synthesis of backbone cyclization amine building units

In this work, N-backbone cyclization building units based on Gly(Nn) or Gly(Cn) were used (n stands for the length of methylene chain). For instance,
30 a simple synthetic procedure was introduced for the synthesis of building units of the type Fmoc-Gly(Nn)Boc-OH and Fmoc-Gly(Nn)Alloc-OH, also

- 27 -

synthesized was Fmoc-Gly(N6)Alloc-OH building unit (Figure 1). Thus, in this work, glycine building units were synthesized consisting of an alkyl arm of various lengths connected to the backbone nitrogen and carrying a Boc (n=2,3,4,6) or Alloc (n=4,6) protected amine group.

5

SEB proteinomimetics

Analysis of the protein three-dimensional structures was performed (Figure 8).

10 Free amine detection

Qualitative free amine determination tests were conducted after each synthetic step (or Kaiser or chloranil). When these reflected the step was not successful it was repeated under the same or other conditions.

15 Kaiser test was performed as follows: Two drops of the following solutions were added to a borosilicate tube with a small amount of resin: phenol in ethanol, KCN buffer in piperidine, and ninhydrin in ethanol. This was heated to 110°C for a few minutes. A positive answer is when both polymer and solution become deep purple after one minute. For a negative result it is
20 necessary to wait 3 minutes [Kaiser et al.: Anal. Biochem. 34 (1970) 595].

Chloranil test was performed as follows: Two drops of a saturated solution of chloranil in toluene and two drops of 10% acetaldehyde in DMF or acetone are added to a small amount of resin. This is shaken for 1-5 minutes. A
25 positive answer is when the polymer is colored green [Christensen: Acta Chem. Scan. B, 33 (1979) 763]

Fmoc-piperidine assay was performed as follows: 3-5 mg of dry peptide-resin were weighed (required accuracy: 0.1 mg) followed by Fmoc removal for 30 minutes. Reaction mixture was filtered and the filtrate was diluted to exactly
30 50ml with DMF. Absorption at 301 nm was measured in a spectrophotometer

- 28 -

and compared to blank. The substitution degree of Fmoc-AA-resin was determined according to equation

$$C = \frac{A_{301} \cdot V[ml]}{7800 \cdot m[g]}$$

where A is the average absorbance of 3 measurements, V is the solution
5 volume (50 ml) and m is the polymer weight [Melenhofer: Int. J. Peptide
Protein Res. 23 (1984) 203].

Design of cyclic SEB antagonists based on the sequence of SEB(150-161)

Before designing cyclized fragments homologous to SEB, the three-
10 dimensional structure of SEB was analyzed by crystallography. It was found
that residue Lys 152 forms two intra-molecular hydrogen bonds, one with
Ser245 carbonyl group and the other via a salt bridge with OD¹ of Glu159
carboxylic group. This salt bridge is located deep within the SEB molecule.
The atoms (N ϵ and OD¹) are 2.71Å apart. Therefore the inventors assumed
15 that residues Lys152 and Glu159 contribute more to the peptides
conformation rather than to its interactions with other proteins. Residue
Glu159 forms another hydrogen bond through its oxygen atom OD2 with
Thr150 O γ . In addition its O α atom is proximal to N α atoms of both Tyr162
and Leu163, still more strengthening the above assumption (regarding this
20 role of said residues) and the decision to use these residues in the cyclization.

Examples 1 - 20

In order to conduct backbone cyclizations, appropriate building units were
incorporated into the sequence, replacing the residue at position 152 with
25 Gly building units having alkyl arms of varying length. A suitable linker was
further introduced. This way a library composed of 16 backbone to side chain
cyclic peptides, and 4 side chain to side chain – classical – cyclic peptides.
Cyclization was formed between positions 3 and 10 of p12 (152 and 159 of the
SEB sequence). The peptides comprise a lactam bridge constructed in two

- 29 -

ways: via a single amide bond formed directly between the two functional groups of the appropriate residues or by the insertion of a linker, comprising one α , β , or γ amino acid. Using this method is another way of controlling the ring size. Thus, 12 peptides possess a new mode of cyclization: backbone to side chain cyclization through a linker. Free carboxylic and amine groups were capped by amidation and acetylation, respectively. This library was designed to scan both ring size and cyclization mode. Figure 3 shows a schematic structure of library SEB-1. "FG" are groups NH or CO. Table 1 describes the structures of the peptides of library SEB-1, relating to symbols in Figure 3.

Tab. 1 Structures of the peptides of SEB-1 library, relating to symbols in Figure 3.

Peptide	Xaa	N	M	Ring size	FG2	FG1
SEB1	Lys	4	0	30	-	-
SEB2	Lys	4	1	33	NH	CO
SEB3	Lys	4	2	34	NH	CO
SEB4	Lys	4	3	35	NH	CO
SEB5	Gly(Nn)	2	0	29	-	-
SEB6	Gly(Nn)	2	1	32	NH	CO
SEB7	Gly(Nn)	2	2	33	NH	CO
SEB8	Gly(Nn)	2	3	34	NH	CO
SEB9	Gly(Nn)	3	0	30	-	-
SEB10	Gly(Nn)	3	1	33	NH	CO
SEB11	Gly(Nn)	3	2	34	NH	CO
SEB12	Gly(Nn)	3	3	35	NH	CO
SEB13	Gly(Nn)	4	0	31	-	-
SEB14	Gly(Nn)	4	1	34	NH	CO
SEB15	Gly(Nn)	4	2	35	NH	CO
SEB16	Gly(Nn)	4	3	36	NH	CO
SEB17	Gly(Nn)	6	0	33	-	-
SEB18	Gly(Nn)	6	1	36	NH	CO
SEB19	Gly(Nn)	6	2	37	NH	CO
SEB20	Gly(Nn)	6	3	38	NH	CO

- 30 -

Library SEB-1 was prepared by the combination of the SPPS and the "tea bags" SMPS methodologies on 5 g MBHA resin while forming the building units on resin during the peptide synthesis as described above. At least three different orthogonal protecting groups had to be used. Fmoc was used for protection of N α of all residues, except for the residues used as linkers, where Alloc group was used. Boc protecting group for protecting the functional groups at the ends of the alkyl arms of building units and on the Lys152 amine, and the Allyl group to protect the side chain of Glu159, and Z, Bzl and Trt were used for the protection of other amino acids side chains (Figure 2).

10

The coupling reagent used was PyBrOP, the first nine coupling steps were conducted in one vessel, since being identical in all the peptides. After the coupling of Thr156, i.e. after six couplings, an Fmoc-piperidine test was performed, followed by a quantitative amino acid analysis of the growing sequence in order to estimate the progress of the synthesis. The yield obtained from the Fmoc-piperidine test was approximately 37% (meaning an average of 85% yield of each coupling), but the amino acid analysis gave an estimate of around 60% yield. After the coupling of Fmoc-Lys(Z)-OH at position 153 the solid phase was washed, dried and weighed 7.76 g. Thus, according to weight gain after nine couplings the yield was 58%. At this point, the resin carrying the growing peptide was divided into 24 polypropylene bags; about 370 mg per bag for the 20 bags containing the library peptides, and about 90 mg per each control bag. This way from the tenth coupling on, SMPS mode was used. Gly(Nn)Boc building units were prepared directly on the solid phase using bromoacetyl bromide [Friedler (1998) Ibid]. After acetylation of the amine end, the Boc protecting group was selectively cleaved using in situ formed SiCl₃I (described above). This method was chosen rather than the standard TFA method in order to avoid harming the Trt protecting group of Asn and Gln residues. For peptides in which a linker was to be introduced, the now exposed primary amine was coupled to one of the linker amino acids protected beforehand by Alloc on their N α atom.

30

- 31 -

Alloc and Allyl groups were cleaved simultaneously using Pd(PPh₃)₄/NDMBA. Cyclization between the linker N α atoms and side chains or alkylene arms in position 159 was repeated three times in standard coupling conditions using PyBOP (Figure 2). Following these synthetic steps peptides were cleaved off the solid phase by HF, and their purity was determined by HPLC on an RP-C18 analytical column. The peptides purity ranged from 25-40%. The synthesis of this library was quite complex, but the desired peptides were obtained at reasonable quantities. Following ESI-MS characterization, the peptides were purified by preparative HPLC and were again characterized by analytical HPLC, amino acid analysis and ESI-MS. Since the charge of each of the peptides under acidic conditions is +2, the major MS peaks corresponded to MW/2. The synthesis results are detailed in table 1. Amino acid analysis also confirmed the supposed structures (not shown).

Tab. 2 Results of the sythesis of SEB-1 library

Peptide	Calculated molar mass of the product	Molar mass of the product by ESI-MS	Product purity (%)	Product mass (mg)	Yield (%)
SEB1	1458.7	1460.3	94	5.0	2.6
SEB2	1515.7	1516.8	97	5.0	2.5
SEB3	1529.7	1530.7	100	6.0	3.0
SEB4	1543.7	1544.8	38	5.0	2.5
SEB5	1430.6	1431.8	82	6.0	3.2
SEB6	1487.6	1490.8	45	6.0	3.1
SEB7	1501.6	1502.7	93	6.4	3.3
SEB8	1515.7	1519.6	100	7.0	3.6
SEB9	1444.9	1447.4	100	3.5	1.9
SEB10	1501.6	1504.2	58	2.0	1.0
SEB11	1515.7	1516.9	98	1.5	0.8

- 32 -

SEB12	1529.7	1531.7	63	3.0	1.5
SEB13	1458.7	1459.7	96	2.0	1.1
SEB14	1515.7	1516.7	56	2.0	1.0
SEB15	1529.7	1532.8	100	0.8	0.4
SEB16	1543.7	1545.8	100	1.0	0.5
SEB17	1486.7	1487.8	100	2.0	1.0
SEB18	1543.7	1544.8	100	4.0	2.0
SEB19	1557.8	1558.8	100	1.2	0.6
SEB20	1571.8	1572.7	100	3.0	1.5

SEB-1 library cyclic peptides antagonize cytokine induction by SEB. The biological activity of all library peptides was determined according to the methodology developed by two of the present inventors (WO 98/29444).

5 Inhibition of the superantigenic activity by the library peptides of the invention was determined as indicated in experimental procedures. Briefly, mRNA quantities of IFN- γ and IL-2 cytokines were determined by quantitative dot blotting on Th1 cells obtained from healthy donors, at three time points (0, 3h and 6h) following incubation with SEB, with and without

10 the peptides. Peptide p12A (dA-YNKKKATVQELD-dA, where dA denotes D-Ala, SEQ ID NO. 3) served as a linear reference peptide. The lower the cytokines quantity the higher the peptides inhibitory activity, since the release of these cytokines implies a successful antigenic mode of action. The concentrations used for the cyclic peptides were 70 μ M while the linear p12

15 was assayed at a ten times higher concentration (700 μ M), the SEB superantigen concentration was 100 ng/ml. All peptides exhibited inhibitory activity to some extent. It should be noted that this biological assay was conducted on blood obtained from 40 different donors, and each peptide was checked in at least 12 repeated tests. The mean antagonist score from

20 multiple assays was calculated for each peptide of the SEB-1 library for IL-2 mRNA and for and IFN- γ RNA. Scoring was done by a point system: <50%

- 33 -

inhibition (0 points); >50% inhibition at either 3 or 6 h time point of the above assay (1 point); >50% inhibition at 3 and 6 h (5 points); and >75% inhibition at 3 or 6 h (10 points).

5 The results (Figure 4) indicate that at least about 15 peptides of 20 in the SEB-1 library reach, or even surpass, the antagonistic behavior of peptide p12. It should be born in mind that all the peptides were checked at the concentration 10 times lower than the control peptide p12, which itself is good antagonist. Among superior active cyclic peptides in the SEB-1 series
10 were SEB 11, SEB13, SEB18, and SEB19.

Examples 21 - 34

On further mimicking the structure of SEB(150-161), library SEB-2 was designed, with the cyclization comprising residue 159 and one of residues 152
15 and 153. The cyclization site passed from position 152 to position 153 in three peptides, backbone to backbone cyclization was used in 11 peptides, and a new structural element was introduced in 1 peptide, Gly(CH[$(\text{CH}_2)_4\text{NH}_2$] which bears another positive charge near the amino end of the peptide. Further, the direction of the amide bond in the linker was
20 switched in 7 peptides. Figure 5A shows a schematic structure of library SEB-2, the arrows being the sites of linking. "FG" are groups NH or CO. Table 3 describes the structures of the peptides of library SEB-2, relating to symbols in Figure 5A.

25 Tab. 3 Structures of the peptides of SEB-2 library, relating to symbols in Figure 5A.

- 34 -

	n	m	l	Xaa 1	Xaa 2	Xaa 3	Xaa 4	Xaa 5	Xaa 6	Xaa 7	FG1	FG2	FG3	FG4
SEB 21	3	6	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 22	2	6	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 23	3	4	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 24	2	4	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 25	6	2	-	Tyr	Asn	Lys	Gly(N)	Glu	Leu	Asp	NH	CO	-	-
SEB 26	6	2	-	Tyr	Asn	Gly(N)	Lys	Gly(C)	Leu	Asp	NH	CO	-	-
SEB 27	6	3	-	Tyr	Asn	Gly(N)	Lys	Gly(C)	Leu	Asp	NH	CO	-	-
SEB 28	6	2	-	Tyr	Asn	Gly(N)	Lys	Gly(C)	-	-	NH	CO	-	-
SEB 29	6	3	-	Tyr	Asn	Gly(N)	Lys	Gly(C)	-	-	NH	CO	-	-
SEB 30	6	2	-	Tyr	Asn	Lys	Gly(N)	Glu	-	-	NH	CO	-	-
SEB 31	4	2	-	Tyr	Asn	Lys	Gly(N)	Glu	Leu	Asp	NH	CO	-	-
SEB 32	1	6	3	-	-	Spec. Unit	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 33	3	2	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH
SEB 34	3	3	3	Tyr	Asn	Gly(C)	Lys	Gly(N)	Leu	Asp	CO	NH	CO	NH

The SEB-2 peptides were prepared according to the standard procedures in "tea bags", while using previously prepared building units, on a Rink-Amide MBHA resin (loading: 0.55 meq/g). Each bag contained 250 mg of resin. The protection of side chains that did not participate in the cyclization was achieved by using "permanent" protecting groups that were TFA sensitive, such as tBu, Boc and Trt. The residues undergoing cyclization were protected by "semi-permanent" protecting groups of Allyl and Alloc. The same as in SEB-1 library, residues 152 and 159 were bridged by coupling Alloc-AA-OH amino acids to the amine of the side chain or alkyl arm of residue 152. This coupling was conducted after completion of the main sequence. In library SEB-2, the Alloc-Gly-OH amino acids were coupled to the amine of the alkylene arm of residue 159 (Figure 6). The building units used at this position were Fmoc-Gly(Cm)Allyl-OH, Fmoc-Gly(Nn)Alloc-OH and Fmoc-Gly(CH[(CH₂)₄NH-Boc]CO₂-Allyl)-OH. After the coupling of Fmoc-Ala-OH in

- 35 -

position 155, the Alloc group off the alkylene arm of the 159 positioned building units was cleaved. Then, Alloc-Gly-OH was coupled to the now exposed functional group, followed by continued building of the main sequence. This way, the direction of the bridge amide bond could be changed.

5 After removal of the Allyl and Alloc groups from residue 152 and the bridge, cyclization was performed in the standard manner (Figure 6). The couplings of the various amino acids were conducted in standard PyBrOP procedure, except for the Asn which was coupled in the same manner as used in library SEB-1. In case of peptide SEB32, the cleavage of the Fmoc protecting group

10 of the building unit at the amine end was performed only after peptide cyclization.

Peptides were cleaved off the solid support by a standard TFA cocktail. The desired products were obtained as main products and their purity at the

15 crude stage was higher than that of the SEB-1 library, 25-85%. For instance, peptide SEB32 was 47% pure and weighed 15 mg. All peptides underwent further preparative HPLC purification. Their final purity was determined by analytical HPLC on RP-C18 column. Pure peptides were characterized by ESI-MS, HPLC and amino acid analysis. All SEB-2 peptides, except SEB32,

20 have a +2 electrical charge thus most of them show a major MS peak at MW/2, as in the previous library. The yields of the final products are lower compared to SEB-1, but their purities are higher.

Tab. 4 Results of the sythesis of SEB-2 library

25

Peptide	Calculated molar mass of the product	Molar mass of the product by ESI-MS	Product purity (%)	Product mass (mg)	Yield (%)
SEB21	1558.8	1559.4	97	2.6	1.3
SEB22	1544.8	1542.1	95	0.7	0.3
SEB23	1530.8	1528.8	100	1.9	1.0

- 36 -

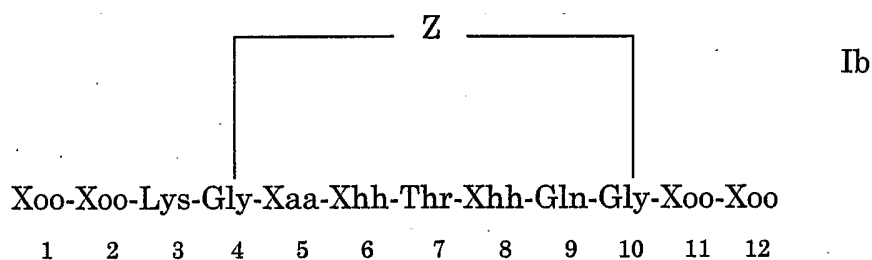
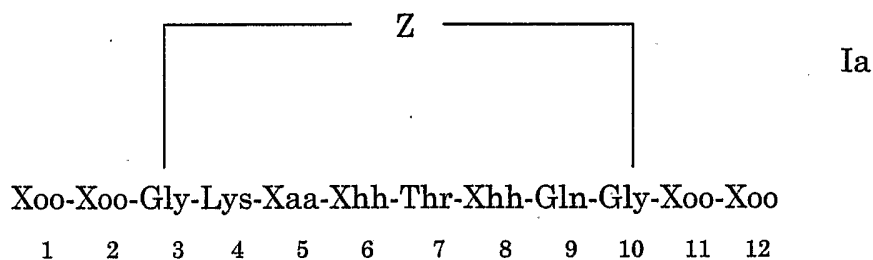
SEB24	1516.7	1514.9	100	0.8	0.4
SEB25	1487.7	1486.8	90	1.5	0.8
SEB26	1487.7	1486.9	100	0.8	0.4
SEB27	1501.8	1504.0	100	0.5	0.3
SEB28	1259.5	1257.7	96	Not determined	N.d.
SEB29	1273.5	1271.5	100	N.d.	N.d.
SEB30	1259.5	1257.9	100	1.4	0.9
SEB31	1459.7	1460.8	100	0.7	0.4
SEB32	1325	1324.5	100	2.0	1.2
SEB33	1502.7	1502.3	100	2.0	1.0
SEB34	1516.7	1517.4	100	1.7	0.9

Peptides of the SEB-2 library were subjected to the same biological assays as the peptides of SEB-1, followed by the same data analysis procedure. The difference was a ten-times raise of their concentration, reaching that for the control peptide, i.e. 700 μ M. Scoring for SEB-2 peptides is shown in Figure 7. More peptides than in SEB-1 had lower activity than control peptide p12A. Nevertheless, several peptides had higher activity than p12A, SEB32 being superior.

While the invention has been described using some specific examples, many modifications and variations are possible. It is therefore understood that the invention is not intended to be limited in any way, other than by the scope of the appended claims.

CLAIMS

1. A cyclized peptide having formula Ia or Ib:



wherein Xoo is any standard amino acid (AA) or a deletion, Xaa is any standard AA; Xhh is a nonpolar AA selected from Ala, Val, Leu, Ile, and Phe; and wherein Z is a linker connecting the two Gly residues, comprising side chains or N-atoms of said Gly residues, which linker has a length from 5 to 21 atoms and is optionally substituted with an alkylamino group;

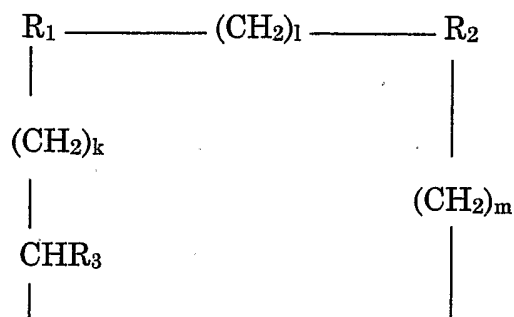
and an analog thereof obtained by one AA replacement with another standard AA or with a modified AA;

and a derivative of said peptide or its analog obtained by their esterification, amidation, acylation, and alkylation;

and a salt of said peptide or its analog or its derivative.

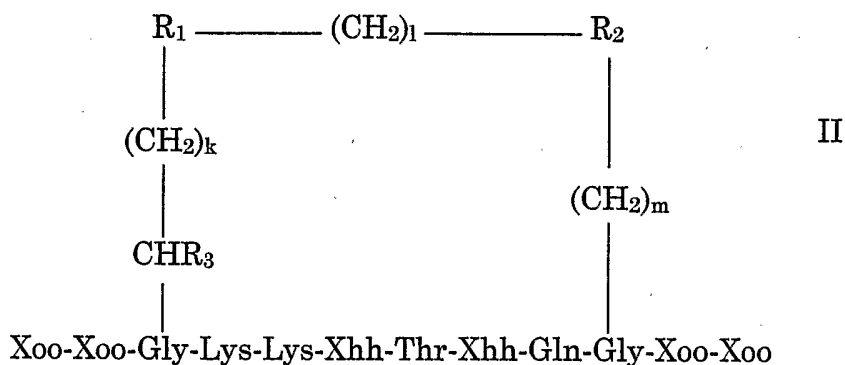
- 38 -

2. A cyclized peptide, analogs, derivatives and salts thereof, according to claim 1, wherein said linker Z has the following structure:



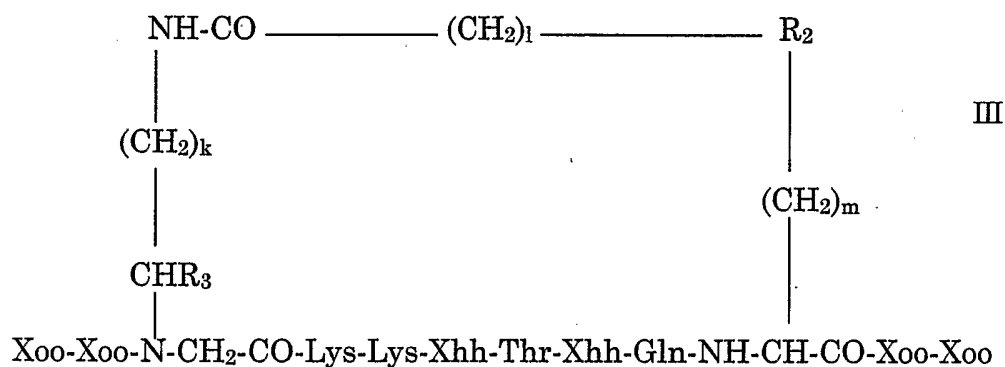
wherein R_1 is a group selected from $-NH-CO-$ and $-CO-NH-$; R_2 is either the same as R_1 , or represents bond when l is zero; R_3 is selected from hydrogen, $-NH_2$, and $-(CH_2)_j-NH_2$; k is an integer from 0 to 6; l is an integer from 0 to 3; m is an integer from 2 to 7; and j is an integer from 0 to 5.

3. A cyclized peptide according to claim 1, having formula II:



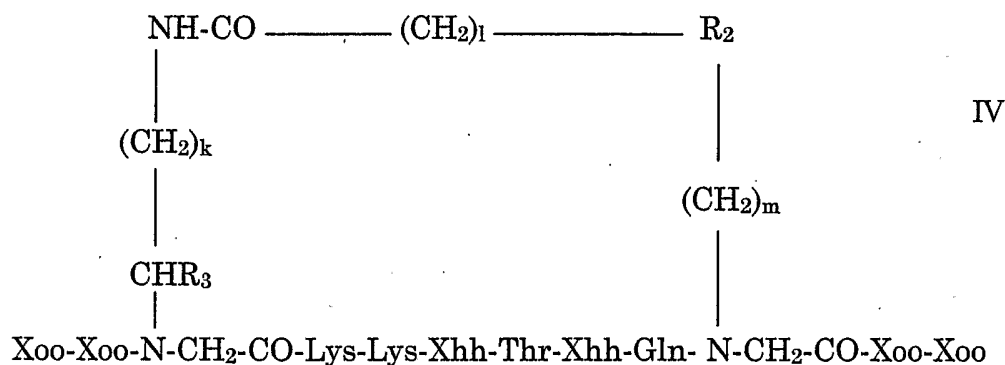
and derivatives and salts thereof; wherein Xoo is any standard AA or a deletion; Xhh is a nonpolar AA selected from Ala, Val, Leu, Ile, and Phe; R_1 is a group selected from $-NH-CO-$ and $-CO-NH-$; R_2 is either the same as R_1 , or represents bond when l is zero; R_3 is selected from hydrogen and $-(CH_2)_j-NH_2$; k is an integer from 0 to 6; l is an integer from 0 to 3; m is an integer from 2 to 7; and j is an integer from 0 to 5.

4. A cyclized peptide according to claim 3, comprising backbone to side chain cyclization, having formula III:



and derivatives and salts thereof, wherein Xoo, Xhh, R₃, k, l, and m have the meaning as defined above, and R₂ is either the same as R₁, or represents bond when l is zero.

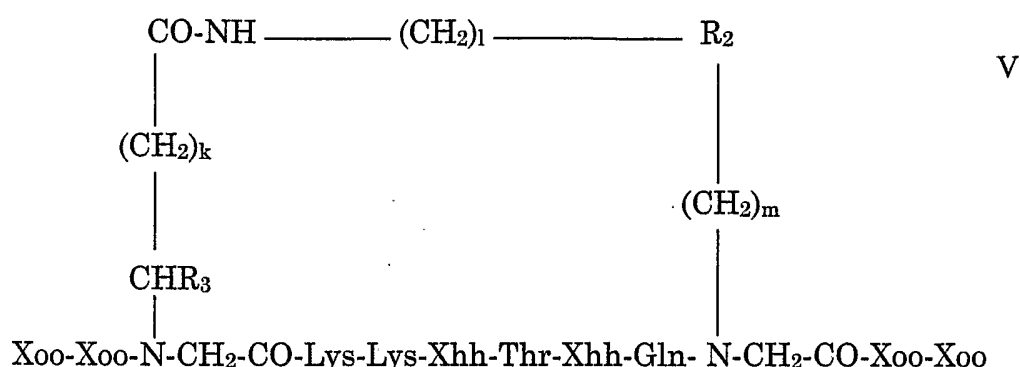
5. A cyclized peptide according to claim 3, comprising backbone to backbone cyclization, having formula IV:



and derivatives and salts thereof, wherein Xoo, Xhh, R₃, k, l, and m have the meaning as defined above, and R₂ is either the same as R₁, or represents bond when l is zero.

6. A cyclized peptide according to claim 3, comprising backbone to backbone cyclization, having formula V:

- 40 -



and derivatives and salts thereof, wherein Xoo, Xhh, R₃, k, l, and m have the meaning as defined above, and R₂ is either the same as R₁, or represents bond when l is zero.

7. A cyclized peptide, derivatives and salts thereof, according to claim 2, wherein AA at positions 1-5 and group R₃ comprise together at least two free amino groups.
8. A cyclized peptide, derivatives and salts thereof, according to claim 2, wherein R₃ is -(CH₂)_j-NH₂, and wherein j is an integer from 0 to 5.
9. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 2, wherein AA at position 5 is Lys.
10. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at position 1 is Tyr.
11. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at positions 1-2 are Tyr-Asn.
12. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at positions 1-2 are deleted.

- 41 -

13. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at position 12 is Asp.
14. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at positions 11-12 are Leu-Asp.
15. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at positions 11-12 are deleted.
16. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 6, wherein AA at positions 6 and 8 are independently selected from Val and Ala.
17. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 16, wherein said linker connecting said two Gly residues has a length from 8 to 16 atoms.
18. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 16, wherein said linker connecting said two Gly residues has a length from 10 to 14 atoms.
19. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 18, acetylated at a free amino group, and amidated at a free carboxyl group.
20. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 19, wherein said modified amino acid comprises a D-amino acid.
21. A cyclized peptide comprising a hexapeptide having sequence Lys-Xaa₁-Xaa₂-Thr-Xaa₃-Gln (SEQ ID NO. 1, wherein Xaa₁ is any standard AA,

- 42 -

and Xaa₂ and Xaa₃ are nonpolar AAs independently selected from Ala, Val, Leu, Ile, and Phe); derivative thereof obtained by acylation, amidation, methylation, and esterification; a homolog thereof obtained by replacing one AA in said sequence by another standard AA; and their salt; for use in antagonizing exotoxin-mediated activation of T-lymphocytes, and in eliciting protective immunity against toxic shock induced by one or more pyrogenic exotoxins.

22. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20, for use in antagonizing toxin-mediated activation of T-lymphocytes, wherein the toxin is a pyrogenic bacterial exotoxin.
23. A cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20, for use in eliciting protective immunity against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins.
24. A cyclized peptide, derivatives and salts thereof, according to claims 22 and 23, wherein said exotoxins are derived from *Staphylococcus aureus* or *Streptococcus pyogenes*.
25. A composition comprising at least one cyclized peptide, derivative and salt thereof, according to claims 1 to 20, for reducing the toxic effect of a bacterial exotoxin or a mixture of bacterial exotoxins.
26. A composition which inhibits pyrogenic exotoxin-mediated activation of T-lymphocytes and protects against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins, comprising a cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20, and optionally further comprising pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

27. An immunogenic composition for eliciting protective immunity against a toxic shock induced by a pyrogenic exotoxin comprising a cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20, and optionally further comprising pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.
28. A pharmaceutical composition for the treatment of incapacitation induced by one or more pyrogenic exotoxins comprising a cyclized peptide, derivatives and salts thereof, as defined in any one of claims 1 to 20, and optionally further comprising pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.
29. A pharmaceutical composition according to any one of claims 25 to 28, comprising a mixture of different cyclized peptides as defined in any one of claims 1 to 20, or derivatives and salts thereof.
30. A method for preventing, treating, or reducing the harmful effects of a bacterial exotoxin or a mixture of bacterial exotoxins in a mammal, comprising administering to said mammal an effective amount of at least one cyclized peptide, derivative and salts thereof, according to claims 1 to 20.
31. A method of inhibiting pyrogenic exotoxin-mediated activation of T-lymphocytes and protecting against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins in a patient in need of such treatment comprising administering to said patient an inhibitory effective amount of at least one cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20.
32. A method of eliciting protective immunity against a toxic shock induced by a pyrogenic exotoxin in a patient in need of such treatment

- 44 -

comprising administering to said patient at least one cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20.

33. A method of preventing or treating incapacitation induced by at least one pyrogenic exotoxin comprising administering to a patient in need of such treatment a therapeutically effective dose of at least one cyclized peptide, derivatives and salts thereof, according to any one of claims 1 to 20, or of a composition comprising the same.
34. A method according to claim 33, wherein said incapacitation is a result of an accidental exposure to a bacterial toxin at situations comprising food poisoning, work environment, combat, or a terrorist attack.
35. A method according to any one of claims 30 to 34, comprising administering a mixture of cyclized peptide of any one of claims 1 to 20, or derivatives and salts thereof.
36. The method of claim 34, wherein said effective dose is administered to said patient repeatedly, at predetermined periods of time.
37. Use of a cyclized peptide, analog, derivative, or salt thereof, according to any one of claims 1 to 20, in the preparation of a medicament.
38. Use of a cyclized peptide, analog, derivative, or salt thereof, according to any one of claims 1 to 20, in the preparation of a medicament for reducing harmful effects of bacterial pyrogenic exotoxins or their analogs.
39. Use of a cyclized peptide, analog, derivative, or salt thereof, according to any one of claims 1 to 20, in the preparation of a medicament for inhibiting pyrogenic exotoxin-mediated activation of T-lymphocytes.

- 45 -

40. Use of a cyclized peptide, analog, derivative, or salt thereof, according to any one of claims 1 to 20, in the preparation of a medicament for eliciting protective immunity against a toxic shock induced by a pyrogenic exotoxin.
41. Use of a cyclized peptide, analog, derivative, or salt thereof, according to any one of claims 1 to 20, in the preparation of a medicament for the treatment of incapacitation induced by one or more pyrogenic exotoxins.
42. A vaccine for protecting against toxic effects of a pyrogenic exotoxin or a mixture of pyrogenic exotoxins comprising as active ingredient an immunologically effective amount of at least one peptide or derivative thereof according to any one of claims 1 to 20.
43. A vaccine for conferring immunity against toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins comprising as active ingredient an immunologically effective amount of at least one peptide or derivative thereof according to any one of claims 1 to 20.
44. A vaccine according to claim 43 further comprising an immunization adjuvant.
45. A vaccine according to claim 43 for enhancing production of antibodies that block T-cell activation.
46. Use of a cyclized peptide of any one of claims 1 to 20, or derivatives and salts thereof in the preparation of a vaccine according to claim 42 or 43.
47. Antibodies directed against a cyclized peptide of any one of claims 1 to 20 or its derivative, which block T-cell activation.

- 46 -

48. An antiserum containing antibodies directed against a cyclized peptide of any one of claims 1 to 20 or its derivative.
49. An antiserum according to claim 48 which is a domestic animal antiserum.
50. An antiserum according to claim 48 capable of alleviating harmful effects and toxic shock induced by a pyrogenic exotoxin or by a mixture of pyrogenic exotoxins.
51. A method for assessing the efficacy of a vaccine for conferring immunity against one or more pyrogenic bacterial toxins comprising determining the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells.
52. A method according to claim 51 wherein the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells is determined by measuring the inhibition of expression of pyrogenic toxin-induced mRNA encoded by the IL-2 or IFN- γ genes.
53. A kit for assessing the efficacy of a vaccine for conferring immunity against one or more pyrogenic toxins comprising determining the ability of serum from an immunized individual to antagonize toxin-mediated activation of T-cells by the method of claim 51.

1/10

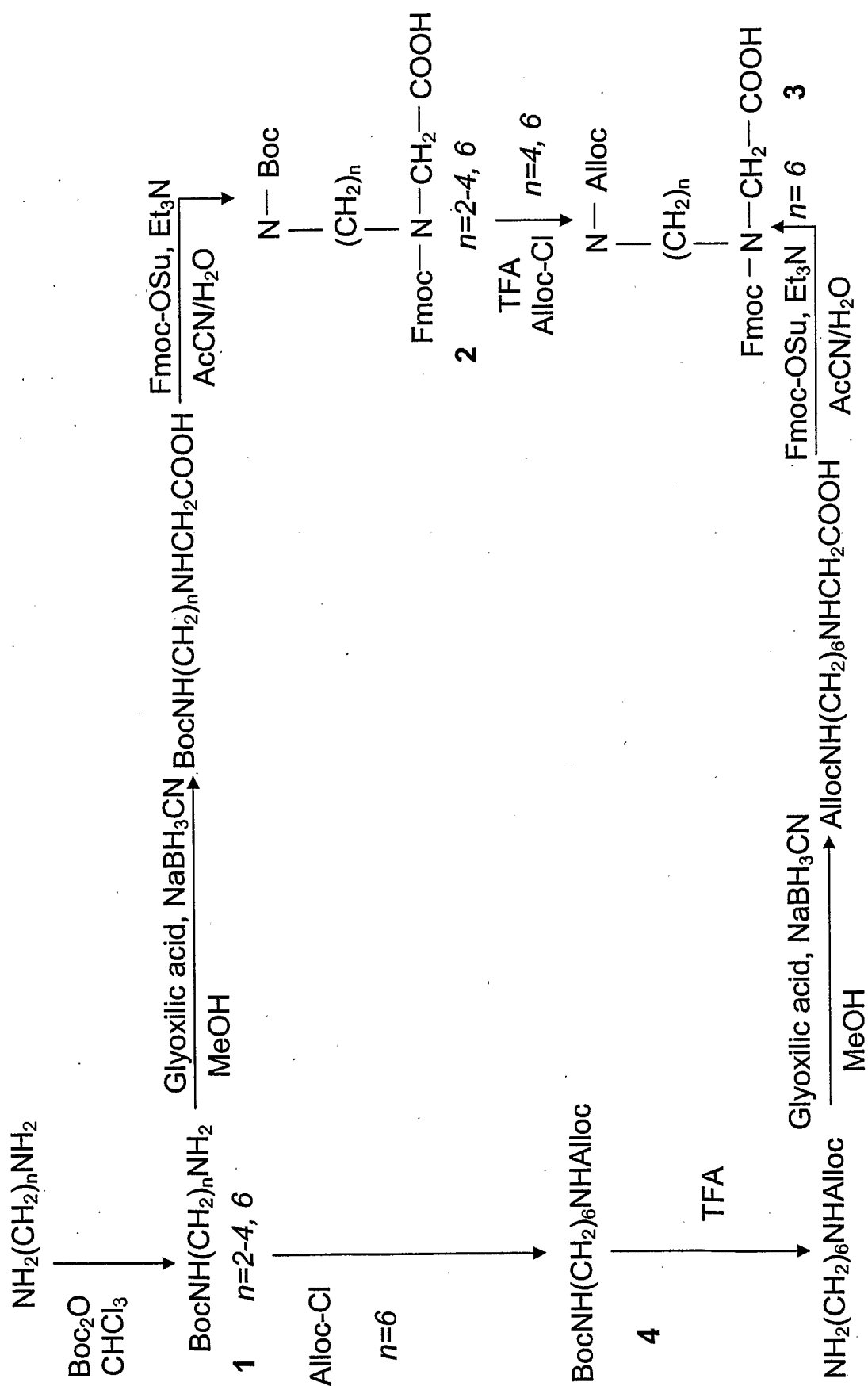
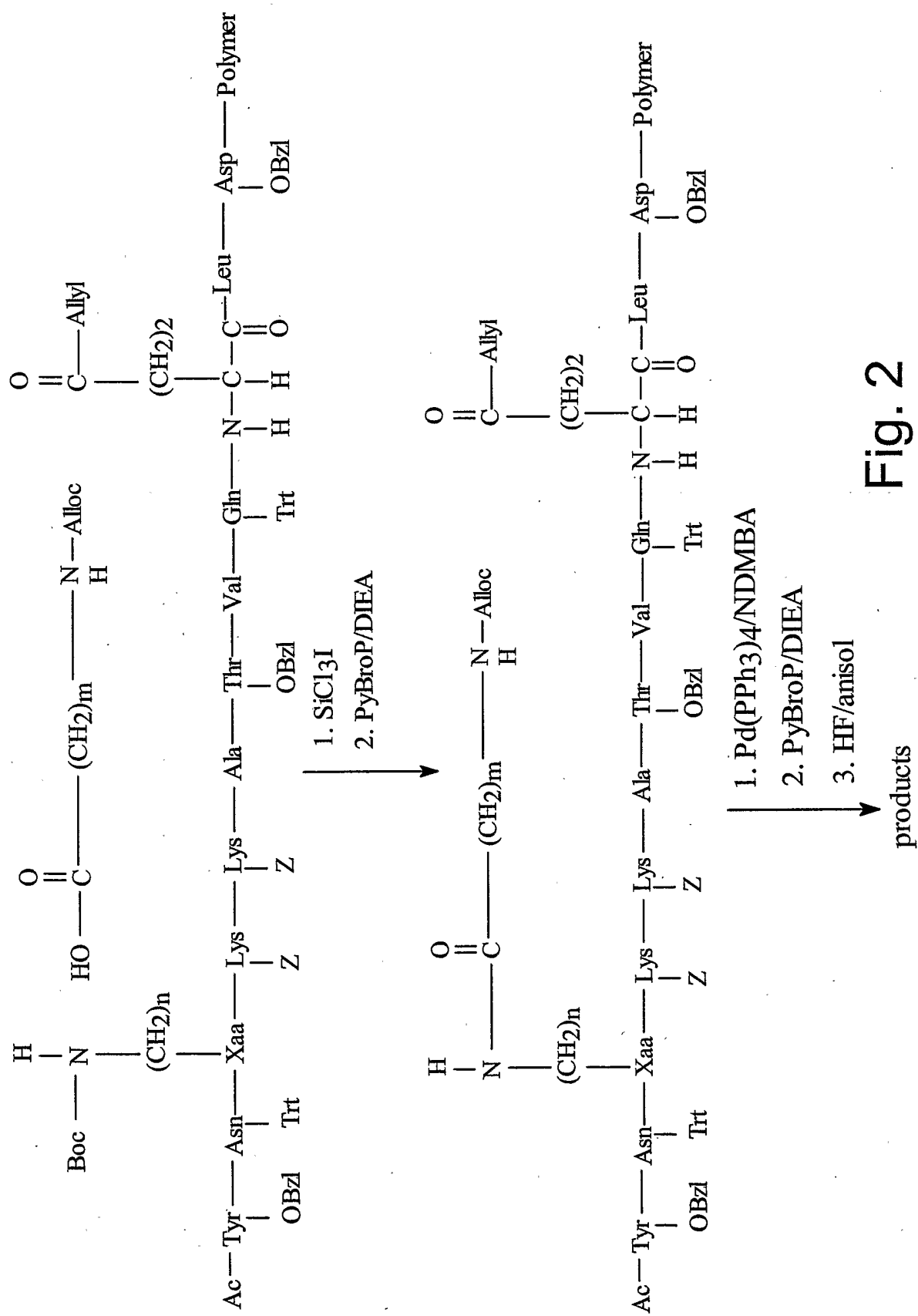


Fig. 1



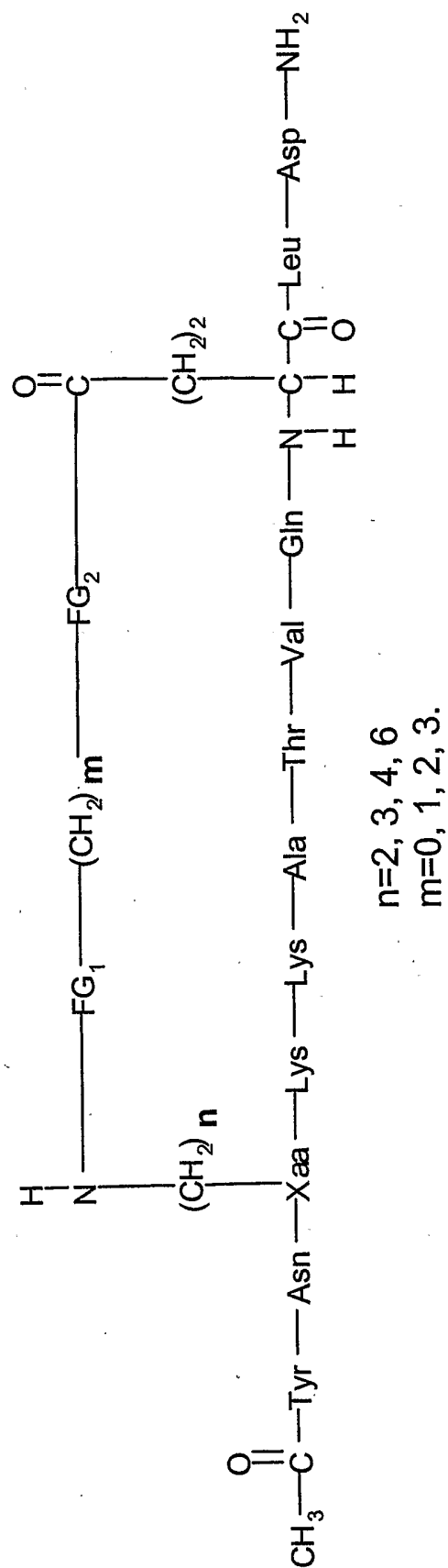


Fig. 3

4/10

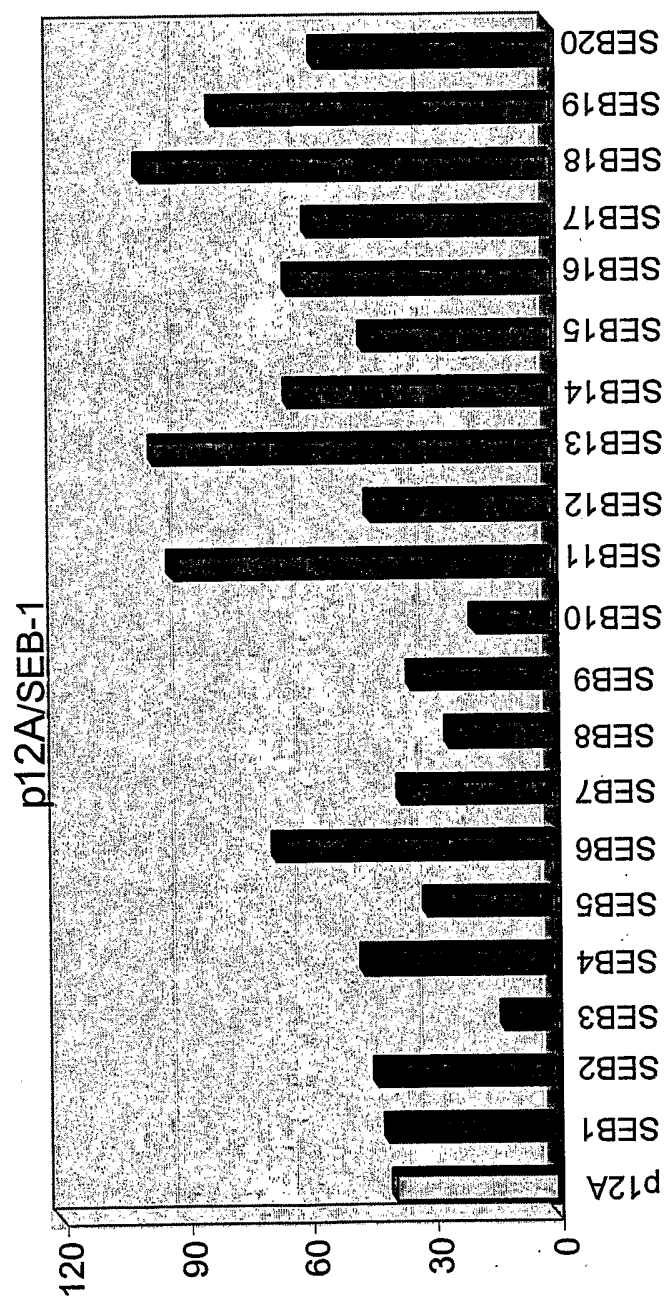


Fig. 4

5/10

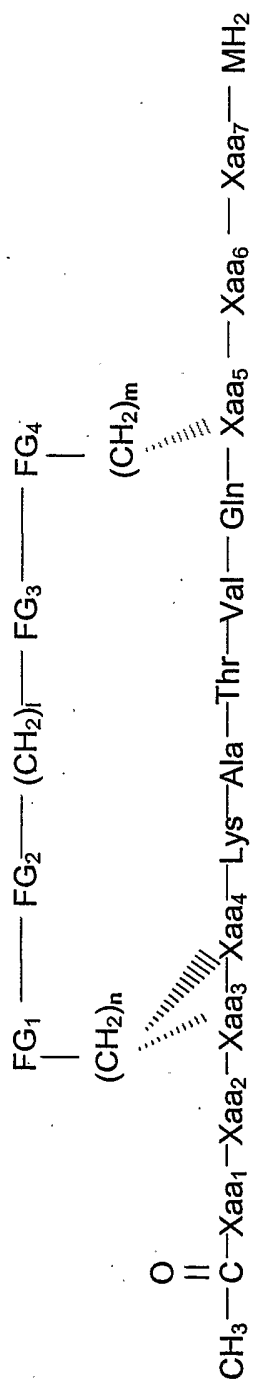


Fig. 5A

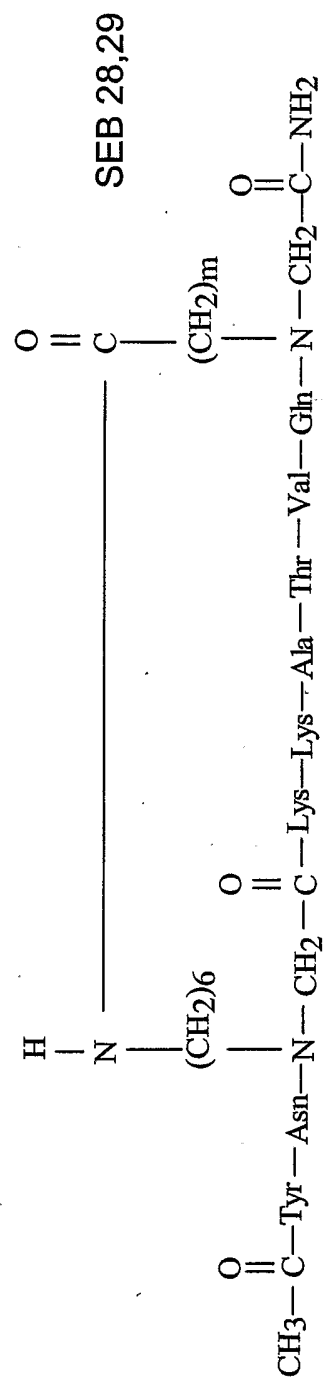
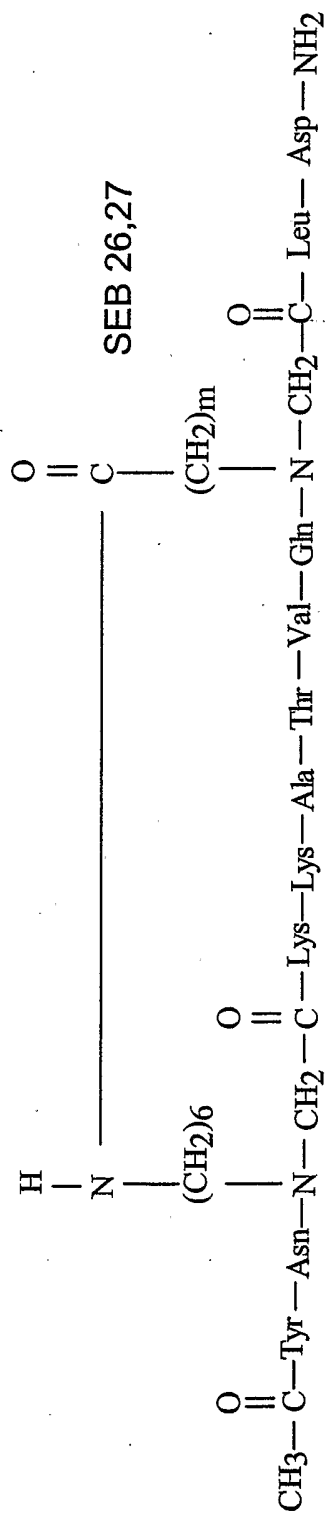


Fig. 5B

6/10

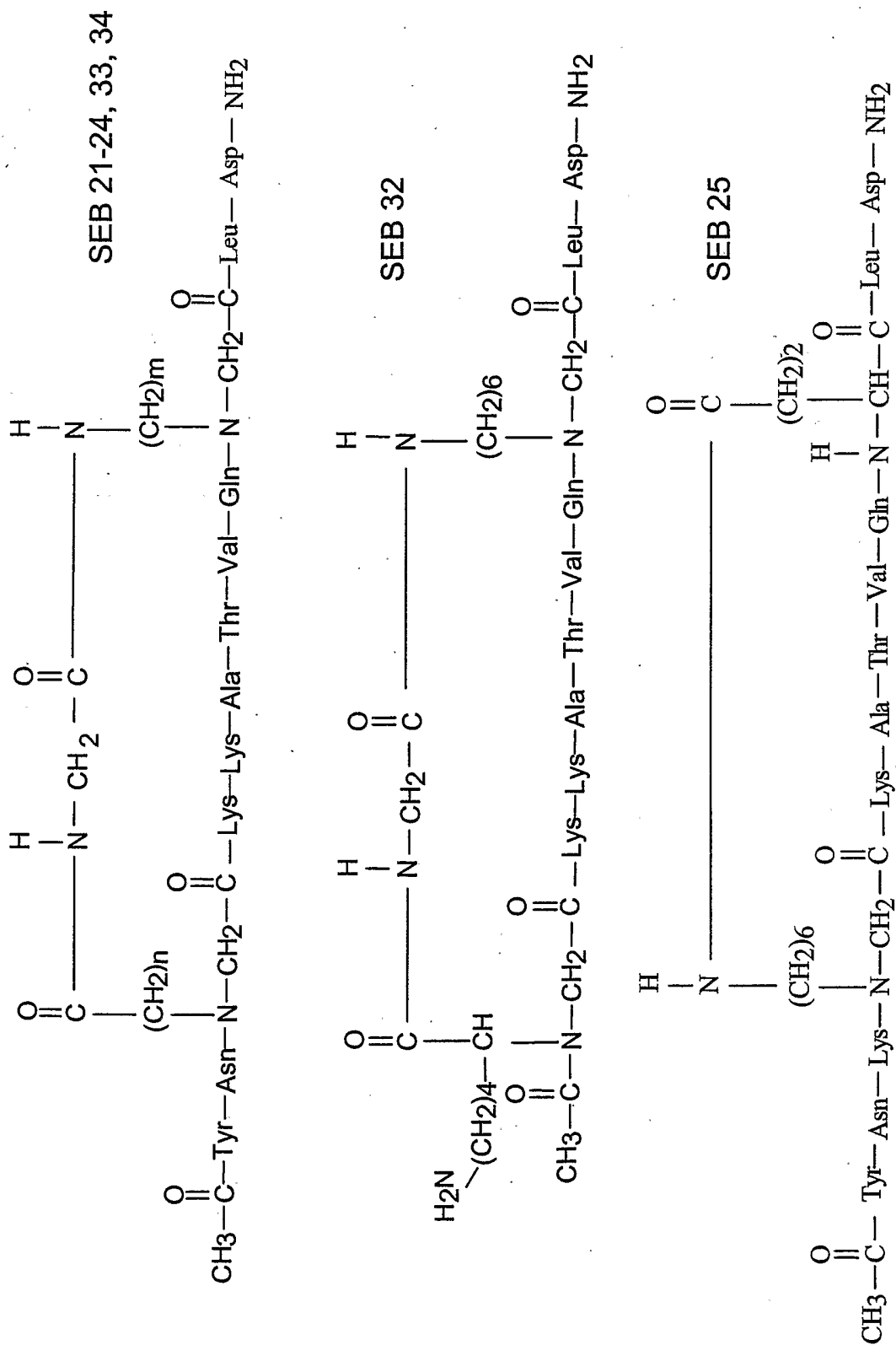


Fig. 5C

7/10

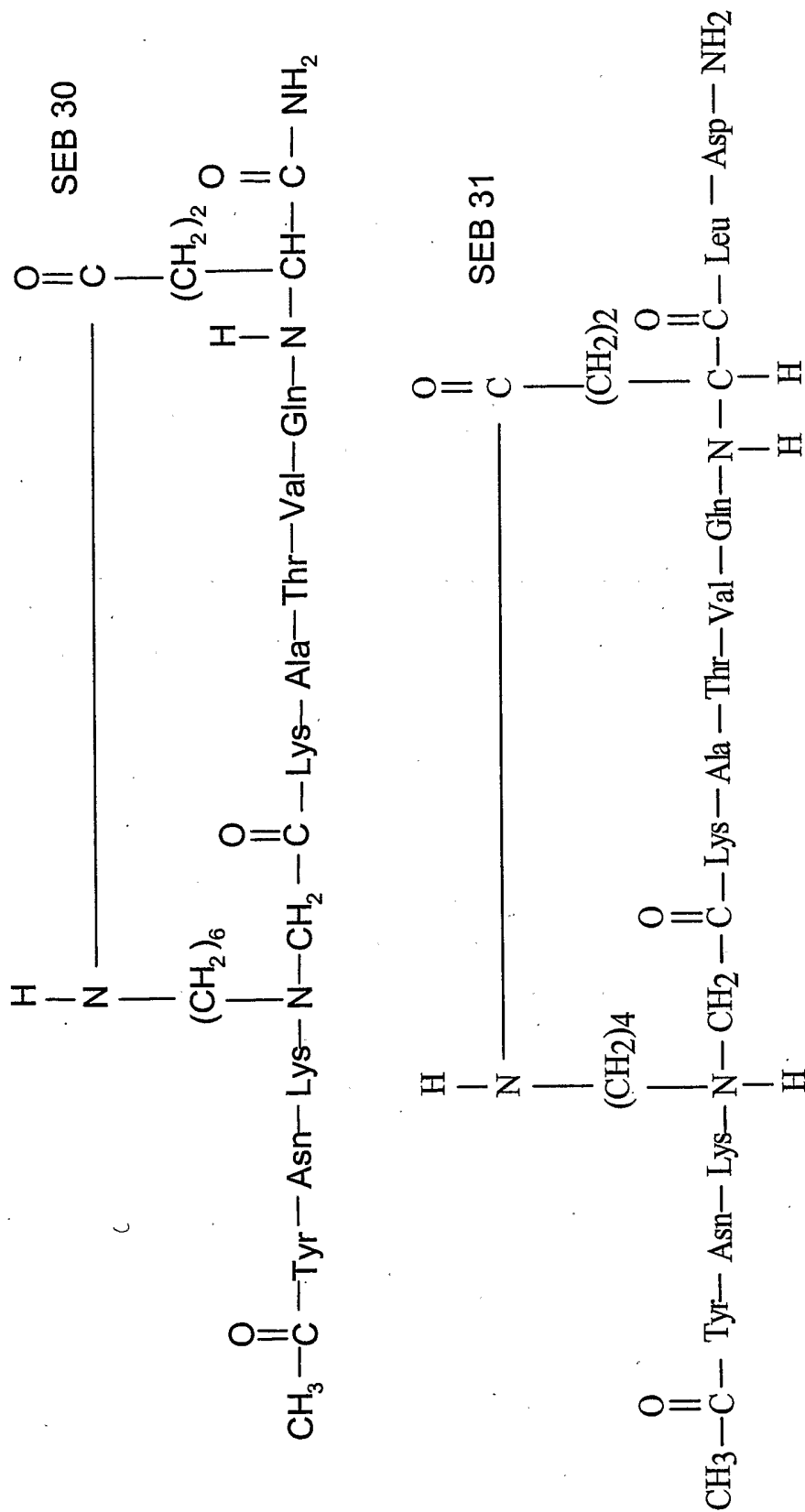


Fig. 5D

8/10

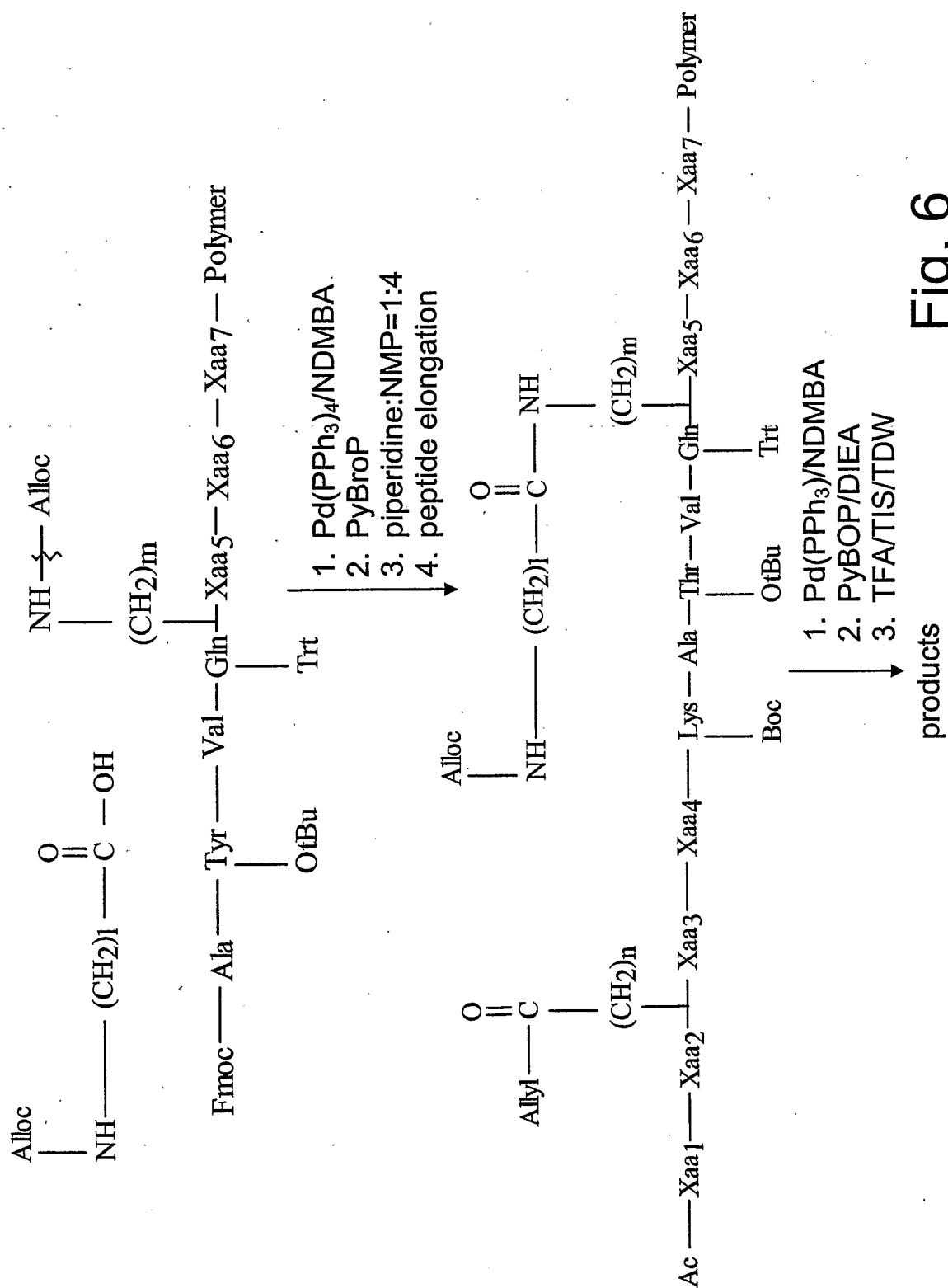


Fig. 6

9/10

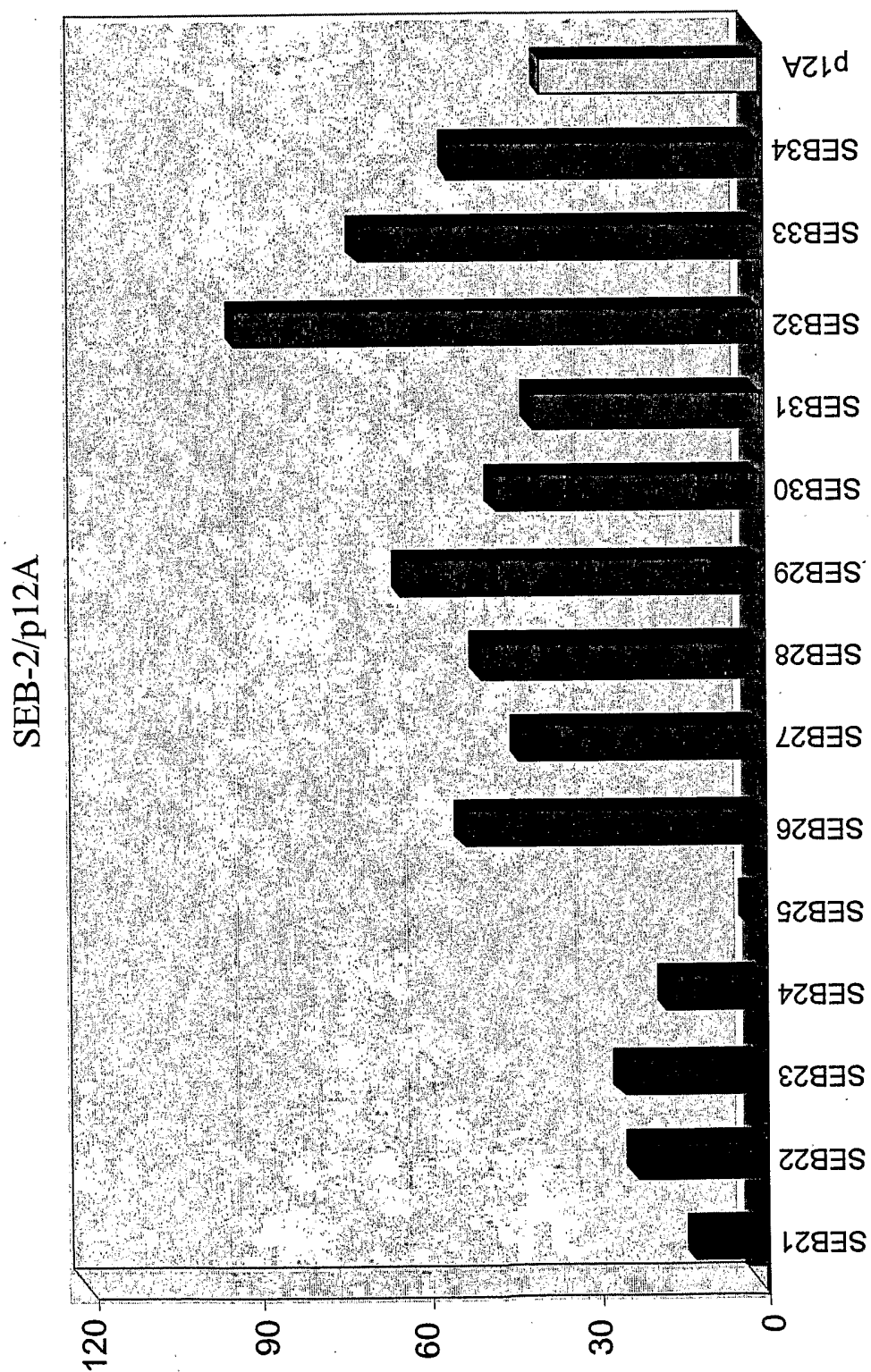


Fig. 7

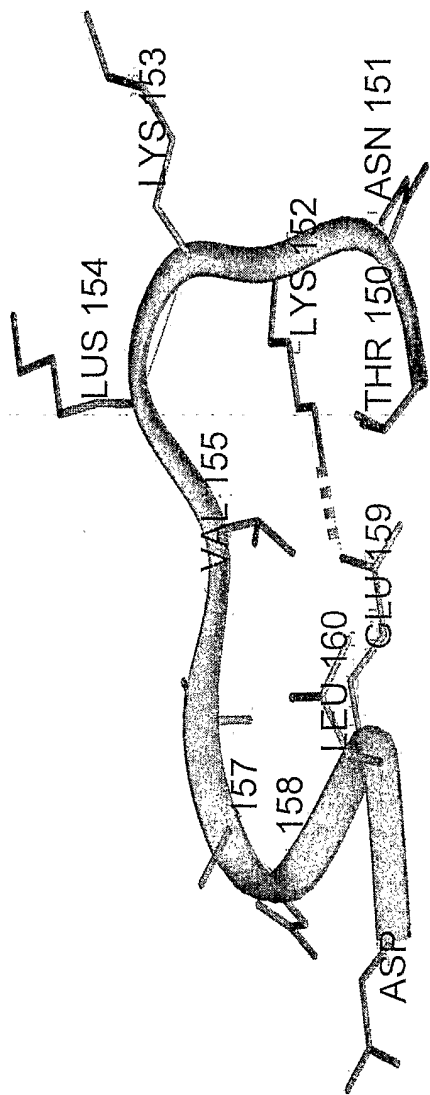


Fig. 8A

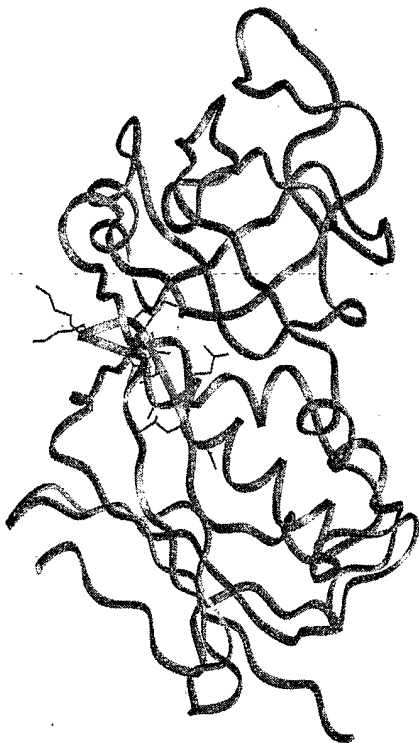


Fig. 8B

SEQUENCE LISTING

<110> YISSUM RESEARCH AND DEVELOPMENT

<120> CYCLIZED PEPTIDES AS EXOTOXIN ANTAGONISTS

<130> 15953WO

<140>

<141>

<160> 3

<170> PatentIn Ver. 2.1

<210> 1

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
peptided

<220>

<221> MOD_RES

<222> (1)..(6)

<223> Xaa in position 2 is any amino acid and Xaa in
positions 3 and 5, is any non-polar amino acid
selected from Ala, Val, Leu, Ile and Phe

<400> 1

Lys Xaa Xaa Thr Xaa Gln
1 5

<210> 2

<211> 12

<212> PRT

<213> staphylococcus

<400> 2

Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu Asp
1 5 10

<210> 3

<211> 14

2/2

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
peptide, wherein the Ala in positions 1 and 14
are D-Ala

<400> 3

Ala Tyr Asn Lys Lys Lys Ala Thr Val Gln Glu Leu Asp Ala
1 5 10