(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 June 2015 (11.06.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/082501 A1

(51) International Patent Classification:
C07K 14/315 (2006.01) *A61K 39/085* (2006.01)
A61K 39/09 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/EP2014/076313

(22) International Filing Date:
2 December 2014 (02.12.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13195402.6 3 December 2013 (03.12.2013) EP

(71) Applicants: **VIROMETIX AG** [CH/CH]; Wagistrasse 14, CH-8952 Schlieren (CH). **SWISS TROPICAL AND PUBLIC HEALTH INSTITUTE** [CH/CH]; Socinstrasse 57, CH-4002 Basel (CH). **UNIVERSITÄT BASEL** [CH/CH]; Vizerektorat Forschung, Petersgraben 35, CH-4003 Basel (CH). **UNIVERSITÄT ZÜRICH** [CH/CH]; Prorektorat MNW, Rämistrasse 71, CH-8006 Zürich (CH).

(72) Inventors: **GHASPARIAN, Arin**; Salomon-Vögelin Strasse 62, CH-8038 Zürich (CH). **ZUNIGA, Armando**; Ottikerstrasse 38, CH-8006 Zürich (CH). **GEIB, Nina**; Neunbrunnenstrasse 161, CH-8050 Zürich (CH). **TAMBORINI, Marco**; Tramstrasse 87, CH-4132 Muttenz (CH). **JUD, Maja**; Schützenstrasse 13, CH-4103 Bottmingen (CH). **PLUSCHKE, Gerd**; Im Vogelsang 3, 79189 Bad Krozingen (DE). **MARRERO NODARSE, Aniebrys**; Bergacker 18, CH-8046 Zürich (CH). **ROBINSON, John Anthony**; Tobelstrasse 24, CH-8615 Wermatswil (CH).

(74) Agent: **LATSCHA SCHÖLLHORN PARTNER AG**; Austrasse 24, CH-4051 Basel (CH).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2015/082501 A1

(54) **Title**: PROLINE-RICH PEPTIDES PROTECTIVE AGAINST S. PNEUMONIAE

(57) **Abstract**: The invention relates to lipopeptides consisting of a peptide chain comprising a parallel coiled-coil domain, a proline-rich peptide antigen, and a lipid moiety, all covalently linked, which aggregate to synthetic virus-like particles. Proline-rich peptide antigens considered contain negatively and positively charged amino acid, and at least 15% of the amino acids are proline. Such synthetic virus-like particles carrying proline-rich antigens derived from pneumococcal proteins are useful as vaccines against infectious diseases caused by Gram-positive bacteria such as *Streptococcus pneumoniae*.

Proline-rich peptides protective against *S. pneumoniae*

Field of the invention

The invention relates to multimeric lipopeptides consisting of a peptide chain comprising a parallel coiled-coil domain, a proline-rich peptide antigen, and a lipid moiety, all covalently linked, which aggregate to synthetic virus-like particles. These synthetic virus-like particles carrying proline-rich antigens are useful as vaccines against infectious diseases caused by Gram-positive bacteria such as *Streptococcus pneumoniae*.

Background of the invention

Gram-positive bacteria, including *Streptococci* or *Staphylococci* are important pathogens and the etiological agent of a number of serious diseases including pneumonia, sepsis, meningitis, wound infections, endocarditis, acute rheumatic fever, neonatal sepsis or toxic shock syndrome. Therefore there is a need to develop vaccines against these pathogens. Vaccines are already available for some *S. pneumoniae* serotypes; these have shortcomings such as a highly complex manufacturing process.

S. pneumoniae is a highly diverse polysaccharide encapsulated alpha-hemolytic *Streptococcus* that frequently colonizes the human nasopharynx and can cause non-invasive pneumococcal diseases such as otitis media, sinusitis and non-bacteraemic pneumonia, and more severe invasive diseases such as bacteraemia/sepsis, meningitis and bacteraemic pneumonia, primarily among young children and the elderly.

The polysaccharide capsule is a major determinant of virulence during invasion and prevents C3b opsonisation and non-opsonic killing by neutrophils. Currently licensed vaccines contain capsular polysaccharide antigens formulated either alone in pneumococcal polysaccharide vaccines (PPSV) or conjugated to a carrier protein such as modified diphtheria toxin CRM 197 in pneumococcal conjugate vaccines (PCV). There are more than 90 different capsular serotypes in 40 serogroups.

Polysaccharide pneumococcal vaccines can provide serotype-specific protection but cross-protection is low even within the same serogroup. Serotype replacement has been observed after introduction of the conjugate vaccine Prevnar® 7 in the US in 2000. Among the emerging serotypes are also multi-drug resistant capsule-switch variants. Therefore

there is a need for next generation pneumococcal vaccines that target other antigens than the capsule.

One potential antigen for inclusion into a next generation pneumococcal vaccine is Pneumococcal Surface Protein A (PspA). PspA is a monomeric polymorphic choline-binding protein and contains an N-terminal alpha-helical part, which forms an antiparallel coiled-coil with itself, a proline-rich region, which is sometimes interspersed by a relatively conserved non-proline block, and a C-terminal region containing multiple repeats of a choline binding domain.

The N-terminal region of PspA contains immunodominant epitopes. Recombinant proteins comprising this region and bacterial vectors expressing this region have shown protective potential in various models. For example, Langermann *et al.* have prepared recombinant Bacille Calmette-Guérin (rBCG) vectors expressing PspA. In order to be able to anchor the PspA in the bacterial membrane, a PspA-derived gene segment was fused to Mtb19 lipoprotein (see Langermann S. *et al.*, *J. Exp. Med.* **1994**, *180*, 2277-2286). There is a safety concern associated with the use of PspA as a vaccine antigen because the N-terminal region may resemble human myosin and thus immunization with an immunogen encompassing this region may lead to tissue cross-reactive antibodies. Therefore recent efforts have been made to use other regions of PspA as antigen. Another PspA region that may be suitable for inclusion into a next generation pneumococcal vaccine is the proline-rich region. Although the proline-rich region (PRR) of PspA is polymorphic, it contains several conserved motifs, including short amino acid motifs like PKP, PAPAP, PEKP, and a highly conserved non-proline block (NPB) that is present in some PspA molecules (Brooks-Walter, A. *et al.*, *Infect Immun* **1999**, *67*, 6533-6542; Hollingshead, S.K. *et al.*, *Infect Immun,* **2000**, *68*, 5889-5900; Daniels, C.C. *et al.*, *Infect Immun,* **2010**, *78*, 2163-2172.). Although the PRR does not contain immunodominant epitopes, antibodies against this part of PspA have been detected in children using an enzyme immunoassay (EIA) with a thioredoxin (Trx) fusion protein as antigen (Melin, M. *et al.,* *Vaccine* **2012**, *30*, 7157-7160). Because the NPB is highly conserved the authors hypothesize that antibodies to the PRR may cross-react with a majority of strains through their recognition of NPB epitopes.

The PRR of PspA has a small size (up to around 100 amino acids) and therefore may not be sufficiently immunogenic when used as an antigen alone. *Escherichia coli* Trx fusion proteins have been produced and their potential for protection has been demonstrated in a mouse model of intravenous infection (WO 2007/089866 and Daniels, C.C. *et al.*, *Infect*

Immun, **2010**, *78*, 2163-2172). However, Trx fusion proteins may not be suitable for human use as a vaccine because of a potential for the induction of immune responses to non-protective Trx epitopes and poor structural representation of native PR epitopes. Moreover NPB or proline-rich (PR) sequences may also contain non-protective epitopes, and hence it may be critical to concentrate immune responses to protective epitopes for efficacy.

Similar PR sequences can be found in other pneumococcal proteins, including the surface proteins PspC (also known as CbpA or Hic), and the PhtX proteins PhtA, PhtB, PhtD and PhtE, and proline-rich regions derived from such other pneumococcal proteins may be suitable for inclusion into a next generation pneumococcal vaccine, like proline-rich sequences from PspA.

Several immunogenic bacterial surface proteins from other Gram-positive bacteria contain proline-rich sequences that can likewise be targeted by vaccines against these pathogens. These proteins include surface proteins from other *Streptococci* such as the M6, SclA and SclB proteins of *S. pyogenes*, CBeta (bac) and BibA of *S. agalactiae* or the P1 adhesin of *S. mutans*, or proteins from *S. aureus*.

Synthetic bacterial lipopeptide analogs have received wide attention in vaccine research, both for their adjuvant effects and as carriers for peptide antigens (Ghielmetti M. *et al.*, *Immunobiology* **2005**, *210*, 211-215). Many lipopeptide constructs have been reported, in which a lipid with known adjuvant effects has been coupled to a peptide to generate self-adjuvanting vaccine candidates. Particularly well studied are tripalmitoyl-S-glyceryl cysteine (N-palmitoyl-S-(2,3-bis-(O-palmitoyloxy)-propyl)-cysteinyl- or Pam3Cys) and dipalmitoyl-S-glyceryl cysteine (2,3-bis-(O-palmitoyloxy)-propyl)-cysteinyl- or Pam2Cys). These lipid moieties are found in lipoprotein components of the inner and outer membranes of gram-negative bacteria. Patent application WO 98/07752 describes the use of lipopeptides for drug targeting purposes, wherein the peptide portion may be a collagen-like sequence capable of inducing triple helical structures. Patent application WO 2008/068017 describes synthetic virus-like particles comprising helical lipopeptide bundles and having a spherical or spheroidal structure with a lipid core and a peptidic outer surface. The peptide chain of the lipopeptides comprises a coiled-coil domain. The properties of the coiled-coil domain in the peptide chain of the lipopeptide building blocks determine the number of building blocks combining to form the synthetic virus-like particle.

Summary of the invention

The invention relates to lipopeptide building blocks consisting of (1) a peptide chain comprising a parallel coiled-coil domain which, as a self-standing lipid-free peptide, forms a parallel dimeric, trimeric or higher order oligomeric helical bundle, (2) a proline-rich (PR) peptide antigen comprising at least one negatively and at least one positively charged amino acid, and wherein at least 15% of the amino acids are proline, optionally linked to a further antigen, and (3) a lipid moiety comprising two or three long hydrocarbyl chains; wherein the peptide chain, the PR peptide antigen and the lipid moiety are covalently linked, either directly or through a linker. Preferably, the peptide chain comprising a parallel coiled coil is linked at one end to the PR peptide antigen and at the other end to the lipid moiety.

These lipopeptide building blocks aggregate to helical lipopeptide bundles and synthetic virus-like particles (SVLP). The presentation of the PR antigen on the SVLP surface enhances the immune response to PR epitopes.

Preferred are lipopeptide building blocks comprising PR peptide antigens derived from *Streptococci* and/or *Staphylococci,* more preferably from *Streptococcus pneumoniae.*

The invention further relates to processes of production of lipopeptide building blocks, helical lipopeptide bundles and synthetic virus-like particles; to the use of lipopeptide building blocks, helical lipopeptide bundles and synthetic virus-like particles carrying PR peptide antigens in the preparation of vaccines; and to methods of vaccination using such vaccines. The invention likewise relates to pharmaceutical preparations containing synthetic virus-like particles carrying PR antigens.

The compositions of the invention comprising PR peptide antigens derived from *Streptococci* and/or *Staphylococci,* in particular from *S. pneumoniae,* are useful for inducing immune responses against *S. pneumoniae* or other Gram-positive bacteria, and for the prevention or treatment of infectious diseases such as pneumococcal diseases caused by *S. pneumoniae.*

Brief description of the figures

Figure 1: IgG ELISA endpoint titers in sera from BALB/c mice immunized two times with lipopeptide **15** (closed circles and squares) or alum adjuvanted recombinant PspA (rPspA, closed triangles) and sera from non-immunized controls (open symbols). Symbols indicate endpoint titers sera from individual mice, lines indicate median values. Titers were measured against a peptide representing the proline-rich region (PR peptide) and recombinant PspA protein comprising the entire N-terminal alpha-helical part, the proline-rich region and the non-proline block (rPspA).

Figure 2: Survival times in days (D) post challenge of immunized and non-immunized BALB/c mice with increasing doses of *S. pneumoniae* serotype 1. The percent Survival (%S) is shown on the y-axis and immunogens and challenge doses (in CFU) are indicated on the right. Mice were immunized two times with lipopeptide **15** or rPspA + alum and then challenged intravenously.

Detailed description of the invention

The invention relates to lipopeptide building blocks consisting of
(1) a peptide chain (PC) comprising a parallel coiled-coil domain which, as a self-standing lipid-free peptide, forms a parallel dimeric, trimeric or higher order oligomeric helical bundle,
(2) a proline-rich (PR) peptide antigen, comprising at least one negatively and at least one positively charged amino acid, and wherein at least 15% of the amino acids are proline, optionally linked to a further antigen, and
(3) a lipid moiety (LM) comprising three or preferably two long hydrocarbyl chains,
wherein the peptide chain, the PR peptide antigen and the lipid moiety are covalently linked, either directly or through a linker, in particular two different linkers.

Preferably, the peptide chain comprising a parallel coiled coil is linked at one end to the PR peptide antigen and at the other end to the lipid moiety.

The peptide chain (PC) comprises a parallel coiled-coil domain. Such coiled-coil domains will associate into a defined helical bundle, e.g. into a dimeric, trimeric, tetrameric, pentameric, hexameric or heptameric bundle. Parallel coiled-coil domains differ from antiparallel coiled-coils, wherein a monomeric peptide chain loops back to form a helical substructure by aligning two (or more) partial domains of the monomeric peptide in an

antiparallel alignment. The parallel coiled-coil domain may contain between 12 and 120 amino acid residues, preferably between 21 and 80 amino acid residues. Coiled-coil domains contain two or more consecutive repeat patterns (usually heptad repeats in which the seven structural positions are labeled *a-g*, with *a* and *d* denoting hydrophobic residues), which as self-standing lipid-free peptides possess the property of self-assembly into a parallel coiled-coil helical bundle (Lupas A.N., Gruber M.; The structure of alpha-helical coiled coils, *Adv. Protein Chem.*. **2005**, *70*, 37–78). The peptide chain must multimerize to form a parallel coiled-coil helical bundle of defined oligomerization state (e.g. dimer, trimer, tetramer, pentamer, hexamer or heptamer, in particular dimer, trimer, tetramer or pentamer). Preferred peptide sequences are non-human sequences to avoid the risk of autoimmune disorders when applied in the vaccination of humans.

The lipopeptide building block further comprises a proline-rich (PR) peptide antigen comprising at least one negatively and at least one positively charged amino acid. Charged amino acids considered herein are amino acids with side chains that are positively or negatively charged at physiological pH. Among the naturally occurring amino acids the most frequent positively charged amino acids considered here are lysine, arginine and histidine; the most frequent negatively charged amino acids are glutamic acid and aspartic acid. A peptide is considered "proline-rich" if at least 15% of the amino acids are proline. Preferred are proline-rich peptides comprising at least one glutamic acid residue and at least one lysine or arginine residue.

Preferred PR peptide antigens are derived from *Streptococci* and/or *Staphylococci,* e.g. from Gram-positive bacteria selected from the group consisting of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mutans*, *Streptococcus suis, Streptococcus equi, Streptococcus dysgalactiae, Peptostreptococcus magnus* and *Staphylococcus aureus.*

Preferably this PR peptide antigen is derived from proteins PspA and/or PspC or other proteins that are protective against pneumococcal infection, including PhtX proteins.

"Derived" means that the amino acid sequence or a substantial portion (i.e. 50% or more) of the amino acid sequence of the peptide antigen originates from one or more naturally occurring protein(s), whereas 0% up to 50% of the amino acid sequence is designed *de novo*. Included are also PR peptide antigens that comprise combinations of proline-rich sequences from different PspA/PspC molecules and/or from other proteins containing

proline-rich segments. "Proline-rich segment" means that at least 15% of the amino acids contained in the segment are proline.

Proline-rich (PR) peptide antigens of particular interest are derived from *S. pneumoniae* and are located immediately after the C-terminal end of the helical region of PspA or PspC and before the non-proline block (if the PspA or PspC sequence comprises a non-proline block) or the repeat region. Alternatively the PR peptide antigen is located in the central region of a pneumococcal polyhistidine triad protein (PhtX). Alternatively the PR peptide antigen is located in a region of PR proteins of Gram-positive bacteria selected from the group consisting of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mutans*, *Streptococcus suis, Streptococcus equi, Streptococcus dysgalactiae, Peptostreptococcus magnus* and *Staphylococcus aureus*, such as the central region of the P1 adhesin of *Streptococcus mutans*.

Protective PR peptide antigens are identified by sequencing PspA and/or PspC and/or other genes from clinical isolates, selecting a portion of the PR region, synthesizing the PR peptide, and conjugating it to a peptide chain (PC) to be incorporated or being part of a lipopeptide.

The efficacy of PR peptide antigens is tested by administering lipopeptide conjugates and synthetic virus-like particles obtained therefrom and observing the activity and efficacy in animal models of pneumococcal sepsis or other diseases caused by streptococcal infection.

The PR peptide antigen is optionally linked to a further antigen. Further antigens considered are other pneumococcal peptides or polysaccharides, in particular the peptides with an amino acid sequence SEQ ID NO: 113 to 119, and other antigens described below.

The PR peptide antigen is conjugated directly or through a linker, either at the N- or C-terminus of the PR peptide antigen, and is connected either to the N- or to the C-terminal of the peptide chain (PC) comprising the coiled coil domain, or optionally to an amino acid side chain. Alternatively the PR peptide antigen is conjugated to the peptide chain (PC) comprising the coiled coil domain through a side chain residue of the PR peptide antigen, such as a terminal or internal aspartic acid, glutamic acid, lysine, ornithine or cysteine side chain. Linkers considered are short peptides of 2 to 20 amino acids, hydroxyalkyl- or aminoalkyl-carboxylic acids, substituted or unsubstituted polyalkylenoxy glycols,

preferably containing one to twelve C_2 and/or C_3 alkylenoxy units, polyalkylenoxy glycol block co-polymers (e.g. pluronics), mono-, di-, tri- and oligosaccharides, which may comprise acetyl, glycerol-phosphate or other substituents at one or more positions, polysaccharides such as poly(sialylic acid) and derivatives (e.g. peptide conjugates) thereof, proteinogenic or non-proteinogenic amino acids, and C_1-C_8 saturated or unsaturated hydrocarbons, and may comprise one or more of the following functional groups: a disulphide bond, amine, amide, acetal, ester, ether, thioether, hydrazone, hydrazide, imine, oxime, urea, thiourea, carbonate, iminocarbonate, amidine, amide, imide, an alkyl succinimide, which may also be hydrolyzed to an amide, sulphonamide, sulfone, or a heterocyclic ring comprising one or more atoms selected from nitrogen and oxygen, preferably a triazole. Also considered are combinations of the aforementioned linkers, including those used in the Examples.

Any method used for conjugating peptides or other antigens to an antigen delivery system such as carrier protein, polymer, dendrimer, nanoparticle or virus-like particle, can be used to conjugate the PR peptide antigens to the peptide chain (PC) comprising the parallel coiled-coil domain. Such methods are well-known to those skilled in the art, see for example Hermanson, G.T, Bioconjugate Techniques, 2nd edition, Academic Press, 2008.

PR peptide antigens consist of 5-200 amino acids, preferably 8-80 amino acids. Multiple PR peptide conjugates can be used in combination in a vaccine formulation. PR peptide antigens can also be fused together in order to produce a longer artificial PR peptide. PR conjugates can also be combined with conjugates comprising other pneumococcal peptides or polysaccharides. Amino acids and derivatives thereof comprising a functional group (e.g. an amino-, halo-, hydrazino-, hydroxylamino- or sulfhydryl group) can be incorporated into the PR peptide in order to facilitate conjugation of the PR peptide and enhance stability.

The peptide chain (PC) may further comprise an amino acid sequence which includes one or more T-helper cell epitopes, and/or strings of polar residues that promote the solubility of the lipopeptide building block in water.

T-helper epitopes that may be incorporated into the peptide chain (PC) include those listed in Table 1 below, and variants thereof in which one, two, or three amino acids are replaced by other amino acids or are deleted.

Table 1

T-helper epitope	SEQ ID NO:	Sequence [a)]
TT830-843	1	QYIKANSKFIGITE
TT1064-1079	2	IREDNNITLKLDRCNN
TT1084-1099	3	VSIDKFRIFCKANPK
TT947-968	4	FNNFTVSFWLRVPKVSASHLET
TT1174-1189	5	LKFIIKRYTPNNEIDS
DTD271–290	6	PVFAGANYAAWAVNVAQVID
DTD321–340	7	VHHNTEEIVAQSIALSSLMV
DTD331–350	8	QSIALSSLMVAQAIPLVGEL
DTD351–370	9	VDIGFAAYNFVESIINLFQV
DTD411–430	10	QGESGHDIKITAENTPLPIA
DTD431–450	11	GVLLPTIPGKLDVNKSKTHI
TT632-651	12	TIDKISDVSTIVPYIGPALN
CTMOMP36-60	13	ALNIWDRFDVFCTLGATTGYLKGNS
TraT1	14	GLQGKIADAVKAKG
TraT2	15	GLAAGLVGMAADAMVEDVN
TraT3	16	STETGNQHHYQTRVVSNANK
HbcAg50-69	17	PHHTALRQAILCWGELMTLA
HbSAg19-33	18	FFLLTRILTIPQSLD
HA307-319	19	PKYVKQNTLKLAT
MA17-31	20	YSGPLKAEIAQRLEDV
MVF258-277	21	GILESRGIKARITHVDTESY
MVF288-302	22	LSEIKGVIVHRLEGV
CS.T3*	23	IEKKIAKMEKASSVFNVVNS
SM Th	24	KWFKTNAPNGVDEKIRI
PADRE1 [b)]	25	aKFVAAWTLKAAa
PADRE2 [b)]	26	aK-Chx-VAAWTLKAAa

[a)] References: SEQ ID NO: 1-5 and 17-20: *Eur. J. Immunol.* **2001**, *31,* 3816–3824; SEQ ID NO: 6-12: *JID* **2000**, *181,* 1001-1009; SEQ ID NO: 13-16, 21-22 and 24: US 5,759,551; SEQ ID NO: 23: *Nature* **1988**, *336*, 778-780; SEQ ID NO: 25-26: *Immunity* **1994**, *1*, 751–761.

[b)] „a" denotes D-Ala and „Chx" denotes cyclohexylalanine.

The total length of the peptide chain (PC) is preferably between 21 and 200 amino acid residues, more preferably between 21 and 120 amino acid residues.

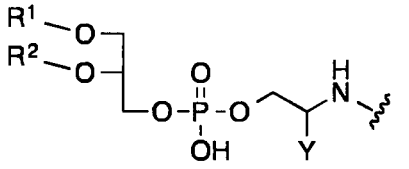
5

The lipid moiety (LM) contains a lipid anchor with two or three, preferably two, long hydrocarbyl chains and a structure combining these hydrocarbyl chains and connect it to the peptide chain (PC), either directly or via a linker. The lipid moiety can also be connected to the PR peptide again, which in turn is conjugated to the peptide chain comprising the parallel coiled-coil, however, connection of the lipid moiety to the peptide chain is preferred. Preferred lipid moieties are lipids containing two or three, preferably two extended hydrocarbyl chains.

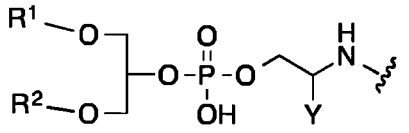
"Long hydrocarbyl" means a straight alkyl or alkenyl group of at least 7 carbon atoms, for example straight alkyl or alkenyl consisting of between 8 and 50 C atoms, preferably between 8 and 25 C atoms. Alkenyl has preferably one, two or three double bonds in the chain, each with E or Z geometry, as is customarily found in natural fatty acids and fatty alcohols. Also included in the definition of "long hydrocarbyl" is branched alkyl or alkenyl, for example alkyl bearing a methyl or ethyl substituent at the second or third carbon atom counted from the end of the chain, as e.g. as in 2-ethyl-hexyl.

"Lower alkyl" means alkyl with 1 to 7 carbon atoms (C_1-C_7), preferably 1 to 4 carbon atoms (C_1-C_4), such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl or tert-butyl.

Particular preferred lipid moieties according to the invention are those of formula Z^1 to Z^8

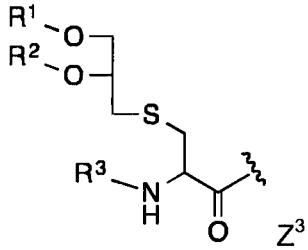


Z^1



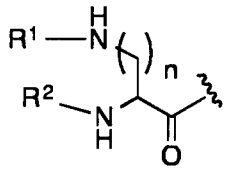
Z^2

wherein R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and Y is H or COOH,

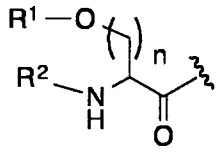


Z^3

wherein R^1, R^2 and R^3 are long hydrocarbyl or long hydrocarbyl-C=O or R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and R^3 is H or acetyl or lower alkyl-C=O,

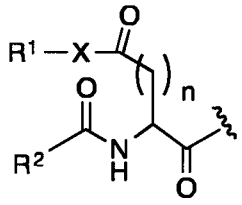


Z^4



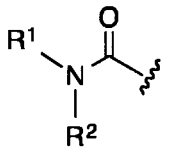
Z^5

wherein R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and n is 1, 2, 3 or 4,

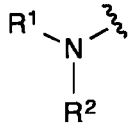


Z^6

wherein R^1 and R^2 are long hydrocarbyl, X is O or NH, and n is 1, 2, 3 or 4, or



Z^7



Z^8

wherein R^1 and R^2 are long hydrocarbyl.

The lipid moiety contains at least two long hydrocarbyl chains such as found in fatty acids, e.g. as in Z^1 to Z^8. One preferred lipid moiety is a phospholipid of various types, e.g. of formula Z^1 or Z^2, that possess either ester- or ether-linked extended alkyl or alkenyl chains, such as either enantiomer of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, or achiral analogues such as 1,3-dipalmitoyl-glycero-2-phosphoethanolamine. A preferred lipid moiety is a tri- or di-palmitoyl-S-glycerylcysteinyl residue (type Z^3) or lipid moieties of types Z^4 to Z^8. Most preferred are the lipid moieties described in the Examples.

The peptide chain (PC) is covalently linked to the lipid moiety (LM) at or near one terminus, i.e. the N terminus or the C terminus, preferably the N terminus. The lipid moiety may be directly attached as in

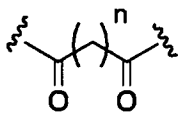
LM – PC (1)

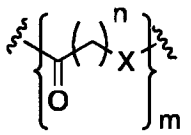
or *via* a linker (L) as in

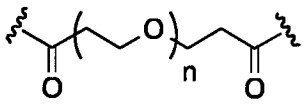
LM – L – PC (2).

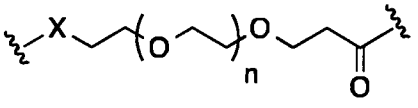
If the peptide chain (PC) and the lipid moiety (LM) are directly linked, this is preferably accomplished through an amide bond between a lipid moiety carbonyl function and an amino function, e.g. the N terminal amino function, of the peptide chain (PC). Particular lipid moieties Z^1, Z^2 and Z^8 are preferably connected through an amide bond between their amine function and a carboxy function, e.g. the C terminal carboxy function, of the peptide chain (PC).

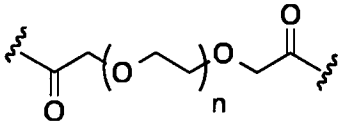
It will be apparent to those knowledgeable in this area, that a large variety of suitable linkers and coupling strategies exist, which include but are not limited to linkers based on dicarboxylic acid derivatives, linkers containing one or multiple ethylene glycol units, amino acid residues (including α-, β-, γ-, δ-amino acids), or sugar (carbohydrate) units, or containing heterocyclic rings. Particular linkers considered are linkers L^1 to L^{16}, wherein n is between 1 and 45 and m is between 1 and 45, for example wherein n is between 1 and 20 and m is between 1 and 20, shown with the connecting functional group C=O and/or X wherein X is O or NH:

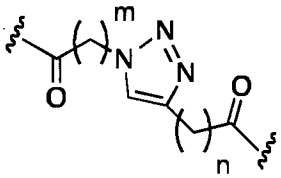

L1

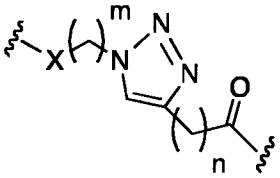

L2

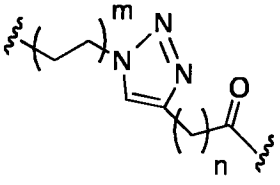

L3

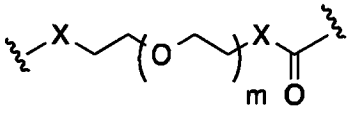

L4

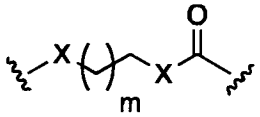

L5

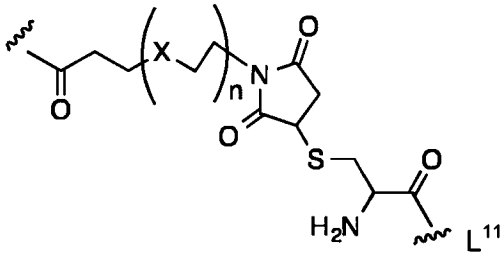

L6

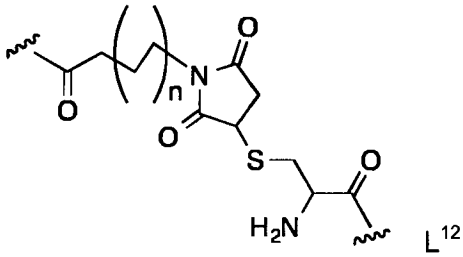

L7

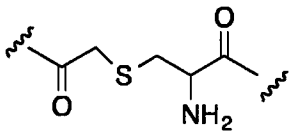

L8

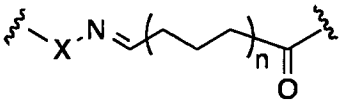

L9

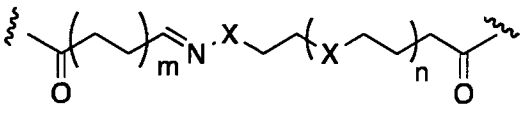

L10

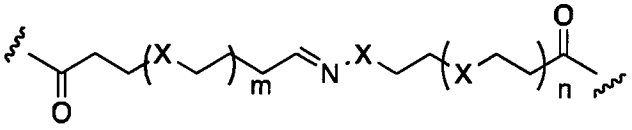

L11


L12


L13


L14


L15


L16

Most preferred are the linkers described in the Examples.

Particular linkers L^1 to L^{16} may be connected to LM and PC as follows:

A carbonyl function shown for L^1 to L^{16} may be connected to an amino function of a suitable lipid moiety (LM) and/or an amino function, e.g. the N terminal amino function, of the peptide chain (PC) through an amide bond. Alternatively a carbonyl function shown for L^1 to L^{16} may be connected to a lipid moiety (LM) by replacement of the corresponding carbonyl function in particular lipid moieties Z^3 to Z^7.

A functional group X shown for L^1 to L^{16} (with the meaning NH or O) may be connected to a carbonyl function of a suitable lipid moiety (LM) and/or a carboxy function, e.g. the C terminal carboxy function, of the peptide chain (PC) through an amide bond (for X = NH) or through an ester bond (for X = O).

The terminal CH_2 group of L^8 may be connected to an amino function of a suitable lipid moiety (LM), an amino function, e.g. the N terminal amino function, of the peptide chain (PC), or a carbonyl function of a suitable lipid moiety (LM).

"Near one terminus" as understood in this connection means that the lipid moiety or the linker is bound to the first, second, third, fourth or fifth amino acid calculated from the N terminal or C terminal end, respectively, of the peptide. The lipid moiety may be attached, directly or through a linker, to the backbone of the peptide structure or to the side chain of one of these amino acids near to the terminus.

"Coiled-coil domains" are designed by careful selection of appropriate amino acid sequences that form a thermodynamically stable, alpha-helical, parallel bundle of helices by spontaneous self-association.

A coiled-coil domain includes peptides based on canonical tandem heptad sequence repeats that form right handed amphipathic α-helices, which then assemble to form helical bundles with left-handed supercoils. Also included are peptides built from non-canonical, non-heptad-based repeats that form coiled-coils that are not necessarily left-handed or even regular supercoils.

Canonical coiled-coils occur widely in naturally occurring biologically active peptides and proteins, and have also been designed *de novo*. A set of rules has been elucidated for designing coiled-coil peptides that adopt helical bundles of defined oligomerization state, topology and stability (e.g. dimer, trimer, tetramer, pentamer, hexamer or heptamer).

These rules allow designers to build a peptide sequence compatible with a given target structure. Most important, the sequences of canonical coiled-coil peptides contain a characteristic seven-residue motif, which is repeated typically 3-10 times. The positions within one heptad motif are traditionally denoted *abcdefg*, with mostly (but not exclusively) hydrophobic residues occurring at sites *a* and *d* and generally polar, helix-favoring residues elsewhere. Tandem heptad motifs along a peptide chain have an average separation between the *a* and *d* residues that allows them to fall on one face of the alpha-helix. When two or more helices pack together into a coiled-coil bundle the hydrophobic faces of the helices associate and wrap around each other in order to maximize contacts between hydrophobic surfaces. The type of residue that may occur at each position within a heptad repeat will influence the stability and oligomerization state of the helical bundle. In general, mostly hydrophobic residues (Ala, Ile, Leu, Met, Val), or aromatic hydrophobic side chains (Phe, Trp and Tyr), are used at the *a* and *d* sites. The remaining *b*, *c*, *e*, *f* and *g* sites tend to be more permissive than the *a* and *d* sites, though polar and helix-favoring residues (Ala, Glu, Lys and Gln) are favored. The choice of residues at the *a* and *d* sites can influence the oligomerization state of the coiled coil (i.e. dimer *vs.* trimer). Thus, dimers are favored when non-β-branched residues (e.g. Leu) occur at the *d* positions; at these sites β-branched residues (Val and Ile) disfavor dimers. On the other hand, in dimers β-branched residues (Ile, Val) are preferred at the *a* sites. Another rule is that *a* = *d* = Ile or Leu favors trimers, which is useful in designing coiled coils that specifically form parallel trimers. These and other design rules are discussed in more detail in Woolfson, D.N., Adv. Prot. Chem. **2005**, *70*, 79-112.

The heptad motif codes for amphipathic alpha-helices that oligomerize through their hydrophobic faces. The coiled-coil domain includes at least three tandem heptad repeat motifs. The upper number of heptad repeats in each chain will influence the stability of the helical bundle. It is limited mainly by the feasibility of chemical synthesis of long peptides, but sequences containing more than three heptad repeats (e.g. four, five, six, seven and eight heptad repeats) are preferred. Examples discussed below form trimeric alpha-helical coiled-coils, but the invention likewise concerns dimeric, tetrameric, pentameric, hexameric and heptameric coiled-coil domains.

Coiled-coil domains according to the invention may have longer repeat units, for example 11-residue repeats and 15-residue repeats such as are present in naturally occurring coiled-coils. Thus the helical bundles required for the formation of aggregate structures may also arise when using coiled-coil motifs with periodicities other than seven. Coiled coils with unusual periodicities are also possible. In many naturally occurring coiled-coils

the unbroken heptad repeat pattern may contain various discontinuities. Two common discontinuities are insertions of one residue into the heptad pattern, as well as insertions of three or four residues. For example, a one residue insertion is seen in the trimeric coiled coil of influenza hemagglutinin. Other naturally occurring coiled coils display a periodicity other than seven, for example, the regular periodicity of 11 residues (termed hendecads) found in the surface layer protein tetrabrachion of *Staphylothermus marinus*.

Other examples of coiled-coil peptide sequences occurring naturally in viral coat proteins are coiled-coil motifs forming trimeric helical bundles in the gp41 coat protein of HIV-1, and the F-glycoprotein of RSV. These coiled-coil domains are included in the definition of coiled-coil domain according to the invention.

The preferred coiled-coil peptides should contain between 3-8 tandemly linked heptad motifs. The heptad motifs within the coiled-coil may have identical sequences, or they may each have different sequences. In all cases, the seven positions of the seven amino acid residues within one heptad motif are designated with letters: *a b c d e f g*. The coiled-coil peptide, therefore, comprises an amino acid sequence having the positions $(abcdefg)_{3-8}$.

Preferred are coiled-coil peptide sequences containing between 3-8 tandemly linked heptad motifs, wherein positions *a* and *d* in each heptad motif (*abcdefg*) contain alpha-amino acids belonging to the Group 1 and/or to the Group 2 as defined hereinbelow. In addition, not more than *two* of all the *a* and *d* positions may be occupied by any amino acid residue belonging to the Group 3, and not more than *one* of all the *a* and *d* positions may be occupied by any amino acid residue belonging to the Group 4 or Group 5 or by glycine. In addition, in positions *b*, *c*, *e*, *f* and *g*, alpha-amino acids belonging to the Groups 3, 4 and 5 are preferred, but amino acids belonging to the Groups 1 and 2 are allowed, with the addition that not more than one of these positions within any one heptad motif may be glycine, but none may be proline.

Group 1 comprises alpha-amino acid residues with small to medium sized hydrophobic side chains. A hydrophobic residue refers to an amino acid side chain that is uncharged at physiological pH and that is repelled by aqueous solution. These side chains generally do not contain hydrogen bond donor groups, such as primary and secondary amides, primary and secondary amines and the corresponding protonated salts thereof, thiols, alcohols, ureas or thioureas. However, they may contain hydrogen bond acceptor groups such as ethers, thioethers, esters, tertiary amides, or tertiary amines. Genetically encoded amino acids in this group include alanine, isoleucine, leucine, methionine and valine.

Group 2 comprises amino acid residues with aromatic or heteroaromatic side chains. An aromatic amino acid residue refers to a hydrophobic amino acid having a side chain containing at least one ring having a conjugated aromatic π(pi)-electron system. In addition it may contain additional hydrophobic groups such as lower alkyl, aryl or halogen, hydrogen bond donor groups such as primary and secondary amines, and the corresponding protonated salts thereof, primary and secondary amides, alcohols, and hydrogen bond acceptor groups such as ethers, thioethers, esters, tertiary amides or tertiary amines. Genetically encoded aromatic amino acids include phenylalanine and tyrosine. A heteroaromatic amino acid residue refers to a hydrophobic amino acid having a side chain containing at least one ring having a conjugated aromatic π-system incorporating at least one heteroatom such as O, S and N. In addition such residues may contain hydrogen bond donor groups such as primary and secondary amides, primary and secondary amines and the corresponding protonated salts thereof, alcohols, and hydrogen bond acceptor groups such as ethers, thioethers, esters, tertiary amides or tertiary amines. Genetically encoded heteroaromatic amino acids include tryptophan and histidine.

Group 3 comprises amino acids containing side chains with polar non-charged residues. A polar non-charged residue refers to a hydrophilic side chain that is uncharged at physiological pH, but that is not repelled by aqueous solutions. Such side chains typically contain hydrogen bond donor groups such as primary and secondary amides, primary and secondary amines, thiols, and alcohols. These groups can form hydrogen bond networks with water molecules. In addition, they may also contain hydrogen bond acceptor groups such as ethers, thioethers, esters, tertiary amides, or tertiary amines. Genetically encoded polar non-charged amino acids include asparagine, cysteine, glutamine, serine and threonine.

Group 4 comprises amino acids containing side chains with polar cationic residues and acylated derivatives thereof, such as acylamino-derived residues and urea-derived residues. Polar cationic side chains refer to a basic side chain, which is protonated at physiological pH. Genetically encoded polar cationic amino acids include arginine, lysine and histidine. Citrulline is an example for a urea-derived amino acid residue.

Group 5 comprises amino acids containing side chains with polar anionic residues. Polar anionic refers to an acidic side chain, which is deprotonated at physiological pH.

Genetically encoded polar anionic amino acids include aspartic acid and glutamic acid. A particular polar cationic residue is $-(CH_2)_aCOOH$ wherein a is 1 to 4.

More preferred are coiled-coil peptide sequences containing between 3 to 8 tandemly linked heptad motifs, wherein each heptad motif (*abcdefg*) may have any one of the following sequences:
1xx1xxx (referring respectively to the positions *abcdefg*);
1xx2xxx (referring respectively to the positions *abcdefg*);
2xx1xxx (referring respectively to the positions *abcdefg*); or
2xx2xxx (referring respectively to the positions *abcdefg*);
wherein *1* is a genetically encoded amino acid from Group 1, *2* is a genetically encoded amino acid from Group 2, and wherein *x* is a genetically encoded amino acid from Groups 1, 2, 3, 4 or 5 or glycine.

Equally preferred are coiled-coil peptide sequences identified in naturally occurring peptides and proteins, but excluding those of human origin. These are, for example, coiled-coils identified in viral and bacterial proteins.

The invention also relates to synthetic virus-like particles carrying PR peptide antigens, and to a method of preparing such synthetic virus-like particles involving dissolving the lipopeptide building blocks in a suitable carrier, preferably an aqueous buffer system (e.g. buffered saline or unbuffered saline). The solvent may be removed after preparation of the synthetic virus-like particles, for example by lyophilization or spray drying.

The invention further relates to a method of eliciting an immune response wherein an immunogenically effective amount of a synthetic virus-like particle carrying PR peptide antigens as described herein is administered to an animal. Any animal can be used, although warm-blooded animals, especially humans are considered here the most.

The invention also relates to a vaccine (or likewise to any other pharmaceutical preparation or medicine) comprising as principal or further active ingredient one or more synthetic virus-like particles carrying PR peptide antigens, alone or in combination with a pharmaceutically acceptable carrier.

The vaccine may also comprise one or more adjuvants such as a mineral salt (e.g. aluminium hydroxide, aluminium phosphate, aluminium sulfate, calcium phosphate), monophosphoryl lipid A (MPL), plant extracts containing saponins (e.g. QS-21), imidazo-

quinolines (e.g. Imiquimod), muramyl dipeptides and tripeptides, lipopeptides, oil-in-water emulsions (e.g. Montanide ISA 720), cytokines (e.g. IL-2 or GM-CSF), mycobacterial and bacterial derivatives (e.g. Freund's complete adjuvant), BCG, nucleic acid derivatives (e.g. polyIC) and other adjuvants known to those skilled in the art.

Some components of the vaccine may also be encapsulated in or attached to bio-degradable polymers, which may for example be useful for controlled release, for example polylactic acid, poly-epsilon-caprolactone, polyhydroxybutyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates, and cross-linked or amphipathic block copolymers of hydrogels, or may be formulated in liposomes.

The vaccine is prepared in a manner known *per se*, for example by means of conventional dissolving and lyophilizing processes and/or may comprise excipients, for example preservatives, stabilizers, wetting agents, tonicity adjusting agents and/or emulsifiers, solubilizers, salts for regulating osmotic pressure and/or buffering substances to stabilize the pH.

The vaccine may be in liquid form or solid (e.g. lyophilized) form and can be sterilized by conventional, well-known sterilization techniques or sterile filtered. The resulting aqueous solution can be packaged for use as it is, or lyophilized, spray dried, or the solvent can be removed in another way. The solid form may be combined with a sterile diluent (e.g. water) prior to administration or may be administered as it is. Likewise the vaccine may comprise an emulsion, dispersion or suspension or any other form suitable for the intended route of administration.

The vaccine may be administered by any suitable enteral or parenteral route such as the intranasal, oral, sublingual, intramuscular, intradermal, transdermal, and subcutaneous or transcutaneous route. Other routes are known in the art that could also be employed.

A device may be used for administration such as conventional needles and syringes, micro needles, ballistic devices for administration of solids (e.g. as in WO 99/27961), patches (e.g. as in WO 98/20734), needle free injection systems (e.g. as in WO 01/054539), spray devices and the like, depending on the dose form and administration route. The device may be pre-filled or coated with the vaccine.

The vaccine comprises from approximately 0.05% to approximately 50% of the active ingredient in an appropriate dose form. Unit dose forms for parenteral administration are,

for example, ampoules, pre-filled syringes or vials, e.g. vials containing from about 0.0001 mg to about 0.75 g of the active ingredient in a dose volume between approximately 0.25 ml and 1.5 ml.

The dosage of the active ingredient depends upon the intended recipient (e.g. species), its age, weight, and individual condition, and the administration route. An optimal dosage for a particular active ingredient and a particular target population can be determined by standard studies involving observation of appropriate immune responses in subjects.

The amount of vaccine sufficient to confer immunity to pathogenic *pneumococci* or other Gram-positive bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the desired level of immunity to a disease caused by infection with *pneumococci* or other Gram-positive bacteria.

The vaccine may be administered as a single dose or as two or more doses at adequately spaced time points. The vaccine may also be administered together with other vaccines. For example, the vaccine may be used in prime-boost regimens in combination with other vaccines.

The vaccine is used for prophylactic or therapeutic purposes, or both, for the prevention and/or treatment of bacteraemia and other diseases caused by *S. pneumoniae* infections, such as pneumonia, acute sinusitis, otitis media, meningitis, bacteraemia, septicemia, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis or brain abscesses, or carriage. The vaccine is likewise useful for prophylactic or therapeutic purposes, or both, for the prevention and/or treatment of diseases caused by Group A *Streptococci* or *S. mutans*, such as pharyngitis, pyoderma, rheumatic fever, glomerulonephritis or caries.

For example, the vaccine protects against bacteraemia, pneumonia and meningitis, or against invasive pneumococcal disease (IPD), an infection in which *S. pneumoniae* can be isolated from the blood or another normally sterile site, or against bacteraemia, pneumonia and otitis media. Alternatively the vaccine protects against pharyngitis, pyoderma, rheumatic fever and glomerulonephritis or caries.

The vaccine may be administered to different target populations, including populations that are naive or have failed to respond previously to infection or vaccination, elderly

persons suitably aged (e.g. over 65, 75 or 85 years old), adults at elevated risks such as people working in health institutions, young adults with a risk factor, immune-compromised persons or pediatric populations.

The invention furthermore relates to a method of making a vaccine comprising mixing synthetic virus-like particles carrying PR peptide antigens with synthetic virus-like particles carrying other antigens or antigens not carried by synthetic virus-like particles.

The invention also relates to antibodies to synthetic virus-like particles carrying protective PR peptide antigens, especially to antibodies to PR peptide antigens. Antibodies to PR peptide antigens can cross-react with a broad variety of genetically diverse strains and different capsular serotypes, and extend the median survival time in a model of passive transfer.

Protective antibodies to PR peptide antigens can have opsonophagocytic activity (OPA). Antibodies with OPA may be detected *in vitro* by a suitable assay, for example an opsonophagocytic killing assay (OPKA) using a cell line or whole blood from a donor. Protective antibodies can also mediate protection by other mechanisms that do not involve OPA.

The invention also relates to the use of protective antibodies to PR peptides as described herein for the manufacture of a pharmaceutical preparation or medicine for therapeutic and/or prophylactic purposes and also for the manufacture of a diagnostic kit.

Not all PR peptides may be equally protective as vaccine antigens. Protective PR peptides are preferred. They may be identified by using well-known methods such as challenge studies in a suitable animal model of streptococcal diseases. For example, in a pneumococcal sepsis model, mice may be immunized with synthetic virus-like particles carrying PR peptide antigens as described herein, and subsequently challenged intravenously with a lethal dose of *S. pneumoniae*. In this model mice immunized with synthetic virus-like particles carrying protective PR peptide antigens then have significantly longer survival time compared to that of immunized control mice.

PR peptide antigens can also be derived from other pathogenic streptococci such as *S. pyogenes*, *S. agalactiae*, *S. mutans*, *S. equi*, *S. suis*, *S. dysgalactiae*, *Peptostreptococcus magnus* or other pathogenic Gram-positive bacteria, such as

Staphylococcus aureus, and synthetic virus-like particles carrying these PR peptide antigens are likewise useful for vaccines against these bacteria.

Preferred PR peptide antigen sequences from *S. pneumoniae* PspA and PspC are collected in Table 2 below. Peptide antigen P3 is a synthetic construct combining two sequences.

Table 2

Name	SEQ ID NO:	Sequence
P1	27	PAPKPEQPAEQPKPAPAPQPAPAPKPEKT
P2	28	PKPEQPAPAPKPEQPAKPEKPA
P3	29	PAPKPEQPAEQPKPEQPAPAPKPEQPAKPEKP
P4	30	PAPKPEQPAEQPKPA
P5	31	PAPQPAPAPKPEKT
P6	32	QPAEQPKPAPAPQPAP
P7	33	PAPAPKPEQPAEQPKP
P8	34	PAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P9	35	PAEQPKPAPAPQPAPAPKPEKPAEQ
P10	36	PKPAPAPQPAPAPKPEKPAEQPKPEKT
P11	37	KAEKPAPAPQPEQPAPAPKT
P12	38	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPEQPTPA
P13	39	PAPAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPEQPTPAPKT
P14	40	PEQPAPAPKPEQPAPAPKPEQPTPAPKPEQPTPAPKT
P15	41	PKPEQPTPAPKPEQPTPAPKT
P16	42	PKPEQPAEQPKPAPAPQ
P17	43	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPATPKT
P18	44	PKPEQPAKPEKPAEEPTQPEKPATPKT
P19	45	PAPAPQPAPAPKPAPAPQPEKPAEQPKAEKPA
P20	46	PETPAPAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKSA
P21	47	PAPAPKPEQPAPAPKPEKSA
P22	48	PKPEQPAPAPKPEKSA
P23	49	KAEKPAPAPKPEQPVPAPKT
P24	50	PAPAPKPAPAPQPEKPAPAPAPKPEKSA
P25	51	PAPEQPTEPTQPEKPAEETPAPKPEKPAEQPKAEKT
P26	52	PAPKPEKPAEQPKAEKT
P27	53	PAPAPKPEQPAEQPKPAPAPQPEKPAEEPENPAPAP
P28	54	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P29	55	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P30	56	PAPEQPTEPTQPEKPAEETPAPKPEKPAEQPKAEKT
P31	57	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P32	58	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P33	59	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P34	60	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P35	61	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKAEKPA

P36	62	PQPEQPAPAPKPEQPAPAPKPEQPTPAPKPEHP
P37	63	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPE
P38	64	PAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPP
P39	65	PAPAPAPKPEQPAPAPAPKPEQPAPAPAPKPEQPA
P40	66	PAPAPKPEQPAPAPAPKPEQPAPAPAPKPEQPT
P41	67	PAPAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPE
P42	68	PAPAPKPEQPAEQPKPAPAPQPAPAPKPEKQ
P43	69	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPA
P44	70	PKPEQPTPAPKPEQPTPAPKPEQPTPAPKPEQPT
P45	71	PEKPAPAPEKPAPAPEKPAPA
P46	72	PAPKPAPAPKPAPAPAPKPEKPA
P47	73	PAPAPTPEAPAPAPKP
P48	74	PKPEQPAKPEKPAEEPTQPEKPA
P49	75	PAKPEKPAEEPTQPEKPA
P50	76	PAPAPKPEQPAKPEKPAEEPTQPEKPA
P51	77	PKPEQPAPAPNPEQPAKPEKPAEEPTQPEKPA
P52	78	PKPEQPAPAPAPKPEQPAPAPAPKPEQPA
P53	79	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPA
P54	80	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPA
P55	81	PAPAPQPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPA
P56	82	PAPAPKPEQPTPAPKPEQPTPAPKPEQPAPAPKPEQPAPAPKP
P57	83	PARALQPEQPAPAPKPEQPTPAPKPEQPTPAPKPEQPAPAPKP

In the preferred PR peptides of SEQ ID NO: 27 to 83, one, two or three amino acids may be replaced by other amino acids.

Alternative PR peptide antigen sequences from *S. pneumoniae* and from *S. pyogenes*, *S. agalactiae*, *S. mutans*, *S. suis*, *S. equi*, *S. dysgalactiae*, *Peptostreptococcus magnus* and *Staphylococcus aureus* proteins are collected in Table 3 below.

Table 3

Name	SEQ ID NO:	Sequence[a)]
P58	84	SRLEQPSLQPTPEPSPGPQPAPN
P59	85	RPEEPSPQPTPEPSPSPQPAPSNP
P60	86	HWVPDSRPEQPSPQSTPEPSPSPQPAPNPQPAPSNP
P61	87	PKSNQIGQPTLPNNSLATPSPSLPINPGTSHE
P62	88	PEVTPTPETPEQPGEKAPEKSPEVTPTPETPEQP
P63	89	PEVTPTPETPEQPGEKAPEK
P64	90	PEKSPEVTPTPETPEQP
P65	91	KAPEKSPEVTPTPEMP
P66	92	PGKPAPKTPEVPQKPDTAPHTPKTP
P67	93	KPSAPKAPEKAPAPKAPK
P68	94	PAPKAPKASEQSSNPKAPAPKSAP
P69	95	PGPAGPRGLQGPQGPRGDKGET
P70	96	PQAPSTPEKQPEVPESP
P71	97	PETPDAPSTPKDEPQAP
P72	98	PAPVEPSYEAEPTPPTRTPDQAEPNKPT
P73	99	PTYETEKPLEPAPVEPSYEAEPT
P74	100	KPTAPTKPTYETEKPLKPAPVAPNYEKEPT
P75	101	KPVVPEQPDEPGEIEPIP
P76	102	PEVPSEPETPTPPTPEVPAEPGKPVPPAK
P78	103	KYTPKKPNKPIYPEKPKDKTPPTKPDHS
P79	104	PEKPVEPSEPST
P80	105	KPVEPSEPSTPDVPSNPSNPSTPDVPSTPDVPSNPSTPEVPSNP
P81	106	PQVEPNVPDTPQEKPLT
P82	107	KPLTPLAPSEPSQPSIPETPLIPSEPSVPET
P83	108	PEVKPDVKPEAKPEAKPA
P84	109	KPEAKPEAKPA
P85	110	PDVKPEAKPEAKPDVKPEAK
P86	111	PETPDTPKIPELPQ
P87	112	PDTPQAPDTPHVPESPKTPE

[a)] SEQ ID NO of proteins from *S. pneumoniae*: 84-87; *S. pyogenes*: 88-92, 95; *S. equi*: 93-94, *S. suis*: 96-97; *S. mutans*: 98-100; *S. aureus*: 101-103; *Peptostreptococcus magnus*: 104; *S. dysgalactiae*: 105-107; *S. agalactiae*: 108-112.

In the preferred PR peptides of SEQ ID NO: 84 to 112, one, two or three amino acids may be replaced by other amino acids.

Additional sequences, which, when administered alone, offer limited protective potential, may be conjugated to PR peptide antigens, in particular sequences derived from regions of PspA or PspC, or other proteins, which do not comprise proline in every 3rd or 4th position and/or comprise less than 15% proline, in particular the sequences:

QQAEEDYARRSEEEYNRLPQQQPPKAEKP (non-proline block) (SEQ ID NO:113),
and
AEDQKEEDRRNYPTNTYKTLELEIAESDVEV (helical peptide from PspC)
(SEQ ID NO:114).

Other sequences that may be combined with PR peptide antigens include sequences derived from bacterial surface proteins that do not contain a proline rich region, including:

Sequences from StkP, preferably the C-terminal 79-82 amino acids,
SVAMPSYIGSSLEFTKNNLIQIVGIKEANIEVVEVTTAPAGSAEGMVVEQSPRAGEKVDLN
KTRVKISIYKPKTTSATP (SEQ ID NO:115),
and fragments thereof;

sequences from PsaA, preferably amino acids 250–309:
SLFVESSVDDRPMKTVSQDTNIPIYAQIFTDSIAEQGKEGDSYYSMMKYNLDKIAEGLAK
(SEQ ID NO:116);

sequences from cholesterol dependent cytolysins, such as the 4th domain of Ply, amino acids 360-471:
NGDLLLDHSGAYVAQYYITWNELSYDHQGKEVLTPKAWDRNGQDLTAHFTTSIPLKGNV
RNLSVKIRECTGLAWEWWRTVYEKTDLPLVRKRTISIWGTTLYPQVEDKVEND
(SEQ ID NO:117),
and fragments thereof;

streptococcal polyhistidine triad proteins, for example fragments from the C-terminal half of *S. pneumoniae* PhtD, e.g. the amino acids 680–770:
VEHPNERPHSDNGFGNASDHVRKNKVDQDSKPDEDKEHDEVSEPTHPESDEKENHAG
LNPSADNLYKPSTDTEETEEEAEDTTDEAEIPQV (SEQ ID NO:118),
or the amino acids 771–839:
ENSVINAKIADAEALLEKVTDPSIRQNAMETLTGLKSSLLLGTKDNNTISAEVDSLLALLKE
SQPAPIQ (SEQ ID NO:119),
or fragments thereof.

In the preferred sequences SEQ ID NO: 113–119, one, two or three amino acids may be replaced by other amino acids.

Further sequences include the sequences described in PCT/US2012/022127 or US 2005/0020813 A1. Alternative sequences include sequences described in EP 0280576 A2.

Additional antigens may be combined with synthetic virus-like particles carrying PR peptide antigens. For example, *S. pneumoniae* proteins identified in WO 98/18931, WO 98/18930, US 6,699,703, US 6,800,744, WO 97/43303, and WO 97/37026; Lyt family (LytX), Pht family (PhtX), Sp128, type 1 or type 2 pilus proteins, other streptococcal antigens such as those identified in WO 1993/005155, WO 2002/034771, WO 2002/083859, WO 2002/34771, WO 2003/093306, WO 2004/041157, or WO 2005/002619; or other antigens such as Sp101, Sp130, Sp125 or Sp133, may be combined with synthetic virus-like particles carrying pneumococcal PR peptide antigens.

Saccharide antigens may also be combined with synthetic virus-like particles carrying PR peptide antigens, such as capsular saccharides of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 14, 18C, 19A, 19F, 22F, 23F or 33F. Alternatively other saccharides may be combined with PR peptide antigens, such as saccharides derived from other *S. pneumoniae* serotypes, and/or saccharides from other Gram-positive bacteria (e.g. saccharides derived from *S. agalactiae, S. pyogenes, and/or S. aureus*).

Likewise proteins from other Gram-positive bacteria may be combined with synthetic virus-like particles carrying PR peptide antigens, e.g. one or more proteins from *S. pyogenes*, including M protein, fibronectin binding protein (Sfbl), Streptococcal heme-associated protein (Shp), or proteins identified in Streptolysin S (SagA), and/or one or more proteins from *S. aureus*, such as Alpha-toxin, Clumping factor A (ClfA), Collagen binding protein (CNA), Fibronectin-binding protein A (FbA), Extracellular Fibrinogen-binding Protein (Efb), Iron regulated surface determinant (Isd) proteins, Penicillin binding protein 2a (PBP2a), Serine Aspartate repeat proteins (Sdr) and/or binder of IgG (Sbi). Likewise also peptide antigens derived from such proteins may be combined with synthetic virus-like particles carrying PR peptide antigens.

Examples

Abbreviations:

Boc, t-butoxycarbonyl;
BSA, bovine serum albumin;
DIEA, diisopropylethylamine;
DMF, N,N-dimethylformamide;
EDT, ethanedithiol;
Fmoc, 9-fluorenylmethoxycarbonyl;
HATU, 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
HBTU, 2-[1H-benzotriazole-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate;
HOBt, N-hydroxybenzotriazole;
Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl;
NMP, N-methylpyrrolidone;
MBHA, methylbenzhydrylamine;
OD, optical density;
iPr_2O, diisopropylether;
PCR, polymerase chain reaction
PyBOP, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium-hexafluorophosphate;
PEO6, N-Fmoc-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoic acid
r.t., room temperature;
RP-HPLC, reversed-phase high performance liquid chromatography;
TA, thioanisole;
TIS, triisopropylsilane;
Trt, trityl;
TFA, trifluoroacetic acid;
TFE, 2,2,2-trifluoroethanol;
t_R, retention time;
SD, standard deviation.

Example 1: Design and Synthesis of PR peptides

The proline-rich region of the PspA from a highly virulent serotype 1 clinical isolate SP1577 (Leimkugel et al., *JID*, **2005**, *192*, 192-199) was amplified and sequenced using two primers (LSM13 and SKH2) according to Hollingshead, Becker et al., *Infect Immun*, **2000**, *68*, 5889-5900.

LSM13: 5`-GCAAGCTTATGATATAGAAATTTGTAAC-3` (SEQ ID NO:120)
SKH2: 5`-CCACATACCGTTTTCTTGTTTCCAGCC-3` (SEQ ID NO:121)

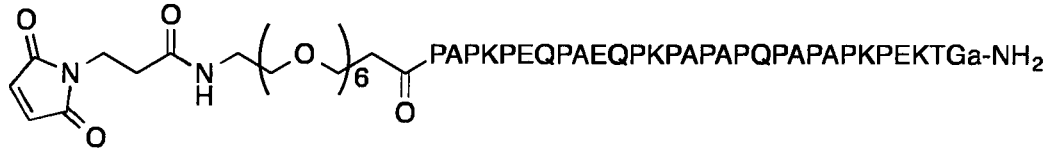
Amplification of the proline-rich region was carried out by PCR using the primers LSM13 and SKH2 and GoTaq Polymerase (PCR conditions: Annealing 48°C for 1 min, Elongation 72°C for 3 min, 30 cycles). The obtained fragments, which were around 1.2 kb in size, were isolated from the PCR reaction and sequenced using the primers LSM13 and SKH2. Around 1'100 bases of the pspA gene could be read. The translated nucleotide sequence is shown below. The proline-rich region, including non-proline block is shown in italics.

XXLGAGFVXX XPTXXXXXEA PVASQXKAEK DXDAXKRDAE NXKKALEEAK XXQKKYEDDQ KKTEEKXKKE KEASKEEQAA NLKYQQELVK YASEKDSVKK AKILKEVEEA EKEHKKKRAE FEKVRSEVIP SAEELKKTRQ KAEEAKAKEA ELIKKVEEAE KKVTEAKQKL DAERAKEVAL QAKIAELENE VYRLETELKG IDESDSEDYV KEGLRAPLQS ELDAKRTKLS TLEELSDKID ELDAEIAKLE KNVEYFKKTD AEQTEQYLAA AEKDLADKKA ELEKTEADLK KAVNEPEKPA EET*PAPAPKP EQPAEQPKPA PAPQPAPAPK PEKTDDQQAE EDYARRSEEE YNRLPQQQPP KAEKPAPAPK PEQPVPAPKT* GWKQENGMWC R (SEQ ID NO:122)

From this sequence the P1 PR Sequence (PAPKPEQPAEQPKPAPAPQPAPAPKPEKT, SEQ ID NO:27) was selected. P1 is located between the helical/coiled-coil region and the non-proline block of the SP1577 PspA.

In order to enable conjugation to SVLP lipopeptides, the following maleimidopeptides were designed and synthesized:

Maleimidopeptide 1:



(1)

In maleimidopeptide **1** 3-maleimidopropionic acid is coupled to the N-terminus in P1 (SEQ ID NO:27) *via* an 21-amino-3,6,9,12,15,18-hexaoxaheneicosan-21-oic acid linker, and a

glycine is added to the C-terminus P1, followed by a D-alanine residue ("a") as the amide ("NH_2") in order to confer stability towards exoproteases.

The synthesis of maleimidopeptide **1** was carried out using Fmoc Solid Phase Peptide Synthesis (SPPS) methods as follows:

The peptide PAPKPEQPAEQPKPAPAPQPAPAPKPEKTGa (SEQ ID NO:27 extended by glycine-D-alanine) was assembled on an ABI 433A peptide synthesizer using Rink Amide MBHA resin (loading: 0.69 mml/g) (362 mg, 0.25 mmol) and standard Fmoc-SPPS protocols. The following amino acids were used (in the correct order): Fmoc-Ala-OH, Fmoc-D-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH and Fmoc-Thr(tBu)-OH. After assembly and removal of the N-terminal Fmoc protecting group, the resin was washed with *N*-methyl-2-pyrrolidone (NMP) and CH_2Cl_2. For coupling of the maleimide, a portion of the resin (ca. 0.1 mmol) was washed with DMF and a solution of Fmoc-PEO6-OH (115 mg, 0.2 mmol) PyBOP (104 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol) and DIEA (66 µl, 0.4 mmol) in 4.5 ml DMF was prepared, mixed for 30 seconds and added to the resin under argon. The mixture was shaken for 16 h. The resin was filtered and washed 4 x with DMF. The Fmoc group was then removed by treatment with 20% piperidine in DMF (6 x 2 min.). The resin was then washed again with DMF and a solution of 3-maleimidopropionic acid (34 mg, 0.2 mmol), PyBOP (104 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol) and DIEA (66 µl, 0.4 mmol) in DMF was prepared and added to the resin under argon. The resin was shaken for 3 h, filtered, washed sequentially 4 times with DMF, CH_2Cl_2 and MeOH, and dried over night *in vacuo* over KOH pellets. For cleavage of the peptide from the resin and removal of side-chain protecting groups, TFA/TIS/TA/phenol 85:5:5:5 (10 ml) was prepared and added to the dry resin under argon atmosphere. The resin was shaken for 3 h, filtered and the maleimidopeptide **1** was precipitated with iPr_2O, pre-chilled to -20°C (50 ml). The peptide was then washed 4 times with iPr_2O, air-dried over night and purified by RP-HPLC using a preparative C18 column (Agilent Zorbax SB300 PrepHT, 250 x 21.5 mm) and a linear gradient of 10 – 40% MeCN in H_2O (+ 0.1% TFA) in 16 min. and lyophilized to afford **1** as a white powder. The peptide was analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 8.53 min.

ESI-MS: MW calculated for $C_{163}H_{259}N_{41}O_{51}$: 3609.1 Da; MW found: 3609.7 (± 0.02%).

Maleimidopeptide **2**:

(**2**)

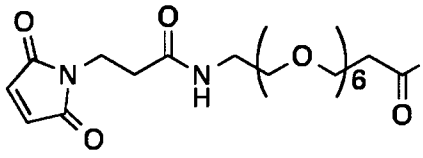
In this maleimidopeptide a glycine is added to the C-terminus in P1 (SEQ ID NO:27) and the maleimide is coupled to the glycine P1 *via* an amino ethyl spacer. The N-terminus is acetylated.

The peptide chain in maleimidopeptide **2** was assembled using Fmoc SPPS on an ABI 433A as described for **1**, except that 2-chlorotrityl resin preloaded with Fmoc-Gly-OH to a resin substitution level of 0.6 mmol/g (416 mg, 0.25 mmol) was used instead of Rink amide MBHA resin as the solid phase support. Following assembly and removal of the N-terminal Fmoc protecting group, the resin was acetylated by treatment with a solution of 0.5 M Ac_2O, 0.05 M HOBt and 0.136 M DIEA in NMP (10 ml) with shaking for 30 min. The resin was then washed 4 times with DMF, 4 times with CH_2Cl_2 and treated with TFE/CH_2Cl_2 2:8 (10 ml) with shaking under argon for 4 h to release the fully side chain protected peptide from the resin. The resin was filtered and washed twice with 10 ml TFE/CH_2Cl_2 2:8, the filtrate was concentrated and the protected peptide was precipitated with 4°C cold Et_2O and washed 4 times with Et_2O. The protected peptide was then dried *in vacuo* over night and stored at -20°C.

For coupling of the maleimide, a portion of the crude side-chain protected peptide (100 mg), HATU (15 mg, 39 µmol), HOAt (5 mg, 39 µmol) were dissolved in DMF (0.8 ml), DIEA (23 µl, 142 µmol) was added and the mixture was stirred for 1 min. A solution of *N*-(2-aminoethyl)maleimide TFA salt (150 mg, 60 µmol) in DMF (0.2 ml) was added and the mixture was stirred for 3 h under argon atmosphere. The DMF was then removed under reduced pressure. The side-chain protected peptide was suspended in 0.3 ml CH_2Cl_2, precipitated with 4°C cold Et_2O, washed 4 times with Et_2O and dried *in vacuo* over night.

The side-chain protecting groups were then removed and the peptide was precipitated and purified as described above for **1** and the final product **2** was analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 7.06 min. MALDI-TOF MS: MW calculated for $C_{146}H_{227}N_{39}O_{43}$: 3216.6 Da; MW found: 3215.7 Da (± 0.05%).

Maleimidopeptide **3**:



QQAEEDYARRSEEEYNRLPQQQPPKAEKPa-NH_2 **(3)**

In maleimidopeptide **3**, 3-maleimidopropionic acid is coupled to the N-terminus of SEQ-ID NO:113 *via* an 21-amino-3,6,9,12,15,18-hexaoxaheneicosan-21-oic acid linker, and the C-terminus is capped with D-alanine ("a") and amidated. SEQ ID NO:113 corresponds to the non-proline block of the P1577 PspA.

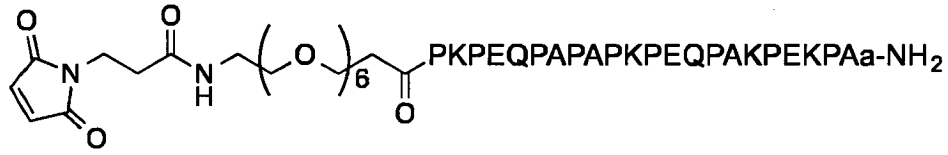
Maleimidopeptide **3** was synthesized and purified as described above for maleimidopeptide **1** and analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 5.31 min. MALDI-TOF MS: MW calculated for $C_{174}H_{272}N_{50}O_{63}$: 4072.3 Da; MW found: 4071.0 Da (± 0.05%).

Other PR sequences can be obtained by sequencing pspA or pspC genes, or, alternatively, may be accessed in public databases, such as UniProtKB. For example, the PspA sequence of serotype 19A isolate TCH8431 (UniProtKB accession no. D6ZPW2) is:

MNKKKMILTS LASVAILGAG FVTSQPTVVR AEESPVASQS KAEKDYDAAV
KKSEAAKKHY EEAKKKAEDA QKKYDEDQKK TEAKAEKERK ASEKIAEATK
EVQQAYLAYL QASNESQRKE ADKKIKEATQ RKDEAEAAFA TIRTTIVVPE
PSELAETKKK AEEAKAEEKV AKRKYDYATL KLALAKKEVE AKELEIEKLQ
YEISTLEQEV ATAQHQVDNL KKLLAGADPD DGTEVIEAKL KKGEAELNAK
QAELAKKQTE LEKLLDSLDP EGKTQDELDK EAEEAELDKK ADELQNKVAD
LEKEISNLEI LLGGADPEDD TAALQNKLAA KKAELAKKQT ELEKLLDSLD
PEGKTQDELD KEAEEAELDK KADELQNKVA DLEKEISNLE ILLGGADSED
DTAALQNKLA TKKAELEKTQ KELDAALNEL GPDGDEEET*P APAPQPEQPA*
PAPKPEQPAP APKPEQPAPA PKPEQPAPAP KPEQPAKPEK PAEEPTQPEK
*PATPKT*GWKQ ENGMWYFYNT DGSMATGWLQ NNGSWYYLNA NGSMATGWVK
DGDTWYYLEA SGAMKASQWF KVSDKWYYVN SNGAMATGWL QYNGSWYYLN
ANGDMATGWL QYNGSWYYLN ANGDMATGWA KVNGSWYYLN ANGAMATGWA
KVNGSWYYLN ANGSMATGWV KDGDTWYYLE ASGAMKASQW FKVSDKWYYV
NGLGALAVNT TVDGYKVNAN GEW (SEQ ID NO:123)

From this sequence the P2 sequence (PKPEQPAPAPKPEQPAKPEKPA, SEQ ID NO:28) was selected. P2 is located immediately after the helical/coiled-coil region of the TCH8431 PspA. In order to facilitate conjugation to SVLP lipopeptides the following maleimido-peptides were designed and synthesized:

Maleimidopeptide **4**



(**4**)

This maleimidopeptide comprises 3-maleimidopropionic acid coupled *via* an 21-amino-3,6,9,12,15,18-hexaoxaheneicosan-21-oic acid linker to the N-terminus of P2 (SEQ ID NO:28). The "a" denotes D-alanine. The C-terminus is amidated.

Maleimidopeptide **4** was synthesized and purified as described above for maleimidopeptide **1** and analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 5.21 min. MALDI-TOF MS: MW calculated for $C_{131}H_{210}N_{32}O_4$: 2889.3 Da, MW found: 2888.8 Da (± 0.05%).

Maleimidopeptide **5**

(**5**)

In this maleimidopeptide a glycine is added to the C-terminus of P2 (SEQ ID NO: 28), and the maleimide is coupled to the glycine *via* an amino ethyl spacer. The N-terminus is acetylated.

Maleimidopeptide **5** was synthesized and purified as described above for maleimidopeptide **2** and analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 6.31 min. ESI MS: MW calculated for $C_{113}H_{178}N_{30}O_{33}$: 2484.8 Da; MW found: 2483.2 Da (± 0.02%).

Maleimidopeptide **6**

Artificial PR sequences can be generated by fusing short PR sequences from two or more distinct PspA proteins. For example the sequence P3 (SEQ ID NO: 29) was designed by fusing the N-terminal residue PAPKPEQPAEQ (SEQ ID NO: 124) in P1 to P2 (SEQ ID NO: 28) and replacing the C-terminal Ala in P2 by a Gly residue. In order to enable conjugation the following maleimidopeptide was designed and synthesized:

6

(**6**)

Maleimidopeptide **6** was synthesized and purified as described above for maleimidopeptide **2** and analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 10 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 10.11 min. MALDI MS: MW calculated for $C_{164}H_{255}N_{45}O_{50}$: 3657.1 Da; MW found: 3654.9 Da (± 0.05%).

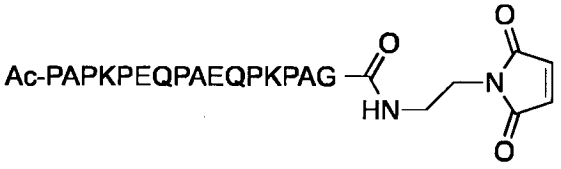
Further examples for PR peptide antigen are described below:

Maleidmidopeptide **7**

(**7**)

Maleimidopeptide **7** was synthesized and purified as described above for maleimidopeptide **2**. ESI MS: MW calculated for $C_{91}H_{137}N_{25}O_{27}$: 2012.0 Da; MW found: 2012.4 Da (± 0.05%).

Maleimidopeptide **8**



(**8**)

Maleimidopeptide **8** was synthesized and purified as described above for maleimidopeptide **2.** ESI-MS: MW calculated for $C_{81}H_{124}N_{22}O_{25}$: 1804.9 Da; MW found: 1805.4 Da (± 0.05%)

Maleimidopeptide **9**

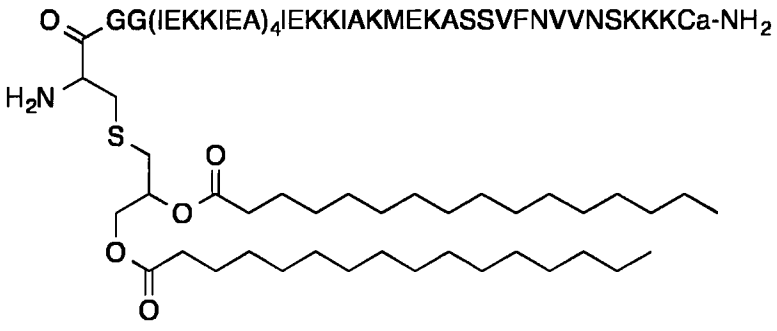
(**9**)

This maleimidopeptide is derived from the PR peptide of PhtD (P60, SEQ ID NO:86). The N-terminus is acetylated. The maleimidopeptide was synthesized and purified as described above for maleimidopeptide **2** and analyzed by analytical RP-HPLC using an Agilent XDB-C18 column (250 x 4.6 mm) and a linear gradient of 20 – 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 3.41 min. ESI-MS: MW calculated for $C_{136}H_{201}N_{37}O_{48}$: 3120.4 Da; MW found: 3120.6 Da (± 0.05%).

Example 2: Conjugation of PR Peptide Antigens to Lipopeptides

In order to prepare lipopeptide conjugates for immunizations the following four lipopeptide building blocks were synthesized.

Lipopeptide Building Block **10**

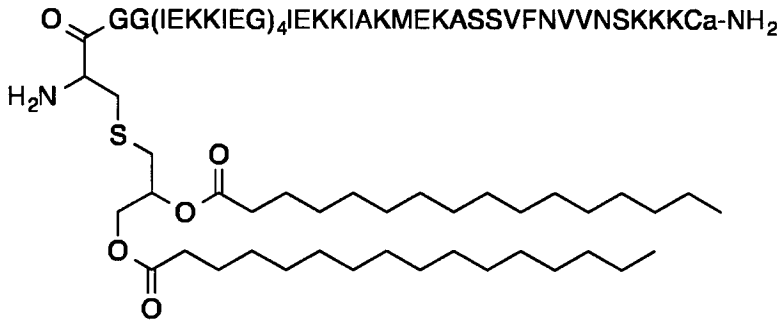


(**10**)

This lipopeptide corresponds to Example 13 in WO 2008/068017. The synthesis was carried out and the product was characterized as described in WO 2008/068017 and Ghasparian, Riedel et al., *Chembiochem*, **2011**, *12*, 100-109. Analytical RP-HPLC

(Interchrom UP5WC4-25QS, 25 to 100% MeCN in H_2O (+ 0.1% TFA) over 25 min.): Purity > 96%, t_R = 22.71 min. MALDI-TOF: MW calculated for $C_{312}H_{552}N_{74}O_{85}S_3$: 6796.4 Da; MW found: 6798.2 Da (± 0.05%).

Lipopeptide Building Block **11**

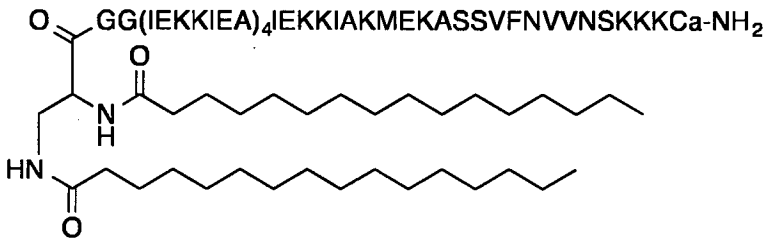


(**11**)

This lipopeptide building block contains a modified coiled-coil domain, which has Gly in the "*c*" positions of the heptad repeat *"defgabc"* IEKKIEG (SEQ ID NO:125).

The modified lipopeptide building block was synthesized and purified as described in WO 2008/068017. Analytical RP-HPLC (Interchrom UP5WC4-25QS, 25 to 100% MeCN in H_2O (+ 0.1% TFA) over 25 min.): Purity > 98%, t_R = 21.41 min. ESI-MS: MW calc. for $C_{308}H_{544}N_{74}O_{85}S_3$ 6740.3 Da; found 6741.7 Da.

Lipopeptide Building Block **12**



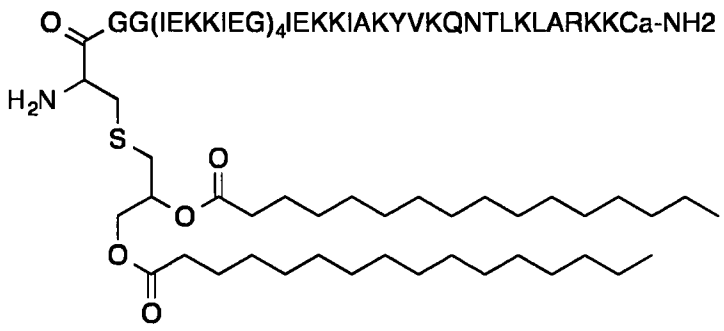
(**12**)

This lipopeptide building block contains a modified lipid *N,N'*-dipalmitoyl.-2,3-diamino-propionamide ("Pam_2Dap"). "a" denotes D-alanine.

The lipopeptide building block was synthesized and purified as described in WO 2008/068017, except that Pam2Dap was incorporated at the end of the synthesis instead of Pam2Cys. The lipopeptide was analyzed by analytical HPLC and MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to

100% B in 25 min.): Purity: >95%. t_R = 22.1 min. ESI-MS: MW calc. 9594.5 Da, found 9596.17 Da.

Lipopeptide Building Block **13**



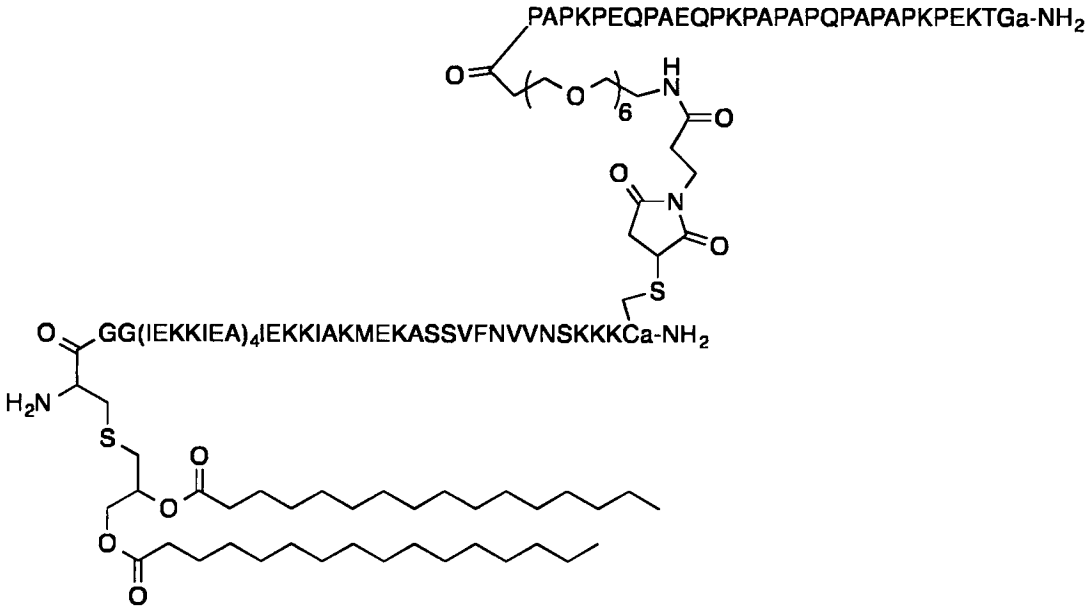
(**13**)

This lipopeptide building block contains a promiscuous T-helper epitope (KYVKQNTLKLARK, SEQ ID NO:126) derived from a HLA-DRB 101 restricted epitope from Influenza hemagglutinin residues 307-309 (SEQ ID NO:19) (Stern, LJ. et al. *Nature* **1994**, *368*, 215). "a" denotes D-alanine.

The lipopeptide building block was synthesized and purified essentially as described in WO 2008/068017 and analyzed by analytical HPLC and MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100%B in 25 min.): Purity: >95%. t_R = 22.35 min. ESI-MS: MW calc. for $C_{302}H_{538}N_{74}O_{79}S_2$: 6530.0 Da; found 6530.4 Da (± 0.05%).

The following conjugates were synthesized:

Conjugate **14** (Maleimidopeptide **1** + Lipopeptide **10**)

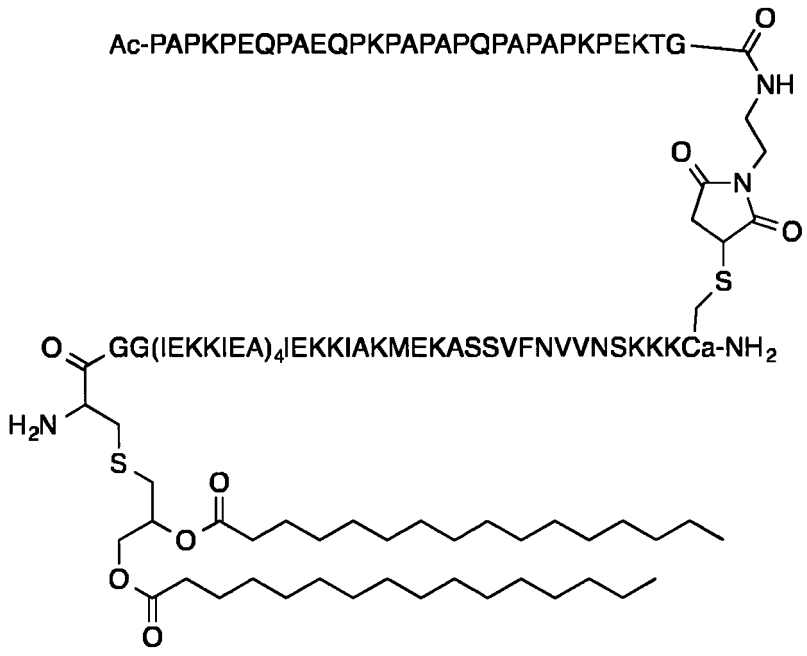


(14)

The conjugation of **1** to **10** was performed essentially as described in WO 2008/068017. To a solution of **10** (6.0 mg, 0.9 µmol) in H_2O/MeCN 1:1 (3 ml) was added a solution of **1** (4.8 mg, 1.3 µmol) in H_2O/MeCN 1:1 (2.4 ml). The pH was carefully adjusted and maintained at pH 6.5 – 7.0 with 0.1 NaOH, and the mixture was stirred for 2 h at room temperature. The conjugate was then purified by RP-HPLC using a C4 preparative column (Interchrom UP5WC4-25M, 250 x 10 mm) and a gradient of 50 to 100% MeCN in H_2O (+ 0.1% TFA) in 17 min. The conjugate **14** was analyzed by analytical RP-HPLC using an Interchrom UP5WC4-25QS column (250 x 4.6 mm) and a gradient of 20 to 100% MeCN in H_2O (+ 0.1 % TFA) in 25 min: Purity > 97%; t_R = 22.34 min. MALDI-TOF MS: MW calc. $C_{475}H_{811}N_{115}O_{136}S_3$: 10406.6 Da; found 10407.8 Da (± 0.1%).

The conjugate was suspended in PBS, equilibrated for 30 min., diluted to 0.5 mg/ml and analyzed by Dynamic Light Scattering (DLS) on a Wyatt DynaPro Titan instrument at 4°C, 25°C and 37°C using a laser intensity of 400'000 counts/s and an acquisition time of 10 s. The size distribution by regularization analysis was monomodal and the size dispersity was small. The mean hydrodynamic radius (R_h) was 12.0 nm, and % Pd value 12.3% at 25°C. Similar values for R_h and % Pd were obtained at other temperatures.

Conjugate **15** (Maleimidopeptide **2** + Lipopeptide **10**)

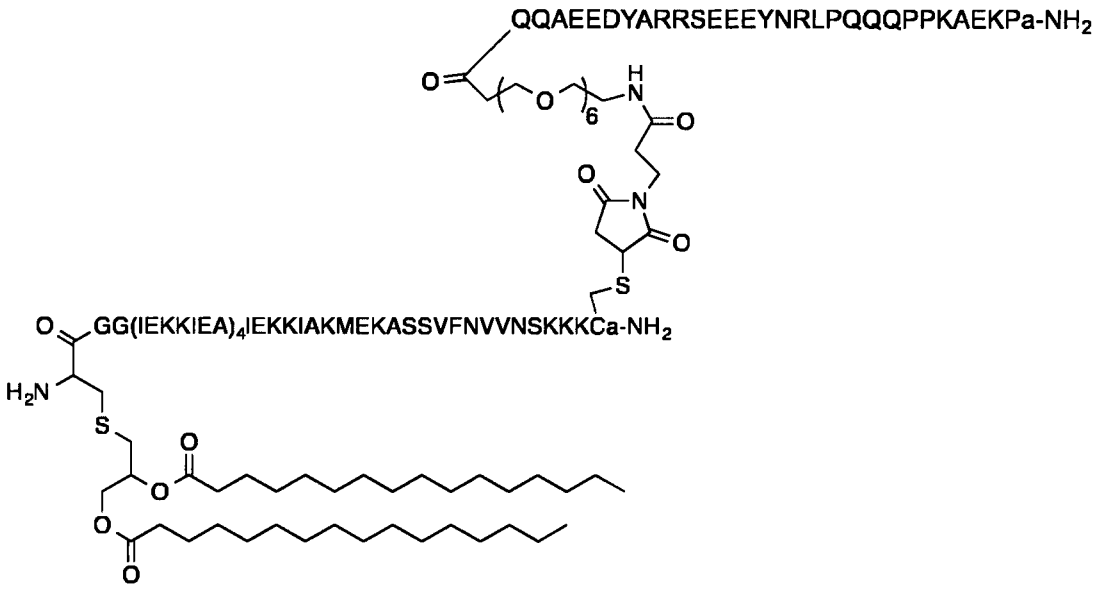


(15)

The conjugation of **2** to **10** and purification of the conjugate was performed essentially as described above for conjugate **14**. Product **15** was analyzed by analytical RP-HPLC using an Interchrom UP5WC4-25QS column (250 x 4.6 mm) and a gradient of 20 to 100% MeCN in H_2O (+ 0.1% TFA) in 25 min: Purity > 97%; t_R = 22.41 min. ESI-MS: MW calc. for $C_{458}H_{779}N_{113}O_{128}S_3$: 10012.9 Da; found 10011.1 Da (± 0.1%).

A suspension of conjugate **12** in PBS was prepared and analyzed using DLS as described above for **14**. R_h values were in the range of 13.2 -14.2 nm, and % Pd values in the range of 12.6 – 18.0% at 25°C.

Conjugate **16** (Maleimidopeptide **3** + Lipopeptide **10**)

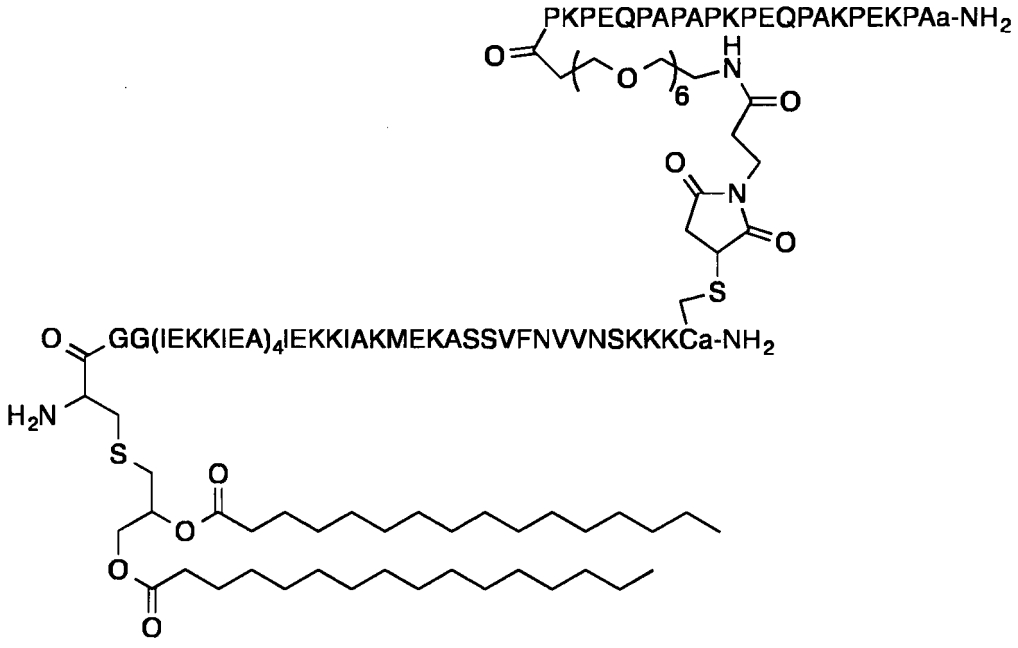


(16)

The conjugation of **3** to **10** and purification of the conjugate was performed essentially as described above for conjugate **14**. Product **16** was analyzed by analytical RP-HPLC using an Interchrom UP5WC4-25QS column (250 x 4.6 mm) and a gradient of 20 to 100% MeCN in H_2O (+ 0.1% TFA) in 25 min: Purity > 97%; t_R = 22.0 min. MALDI-TOF MS: MW calc. for $C_{475}H_{811}N_{115}O_{136}S_3$: 10869.8 Da; found 10'872.3 Da (± 0.1%).

A suspension of conjugate **13** in PBS was prepared and analyzed using DLS as described above for **14**. R_h values were in the range of 14.0 – 15.0 nm, and % Pd values in the range of 13.0 - 13.7%. DLS analysis of a mixture of Conjugate **14** and Conjugate **16** particles yielded an R_h of 12.3 - 13.3 nm and a % Pd values around 25-26%, indicating that mixing the particles did not alter the overall size distribution.

Conjugate **17** (Maleimidopeptide **4** + Lipopeptide **14**)

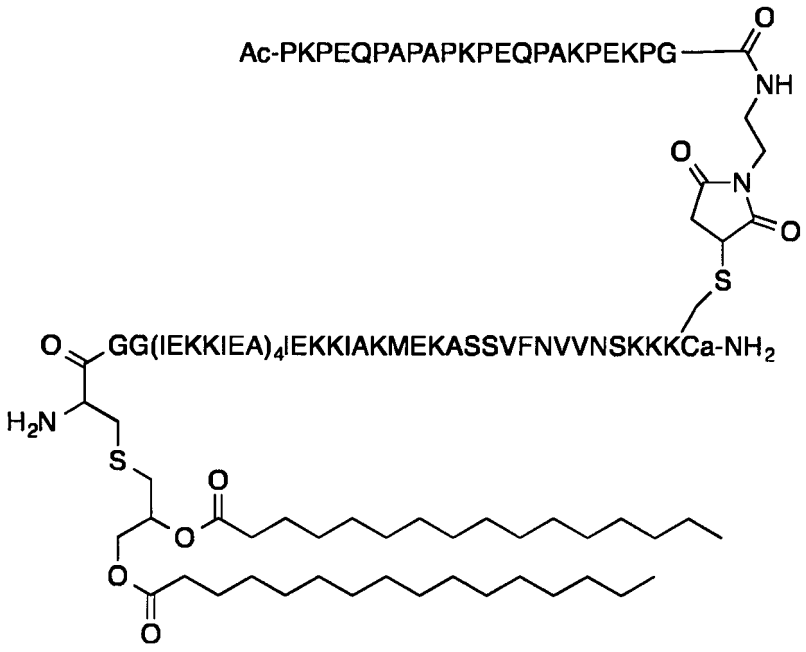


(**17**)

The conjugation of **4** to **10** and purification of the conjugate was performed essentially as described above for conjugate **17**. Product **17** was analyzed by reversed phase HPLC on a C4 analytical column (Interchrom, UP5WC4-25QS, 4.6 mm x 250 mm, 300 Å) and by MALDI-MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >95%. t_R =18.8 min. MALDI-TOF MS: MW calc. for $C_{443}H_{762}N_{106}O_{126}S_3$: 9685.6 Da; found 9686.2 Da (± 0.1%).

A suspension of conjugate **17** in PBS was prepared and analyzed using DLS as described above for **14**. R_h values were in the range of 11.1 - 11.8 nm and % Pd values around 13%.

Conjugate **18** (Maleimidopeptide **5** + Lipopeptide **10**)

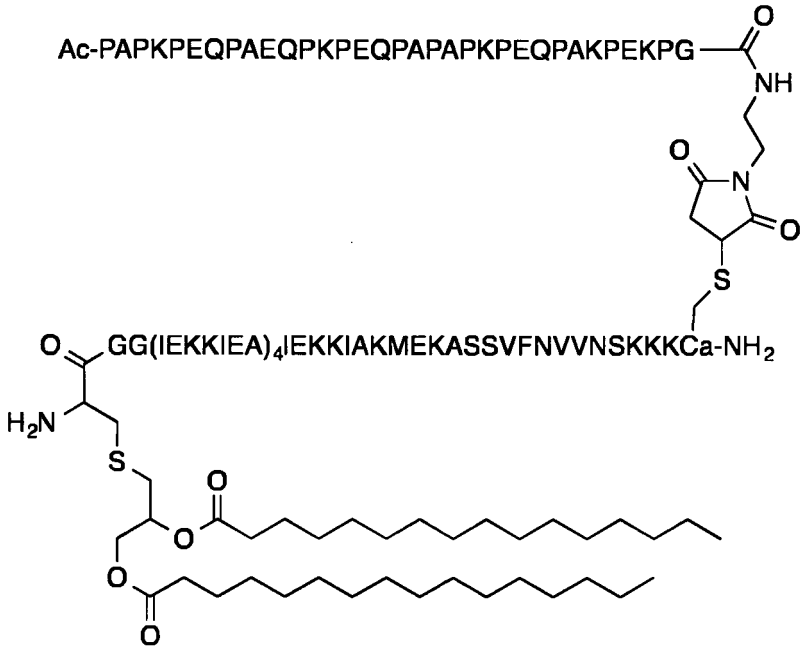


(**18**)

The conjugation of **5** to **10** and purification of the conjugate was performed essentially as described above for conjugate **18**. Product **18** was analyzed by reversed phase HPLC on a C4 analytical column (Interchrom, UP5WC4-25QS, 4.6 mm x 250 mm, 300 Å) and by MALDI-MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >96%. t_R = 22.55 min. MALDI-TOF MS: MW calc. for $C_{425}H_{728}N_{104}O_{118}S_3$: 9279. 2 Da; found 9280.2 Da (± 0.1%).

A suspension of conjugate **18** in PBS was prepared and analyzed using DLS as described above for **14**. R_h values were in the range of 10.0 – 10.5 nm and % Pd values around 16%.

Conjugate **19** (Maleimidopeptide **6** + Lipopeptide **10**)

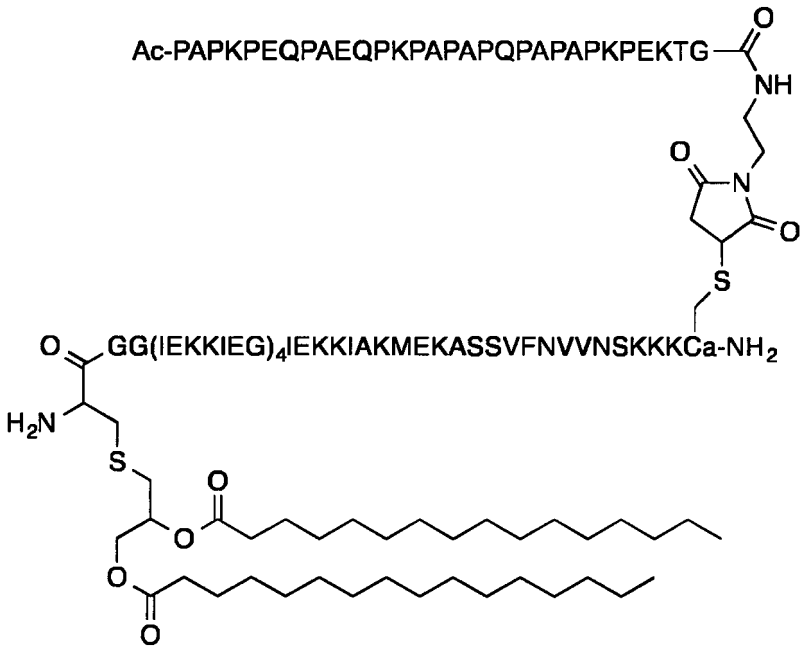


(**19**)

The conjugation of **6** to **10** and purification of the conjugate was performed essentially as described above for conjugate **14**. Product **19** was analyzed by reversed phase HPLC on a C4 analytical column (Interchrom, UP5WC4-25QS, 4.6 mm x 250 mm, 300 Å) and by MALDI-MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >96%. t_R =22.34 min. MALDI-TOF MS: MW calc. for $C_{476}H_{807}N_{119}O_{135}S_3$: 10453.4 Da; found 10452.7 Da (± 0.1%).

A suspension of conjugate **17** in PBS was prepared and analyzed using DLS as described above for **19**. R_h values were in the range of 12.1 - 14.5 nm and % Pd values 12 – 20%.

Conjugate **20** (Maleimidopeptide **6** + Lipopeptide **11**)

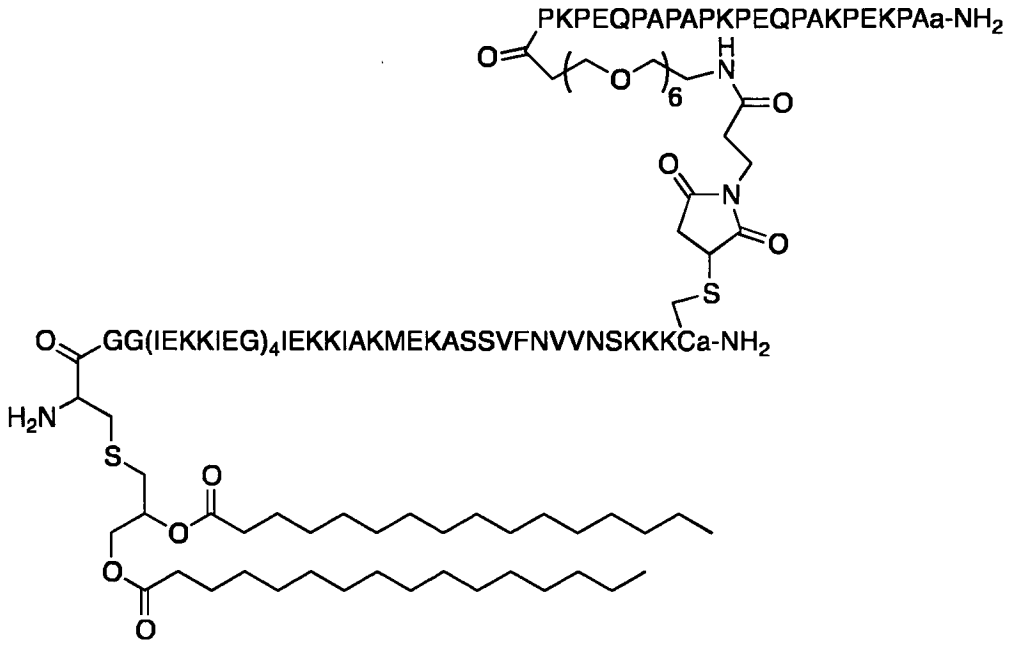


(**20**)

The conjugation of **6** to **11** and purification of the conjugate was performed essentially as described above for conjugate **14**. Product **20** was analyzed by reversed phase HPLC on a C4 analytical column (Interchrom, UP5WC4-25QS, 4.6 mm x 250 mm, 300 Å) and by MALDI-MS. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >98%. t_R =22.34 min. ESI-MS: MW calc. for $C_{454}H_{773}N_{113}O_{129}S_3$ (succinimide ring hydrolysis): 9975.02 Da; found 9974.7 Da (± 0.01%).

A suspension of conjugate **20** in PBS was prepared and analyzed using DLS as described above for **14**. R_h values were in the range of 11.7 - 12.3 nm and % Pd values around 20%.

Conjugate **21** (Maleimidopeptide **4** + Lipopeptide **11**)

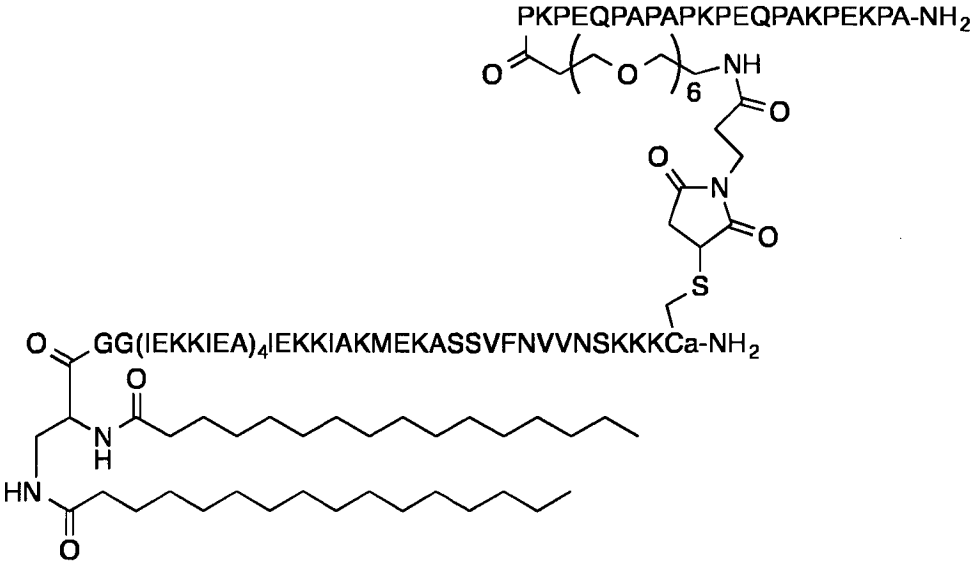


(21)

Maleimidopeptide **4** was conjugated to **11** and the conjugate was purified as described above for conjugate **14**. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >95%. t_R = 17.78 min. MW calc. for $C_{439}H_{753}N_{105}O_{127}S_3$: 9630.5 Da; found 9631.2 Da.

DLS (0.5 mg/ml in PBS, 25°C): R_h = 10.7 nm; % Pd = 12 - 13%.

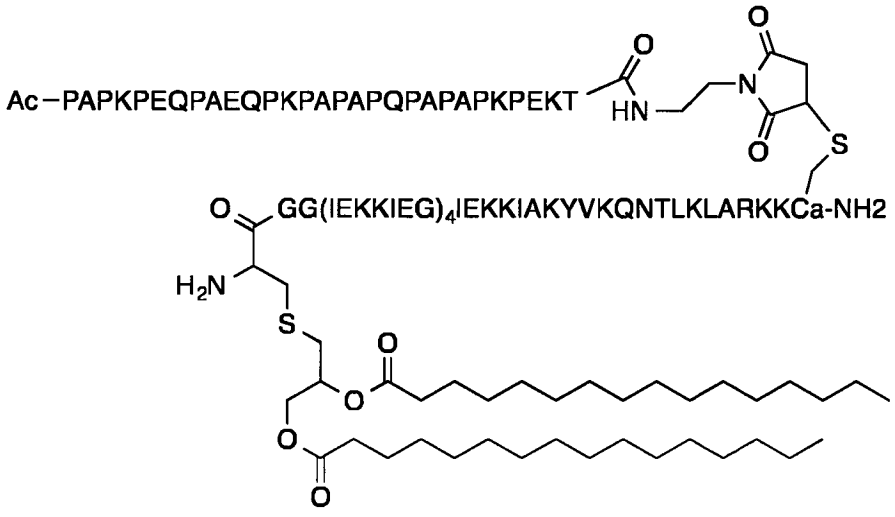
Conjugate **22** (Maleimidopeptide **6** + Lipopeptide **12**)



(22)

The maleimido peptide **4** was conjugated to **12** and the conjugate was purified as described above for conjugate **14**. Analytical RP-HPLC (C4 column, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >95%. t_R = 18.37 min. MALDI-TOF: MW calc. for $C_{440}H_{757}N_{107}O_{124}S_2$,: 9594.5 Da, found 9595.1 (± 0.1%) Da.
DLS measurements (0.5 mg in PBS, 25°) yielded R_h of 10.4 -11.1 nm and % Pd values of 10 - 12%.

Conjugate **23** (Maleimidopeptide **6** + Lipopeptide **13**)

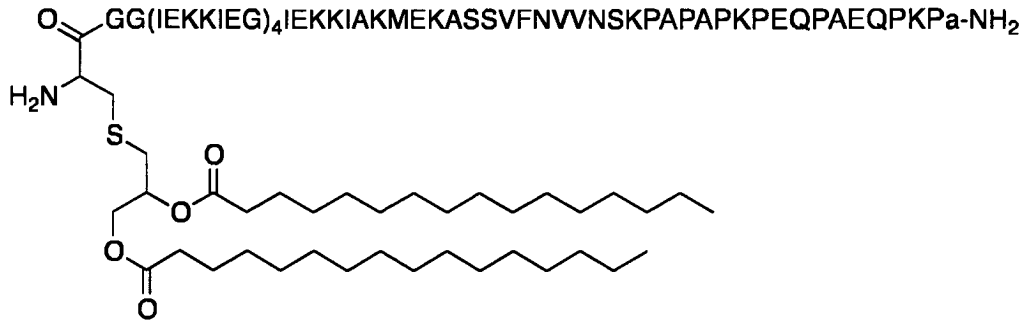


(**23**)

The maleimido peptide **6** was conjugated to **13** and the conjugate was purified as described above for conjugate **14**. Analytical RP-HPLC (C4 column, A = H_2O + 0.1 % TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >95%. t_R = 18.25 min. MALDI-TOF: MW calc. for $C_{448}H_{765}N_{113}O_{123}S_2$ (succinimide ring hydrolysis): 9768.7 Da, found 9767.0 Da (± 0.1%).
DLS measurements (0.5 mg in PBS, 25°) yielded R_h of 9.9 -10.2 nm and %Pd values of 15 - 18%.

Additional Lipopeptides were prepared by fusing the N-terminus of the PR peptide antigen to the C-terminus of the lipopeptide building block. The following fusion lipopeptides were prepared.

Lipopeptide (**24**)

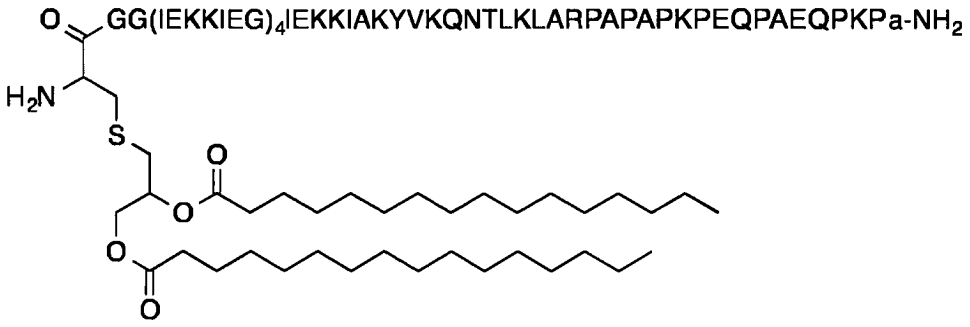


(**24**)

In this example the PR sequence P7, PAPAPKPEQPAEQPKP (SEQ ID NO: 33) was fused directly to the C-terminus of GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSK (SEQ ID NO: 127) to yield the sequence GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSKPAPAPKPEQPAEQPKP (SEQ ID NO: 128). The N-terminus was lipidated by addition of Pam2Cys and D-alanine ("a") was added to the C-terminus in lipopeptide **24.**

The fusion lipopeptide **24** was synthesized using conventional solid-phase peptide synthesis methods (W.C. Chan, P.D. White, Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, Oxford, UK, 2000) and purified as described above for lipopeptide **10**. MALDI-TOF MS: MW calc. for $C_{369}H_{633}N_{89}O_{104}S_2$: 8044.6 Da; found: 8044.6 Da (± 0.1%). Analytical RP-HPLC (Interchrom UP5WC4-25QS, 25 to 100% MeCN in H_2O (+ 0.1% TFA) over 25 min.): Purity > 98%, t_R = 20.48 min.
DLS (0.5 mg/ml in PBS, 25°C): R_h = 10.7 nm; % Pd = 12 - 13%.

Lipopeptide (**25**)



(**25**)

In this example the PR sequence P7, PAPAPKPEQPAEQPKP (SEQ ID NO: 33) was fused directly to the C-terminus of

GG(IEKKIEG)$_4$IEKKIAKYVKQNTLKLAR (SEQ ID NO: 129) to yield the sequence GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSKPAPAPKPEQPAEQPKP (SEQ ID NO: 130). The N-terminus was lipidated by addition of Pam2Cys and D-alanine ("a") was added to the C-terminus in lipopeptide **25.**

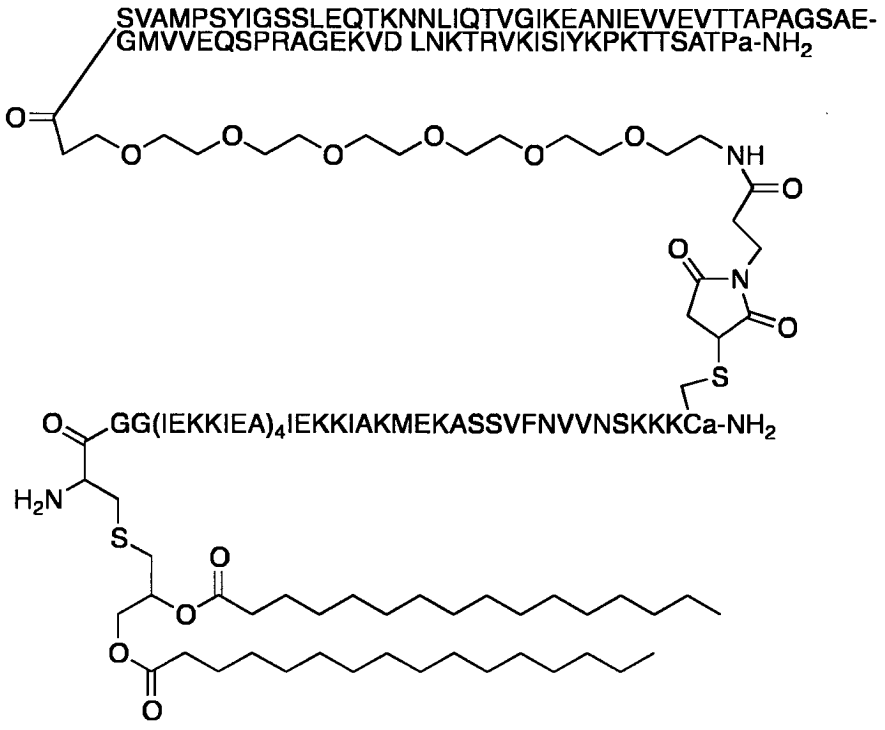
The fusion lipopeptide **25** was synthesized and purified as described above for **24.** ESI-MS: MW calc. for $C_{360}H_{639}N_{91}O_{99}S$: 7966.6 Da; found: 7967.0 Da (± 0.1%). Analytical RP-HPLC (Interchrom UP5WC4-25QS, 25 to 100% MeCN in H_2O (+ 0.1% TFA) over 25 min.): Purity > 98%, t_R = 21.41 min.

DLS (0.5 mg/ml in PBS, 25°C): R_h = 12.2 – 13.7 nm; % Pd = 10 - 15%.

Example 3: Preparation of Controls

The following control compounds were prepared for immunizations and challenge experiments.

Conjugate **26** (non PR conjugate)



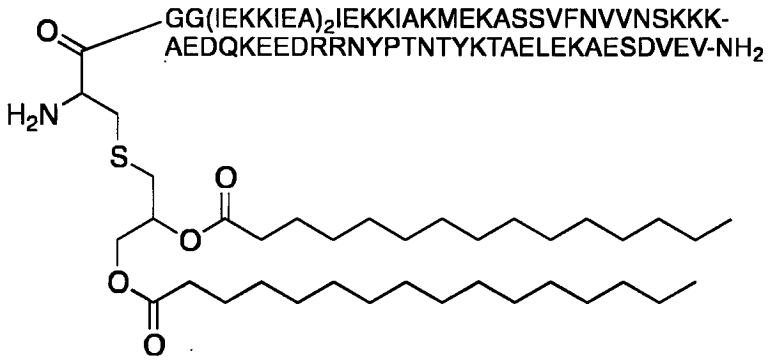
(**26**)

This conjugate contains the C-terminal part (StkP-C; PASTA + C-terminus) of StkP (SEQ ID NO: 115), except that 2 mutations (F594Q and I602T) were incorporated into the StkP

sequence to remove surface-exposed hydrophobic residues, resulting in sequence SVAMPSYIGSSLEQTKNNLIQTVGIKEANIEVVEVTTAPAGSAEGMVVEQSPRAGEKVDL NKTRVKISIYKPKTTSATP (SEQ ID NO: 131). The C-terminus was blocked with a-NH2, where "a" denotes D-alanine. This StkP peptide in the conjugate adopts a regular Penicillin-binding protein and Ser/Thr kinase Associated (PASTA) domain-structure by NMR.

The corresponding maleimidopeptide **27** (3-maleimidopropionyl)-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoyl-(SEQ ID NO: 131)-a-NH_2) was synthesized and conjugated to **10** as described above for **14**. Conjugate **26** was analyzed by HPLC, MALDI-MS and DLS. Analytical RP-HPLC (Interchrom UP5WC4-25QS, 250 x 4.6 mm, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25 min.): Purity: >95%. t_R =18.41 min. MALDI-TOF: MW calc. for $C_{700}H_{1201}N_{177}O_{216}S_5$: 15713.41Da; found 15715.4 Da (± 0.1%). DLS (0.5 mg/ml in PBS, 25°C): R_h = 16.3 nm, % Pd = 16.9%.

Lipopeptide **28**



(**28**)

In this lipopeptide the 31 amino acids at the C terminus correspond to amino acids 344 to 377 from PspC of TIGR4 (SEQ ID NO: 116), except that two mutations were incorporated into the antigen to improve solubility (L366A, I370K; TIGR4 PspC numbering), resulting in peptide AEDQKEEDRRNYPTNTYKTAELEKAESDVEV (SEQ ID NO: 132). The C-terminus was blocked with r-NH2, where "r" denotes D-arginine. This peptide is further coupled through a short linker (KKK) to the universal T-helper cell epitope CST.3* (SEQ ID NO: 23).

Lipopeptide **28** was synthesized and purified as described in WO 2008/068017, and was analyzed by HPLC, MALDI-MS and DLS. Analytical RP-HPLC (Interchrom UP5WC4-25QS, 250 x 4.6 mm, A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA, 20 to 100% B in 25

min.): Purity: >95%. t_R =18.41 min. MALDI-TOF: MW calc. for $C_{386}H_{654}N_{100}O_{121}S_2$: 8696.1 Da; found 8696.0 Da. DLS (0.5 mg/ml in PBS, 25°C): R_h = 7.9 nm; % Pd = 29%.

Recombinant PspA protein (rPspA)

The proline-rich region of the PspA from *S. pneumoniae* strain SP1577 was cloned and expressed as recombinant Trx fusion protein (rPspA). Cloning and expression of recombinant PR from the SP1577 strain was performed as described in WO 2007/089866. The purity and identity were confirmed by SDS-PAGE, dot-blot using anti-PspA antibodies and mass spectrometry. The sequence of the protein is:

MSDKIIHLTD DSFDTDVLKA DGAILVDFWA EWCGPCKMIA PILDEIADEY QGKLTVAKLN IDQNPGTAPK YGIRGIPTLL LFKNGEVAAT KVGALSKGQL KEFLDANLAG SGSGHMHHHH HHSSGLVPRG SGMKETAAAK FERQHMDSPD LGTDDDDKAM ADLKKAVNEP EKPAEET*PAP APKPEQPAEQ PKPAPAPQPA PAPKPEKTDD QQAEEDYARR SEEEYNRLPQ QQPPKAEKPA PAPKPEQPVP APKPEQPVPA PKT*GWKQE (SEQ ID NO: 133)

PspA proline-rich region including non-proline block are shown in italics.

Example 4: Mouse Immunization Studies

Conjugates were tested for immunogenicity against *S. pneumoniae* in mice. All experiments were performed in accordance with the Swiss rules and regulations for the protection of animal rights and have been approved by the responsible authorities.

For analysis of the antibody response outbred six to eight week-old female NMRI outbred mice (10 per group) were subcutaneously immunized two times in three-week intervals on days 0 and 21 with 0.1 ml of the formulations shown in Table 4. Control animals were immunized with PBS or rPspA + alum in saline. Blood was collected before the first and ten days after the second immunization and sera were analyzed using ELISA to determine titers of IgG antibodies to the proline-rich peptide, Western Blot to determine IgG to endogenous proteins and flow cytometry to determine surface binding of IgG to intact pneumococci.

Table 4. Formulations used for immunizations of mice

Formulation	Adjuvant	Concentration (mg/ml)[a)]
14 in PBS	None	0.2
15 in PBS	None	0.2
16 in PBS	None	0.2
17 in PBS	None	0.2
18 in PBS	None	0.2
19 in PBS	None	0.2
16 + **14** in PBS	None	0.2 + 0.2
rPspA in saline	Alum	0.2
PBS	None	-
[a)] Shown is the overall lipopeptide conjugate / protein concentration.		

For ELISA, MaxiSorp 96-well microtitre plates (Nunc, Fischer Scientific) were coated at 4°C overnight with 5 µl/ml solutions of PR peptides or rPspA in PBS, pH 7.2 (50 µl/well). The ELISA was performed essentially as described in WO 2008/068017, using goat anti-mouse IgG (γ-chain-specific) antibodies (Sigma, St. Louis, MO) and 1 mg/ml p-nitrophenyl phosphate (Sigma) for IgG detection. Endpoint titers were defined as the highest serum dilution for which the OD of the test sera was larger than the mean OD of PBS plus two SD.

For Western Blot analysis of immune sera, SP1577 was cultured in blood agar plates at 37°C, 5% CO_2 and total bacterial lysates were prepared. Lysates were separated by SDS-PAGE under reducing conditions and blotted onto nitrocellulose membranes. Blots were incubated with immune or pre-immune sera (1:500 in PBS) and developed using the ECL system. A PspA-specific monoclonal antibody was used as positive control.

For flow-cytometry analysis (FACS), SP1577 was cultured as described above, inactivated with formalin for 30 min., blocked with 5 mg/ml fatty-acid-free BSA in PBS and approximately 7 x 10^5 CFU were incubated with immune or pre-immune sera (1:100 in PBS) for 1 h at room temperature. Surface-bound IgG was detected using an Alexafluor 488-conjugated secondary antibody.

ELISA Geometric Mean Endpoint titers (GMT) ± one Standard Deviation of the Mean (SEM) and results from the Western Blot and FACS analyses (as positive vs. negative reactivity) are summarized in Table 5.

Table 5: IgG Response in mice after two immunizations

Immunogen	ELISA (GMT ± SEM)[a)]	Western Blot (Pos./Neg.)	FACS (Pos./Neg.)
14	26013 ± 30255	9/1	8/2
15	271227 ± 82014	10/0	10/0
16	65606 ± 13592	10/0	10/0
17	82066 ± 36672	8/2	6/4
18	10354 ± 6760	7/3	5/5
19	263069 ± 62452	7/3	7/3
14 + 16[a)]	9027 ± 6150	10/0	10/0
14 + 16[b)]	65606 ± 13592	10/0	10/0
rPspA + alum	212977 ± 65684	10/0	10/0
PBS	< 100	0/10	0/10

[a)] Pre-immune sera showed no significant reactivity in ELISA, Western Blots and FACS.
[b)] IgG response measured in ELISA against **14**
[c)] IgG response measured in ELISA against **16**

Although the antibody response was variable in the out-bred mice, all mice developed high titers of antigen-specific IgG as measured in ELISA and IgG in most of the immune sera bound to endogenous PspA and to intact SP1577 cells. No significant levels of antigen-specific IgG could be detected in pre-immune sera and in sera from PBS-immunized mice.

The cross-reactivity of the elicited IgG to was assessed using Western Blot and FACS and a panel of genetically diverse pneumococcal strains representing different PspA belonging to different clades from PspA families 1-3, which are defined in Hollingshead, Becker et al., *Infect Immun,* **2000**, *68*, 5889-5900, and different pneumococcal capsular serotypes, including serotypes that are not covered by the currently licensed pneumococcal conjugate vaccines.

Bacteria were cultured and Western Blot and FACS analyses were performed with sera from immunized mice as described above for SP1577. The results are summarized below in Table 6.

Table 6: Cross-reactivity of IgG in mouse sera with genetically diverse *Pneumococci*

Immunogen	Test [a)]	Strain /Serotype						
		SP1577 /1	SP920 /8	SP4408 /19A	SP1272 /2	SP1388 /4	SP1260 /4	SP716 /5
14	WBA	++	+	+	++	+	+	++
	FACS	++	+	++	++	+	++	++
15	WBA	++	++	++	++	+	++	++
	FACS	++	+	++	+	+	++	++
16	WBA	++	++	+	++	++	++	++
	FACS	++	++	++	++	++	++	++
17	WBA	++	-	-	+	++	-	-
	FACS	++	++	++	++	++	-	-
18	WBA	++	-	-	+	++	-	-
	FACS	++	++	++	++	++	-	-
19	WBA	+	-	-	+	++	-	+
	FACS	+	+	++	++	++	++	++
14 + 16	WBA	++	++	++	++	++	++	++
	FACS	++	++	++	++	++	++	++
rPspA + alum	WBA	++	++	++	++	++	++	++
	FACS	++	++	++	++	++	++	++

[a)] Sera after two immunizations were used. Pre-immune sera showed no reactivity in the assays. ++ = strong band/signal; + = weak band/signal; - = no binding could be detected.

The results obtained with the genetically diverse panel indicates that immunization with PR peptide antigens elicited broadly cross-reactive IgG, although some PR peptide antigen constructs elicited more broadly cross-reactive IgG than others.

For challenge studies NMRI outbred mice were immunized with different formulations as described above. For comparison of the immunogenicity additional animals were immunized with rPspA + alum, conjugate **44** or lipopeptide **46**. The mice were bled 10 days after the final immunization, sera were analyzed by ELISA as described above in order to determine seroconversion. The mice were then challenged intravenously (iv) *via* the tail vein with 100 times of a pre-determined lethal dose (100 x LD_{100}) *pneumococci*, which were rendered highly virulent by passage in mice prior to the challenge. Bacteria were either passaged by intraperitoneal (ip) or intravenous (iv) injection. After challenge, the health status of the mice was monitored over 14 days. Moribund animals were

euthanized and the time to moribund was recorded. The survival and time to moribund of the animals immunized with lipopeptides or the protein were compared to that of animals immunized with PBS alone.

Results obtained with strain SP1577 are summarized in below in Table 7. The SP1577 strain is highly virulent in mice. Unprotected mice died or became moribund within the first 12-24 h after challenge. Protection, therefore, indicates high efficacy.

Table 7: Protection of NMRI mice from lethal challenge with strain SP1577

Formulation	Passage	Days to moribund		P-value for survival[b]
		Test	Control (PBS)	
14 in PBS	ip	1, 2, 3 x > 14	5 x 1	0.0143
14 in PBS	iv	2 x 1, 5, 2 x > 14	5 x 1	0.0495
15 in PBS	ip	1, 2, 3, 2 x > 14	5 x 1	0.0143
15 in PBS	iv	3 x 2, 2 x > 14	5 x 1	0.0027
16 + **14** in PBS	ip	1, 1.5, 4, 2 x > 14	5 x 1	0.0143
16 + **14** in PBS	iv	3 x 2, 6, 14	5 x 1	0.0027
18 in PBS	ip	1, 1.5, 3 x > 14	5 x 1	0.0143
19 in PBS	ip	2 x 2, 3 x > 14	5 x 1	0.0027
17+26+28 in PBS	ip	2 x 1, 3 x > 14	5 x 1	0.0495
rPspA + alum	ip	3 x 1, 2 x >14	5 x 1	NS
rPspA + alum	iv	3 x 1, 5, >14	5 x 1	NS
16 in PBS	iv	5 x 1	5 x 1	NS
26 in PBS	ip	5 x 1	5 x 1	NS
28 in PBS	ip	4 x 1, 1 x 3	5 x 1	NS

[b] Shown is the P-value for the survival distribution compared to the distribution for mice immunized with PBS by Logrank test. NS = non-significant (P > 0.05).

Mice immunized with PR peptide antigens conjugated to SVLPs were partially protected and the median survival time was prolonged compared to that for PBS immunized mice and mice immunized with rPspA. The survival distributions obtained for mice immunized with SVLPs or rPspA were compared with that for mice immunized with PBS alone (negative control) by the Log-rank test. The results for mice immunized with PR conjugates, alone or in combination with other antigens, were significantly different to those for PBS immunized mice. The P-values were in the range of 0.0027-0.0143. The results for mice immunized with rPspA were not significantly different (P = 0.1336)

compared to that of PBS immunized mice, although some mice were protected. The difference may become more significant for larger groups. Results for StkP and PspC-derived conjugates were not significant from PBS, indicating that these antigens are not protective in this model.

For passive immunization/challenge experiments to determine if antibodies mediate the protection, monoclonal antibodies were generated in mice. In one experiment BALB/c mice were immunized three times with conjugate **17.** Seven B cell hybridoma lines producing antigen-specific monoclonal IgG antibodies (mAbs) were generated from spleen cells of one mouse and tested for cross-reactivity with different pneumococcal strains by FACS analysis. One mAb (5H8) bound to a broad range of strains (clinical isolates) representing different PspA clades and capsular serotypes. Other mAbs bound to the PR antigen in ELISA but not to intact bacteria. Epitope mapping indicated that mAb 5H8 recognized an epitope in the C-terminal part of the PR peptide, which occurs in a broad variety of different PspA sequences, whereas the other 6 mAbs recognized other epitopes, which are less frequently found in different PspA sequences. For the challenge 0.1 to 0.5 mg of purified 5H8 or other mAbs was administered to groups of 5 NMRI mice by iv injection. Animals passively immunized with an StkP-derived mAb (1A7) and naïve animals were used as controls. After sufficient time for equilibration the mice were challenged iv with passaged *pneumococci* and the health status and time to moribund was monitored as described above. Results obtained with strain SP1577 are shown in Table 8 below.

Table 8: Passive protection of NMRI mice from lethal challenge with strain SP1577

Dose (mg/ml)	mAb	Days to moribund		P-value for survival[a)]
		Test	Control	
0.5	5H8	1.5, 2, 3, 2 x >14	5 x 1	0.0027
0.1	5H8	2 x 1, 1 x 1.5, > 14	5 x 1	0.0495
0.5	3H5	5 x 1	5 x 1	NS
0.5	1H9	5 x 1	5 x 1	NS
0.5	1A7	5 x 1	5 x 1	NS

[a)] Shown is the P-value for the survival distribution compared to the distribution for naïve mice by Logrank test. NS = non-significant (P > 0.05).

Only mAb 5H8 gave significantly different results after passive immunization, compared to naïve mice. The protection was dose dependent. MAbs 3H5 and 1H9 did not show a

significant effect in this model of passive protection. Sequencing revealed that the PspA of strain SP1577 contains only the 5H8 epitope but not the epitopes of the other two PR-derived antibodies. No protection was also seen for the StkP-derived mAb 1A7 in this model.

The results demonstrate that immunization with SVLP-forming lipopeptides carrying proline-rich peptide antigens elicits highly *S. pneumoniae* cross-reactive antibodies in mice when administered alone or in combination with other antigens, without co-administration of an adjuvant.

Example 5: Rabbit Immunization Studies

In order to characterize the antibody response in non-rodents, New Zealand White rabbits were sc immunized three times on days 0, 28 and 56 with different concentrations of conjugates **17**, **18** or **19** in 0.4 ml PBS, without or with an adjuvant (R848) (Table 9). Blood samples were taken on days 0, 14, 38 and 66 to determine seroconversion.

Table 9: Formulations used for immunizations of rabbits

Group	Formulation	Adjuvant	Concentration (mg/ml)[a)]
1	**17** in PBS	None	0.35
2	**22** in PBS	None	0.35
3	**21** in PBS	None	0.1
4	**21** in PBS	None	0.025
5	**21** in PBS	R848	0.025
[a)] Shown is the lipopeptide concentration.			

Pre-immune sera and sera after the third immunization were analyzed by Western Blot and FACS using rabbit-IgG-specific secondary antibodies and various pneumococcal isolates as described above for the mice. The development of the IgG response was also analyzed by ELISA using a monoclonal anti-rabbit IgG (γ-chain specific) alkaline phosphatase antibody with 4-nitrophenyl-phosphate for IgG detection, essentially as described above. Results are shown in Table 10. All immunized rabbits developed IgG binding to endogenously expressed PspA and PspA expressed on intact pneumococci in response to the immunization. Pre-immune sera showed no significant reactivity in these assays. High titers of PR peptide-specific IgG antibodies were detected in the immune

sera from immunized rabbits, even after administration of low doses of the conjugate without an adjuvant.

Table 10: Development of the IgG response In NZW rabbits (N = 3)

Group	ELISA (GMT ± SEM)[a)]			Western Blot (Pos./Neg.)[b)]	FACS (Pos./Neg.)[b)]
	1st imm.	2nd imm.	3rd imm.		
1	4.01 ± 0.22	4.92 ± 0.15	4.66 ± 0.16	3/0	3/0
2	2.96 ± 0.05	4.13 ± 0.19	4.15 ± 0.08	3/0	3/0
3	< 2.3	4.47 ± 0.05	4.67 ± 0.16	3/0	3/0
4	< 2.3	2.84 ± 0.25	3.79 ± 0.34	3/0	3/0
5	< 2.3	3.70 ± 0.38	4.56 ± 0.08	3/0	3/0

[a)] Shown are log10 Geometric Mean Titers (GMT) ± one Standard Error of the Mean (SEM) after one (1st imm.), two (2nd imm.) or three (3rd imm.) immunizations.
[b)] Results for strain SP1577 are shown.

The results demonstrate that immunization with SVLP-forming lipopeptides carrying proline-rich peptide antigens elicits broadly cross-reactive antibodies in non-rodents also when administered without an adjuvant.

Example 6: Comparison of antigen-specific antibody responses in BALB/c mice

The antigen-specific antibody response elicited by conjugate **15** SVLPs was compared to that elicited with recombinant PspA protein. Six to eight week-old female BALB/c mice (18 per group) were subcutaneously immunized two times in three-week intervals on days 0 and 21 with 0.1 ml of conjugate **15** in PBS or rPspA + alum in saline prepared as described above in Example 5. Blood was collected ten days after the second immunization.

In order to determine the antigen specificity of the antibody response, ELISA were performed as described in Example 5, using PR peptide SEQ ID NO:27 as the coating antigen for the measurement of PR-specific antibodies and rPspA as the coating antigen for the measurement of total anti-PspA antibodies (i.e. antibodies to N-terminal epitopes, NPB and PR). The results are shown in Figure 1. Sera from non-immunized mice showed no cross-reactivity with either antigen in ELISA.

As expected rPspA was highly immunogenic (anti-PspA IgG GMT ± SEM = 81'969 ± 28'068) but failed to elicit significant levels of anti-PR antibodies (anti-PspA IgG GMT ± SEM = 213 ± 218). This was not due to a failure of antibodies raised against rPspA to bind to the PR peptide, since antibodies raised against conjugate **15** SVLPs recognized both, the PR peptide (anti-PspA IgG GMT ± SEM = 27'583 ± 6'204) and the rPspA antigen (20'919 ± 3'444) in ELISA. It is, therefore, likely that the majority of rPspA-elicited antibodies bind to epitopes in the N-terminal alpha helical part.

In order to determine whether PspA lacking the N-terminal alpha helical would elicit more PR-specific antibodies, mice were immunized with a truncated recombinant PspA-Trx fusion protein (rPspA-delta-N-term; contains PR and NPB). Surprisingly also the truncated protein failed to elicit significant levels of anti-PR antibodies: The GMT ± SEM was 128'496 ± 28'481 for the protein and 213 ± 1'094 for the PR antigen. This was significantly lower than for conjugate **15** SVLP-immunized control animals (GMT ± SEM of 32'776 ± 6974 for the protein and 47'679 ± 23'383 for the PR peptide). Together these results indicate that SVLPs elicit significantly higher levels of PR-specific antibodies than recombinant PspA.

Example 7: Protection from increasing challenge doses

In order to determine the protection of immunized BALB/c mice from increasing challenge doses, mice were immunized with conjugate **15** or rPspA as described above in Example 6, challenged intravenously with increasing doses of serotype 1 bacteria ranging from 10^2 to 10^6 CFU and monitored for survival. Standardized pneumococcal serotype 1 bacterial inocula were prepared as described in Aaberge I.S. *et al.*, *Microbial Pathogenesis*, **1995**, 18, 141-152. LD_{50} in non-immunized mice were verified by intravenously injecting BALB/c mice increasing doses of bacteria and monitoring for survival.

The protection of immunized mice was dependent on the bacterial challenge dose and the type of immunogen. At low challenge doses the protection was comparable for animals immunized with rPspA or lipopeptide building block **15** SVLPs (See Figure 2). At higher challenge doses (100–10'000 x LD_{50}), the mice immunized with conjugate **15** SVLPs were better protected than those immunized with rPspA. Together these results indicate that the anti-PR antibodies elicited by SVLPs protect over a wider range of bacterial challenge doses than antibodies raised against rPspA.

Claims

1. Lipopeptide building block consisting of
(1) a peptide chain comprising a parallel coiled-coil domain which, as a self-standing lipid-free peptide, forms a parallel dimeric, trimeric or higher order oligomeric helical bundle,
(2) a proline-rich peptide antigen comprising at least one negatively and at least one positively charged amino acid, and wherein at least 15% of the amino acids are proline, optionally linked to a further antigen, and
(3) a lipid moiety comprising two or three long hydrocarbyl chains,
wherein the peptide chain, the proline-rich peptide antigen and the lipid moiety are covalently linked, either directly or through a linker.

2. Lipopeptide building block according to claim 1 wherein the peptide chain comprises between 21 and 200 amino acid residues.

3. Lipopeptide building block according to claim 1 or 2 wherein the peptide chain comprises a coiled-coil domain consisting of three to eight heptad motifs.

4. Lipopeptide building block according to claim 3 wherein in the coiled-coil domain positions *a* and *d* in each heptad motif (*abcdefg*) comprise alpha-amino acids with small to medium-sized hydrophobic side chains and/or aromatic or heteroaromatic side chains, in zero, one or two of all the *a* and *d* positions an amino acid with a polar non-charged residue and in zero or one of all the *a* and *d* positions an amino acid with a polar cationic residue or an acylated derivative thereof, or with a polar anionic residue, or glycine.

5. Lipopeptide building block according to claim 4 wherein
alpha-amino acids with small to medium-sized hydrophobic side chain are alanine, isoleucine, leucine, methionine and valine;
alpha-amino acids with aromatic or heteroaromatic side chain are phenylalanine, tyrosine, tryptophan and histidine;
alpha-amino acids with polar non-charged residue are asparagine, cysteine, glutamine, serine and threonine;
alpha-amino acids with polar cationic residue are arginine, lysine and histidine; and
alpha-amino acids with polar anionic residue are aspartic acid and glutamic acid.

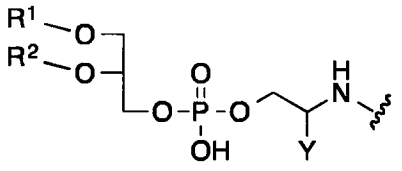
6. Lipopeptide building block according to any one of claims 1 to 5 wherein the proline-rich peptide antigen comprises at least one glutamic acid residue and at least one lysine or arginine residue.

7. Lipopeptide building block according to any one of claims 1 to 6 wherein the proline-rich peptide antigen is derived from proteins of *Streptococci* and/or *Staphylococci.*

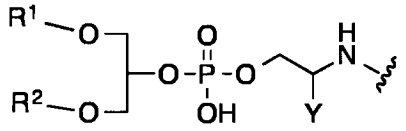
8. Lipopeptide building block according to any one of claims 1 to 6 wherein the proline-rich peptide antigen is derived from proteins PspA and/or PspC.

9. Lipopeptide building block according to any one of claims 1 to 8 wherein the proline-rich peptide antigen comprises a peptide of SEQ ID NO:27 to 112, and such peptides in which one, two or three amino acids are replaced by other amino acids.

10. Lipopeptide building block according to any one of claims 1 to 9 wherein the lipid moiety is one of types Z^1 to Z^8

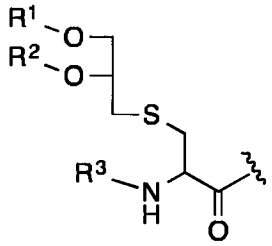


Z^1



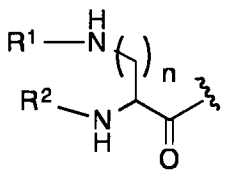
Z^2

wherein R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and Y is H or COOH,

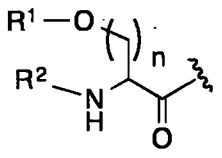


Z^3

wherein R^1, R^2 and R^3 are long hydrocarbyl or long hydrocarbyl-C=O or R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and R^3 is H or acetyl or lower alkyl-C=O,

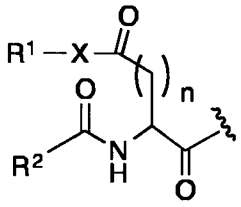


Z^4



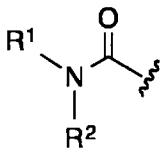
Z^5

wherein R^1 and R^2 are long hydrocarbyl or long hydrocarbyl-C=O and n is 1, 2, 3 or 4,

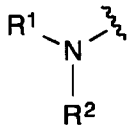


Z^6

wherein R^1 and R^2 are long hydrocarbyl, X is O or NH, and n is 1, 2, 3 or 4, or



Z^7



Z^8

wherein R^1 and R^2 are long hydrocarbyl.

and wherein long hydrocarbyl is straight or branched alkyl or alkenyl consisting of between 8 and 25 carbon atoms and optionally one, two or three double bonds in the chain.

11. Lipopeptide building block according to claim 10 wherein the lipid moiety is di-palmitoyl-S-glycerylcysteinyl of formula **Z³**, wherein R^1 and R^2 are palmitoyl and R^3 is H or acetyl.

12. Lipopeptide building block according to any one of claims 1 to 11 wherein the peptide chain comprising a parallel coiled coil is linked at one end to the PR peptide antigen and at the other end to the lipid moiety.

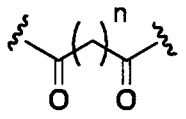
13. Lipopeptide building block according to claim 12 wherein the peptide chain PC is covalently linked to the lipid moiety LM at or near one terminus of the peptide chain either directly as in

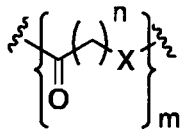
LM – PC (1)

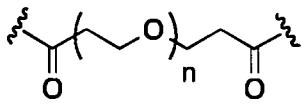
or *via* a linker (L) as in

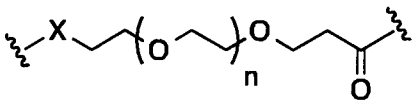
LM – L – PC (2)

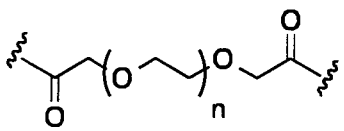
wherein linker L is selected from

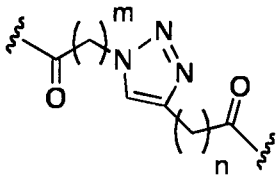

L1

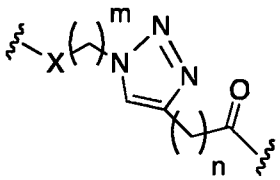

L2

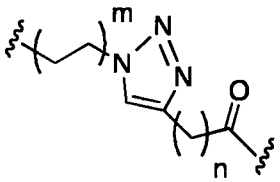

L3

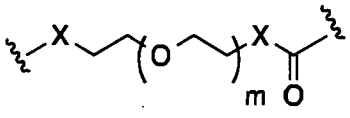

L4

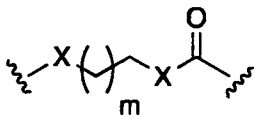

L5

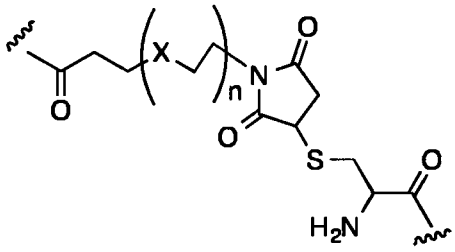

L6

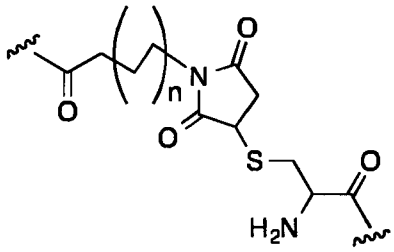

L7

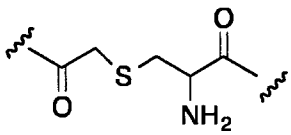

L8

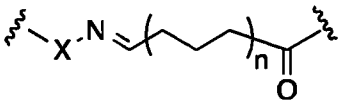

L9

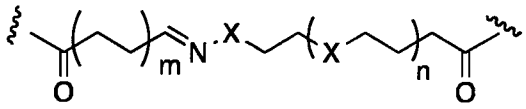

L10

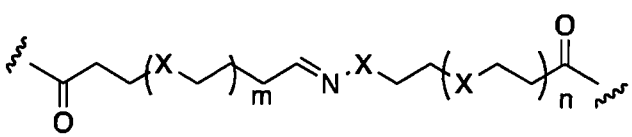

L11


L12


L13


L14


L15


L16

wherein X is O or NH, m is between 1 and 45 and n is between 1 and 45.

14. Synthetic virus-like particles consisting of helical lipopeptide bundles comprising two, three, four, five, six or seven lipopeptide building blocks according to any one of claims 1 to 13.

15. A vaccine comprising a synthetic virus-like particle according to claim 14.

16. A method of vaccination against a disease caused by Gram-negative bacteria wherein an immunogenically effective amount of a synthetic virus-like particle according to claim 14 is administered to a patient in need thereof.

Fig. 1

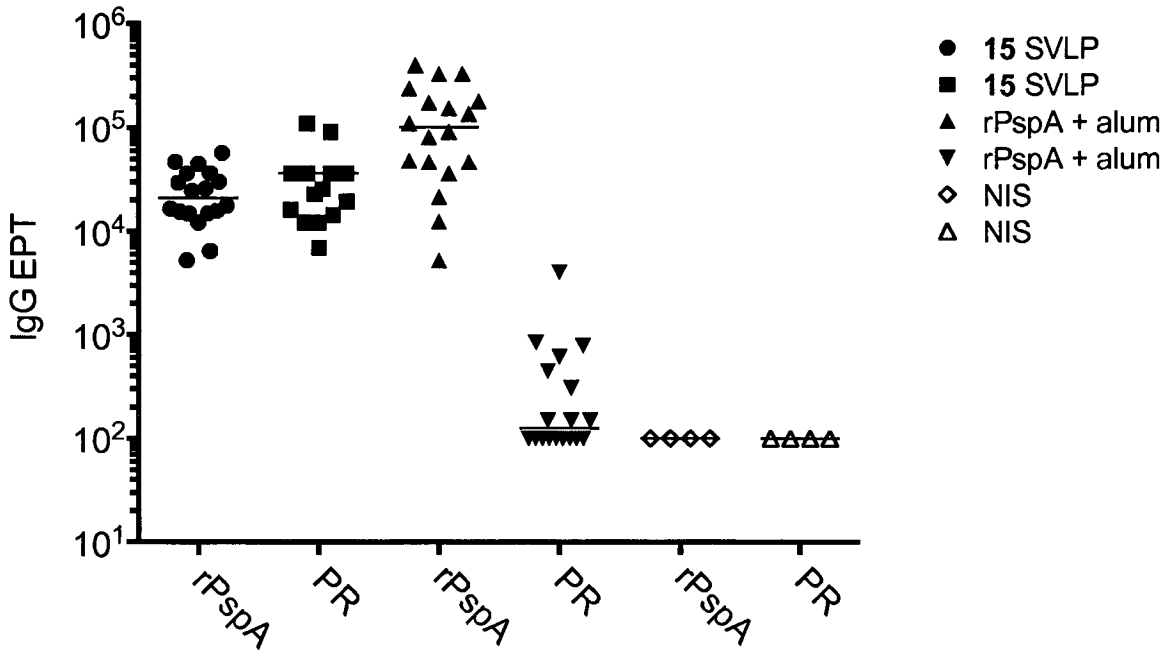
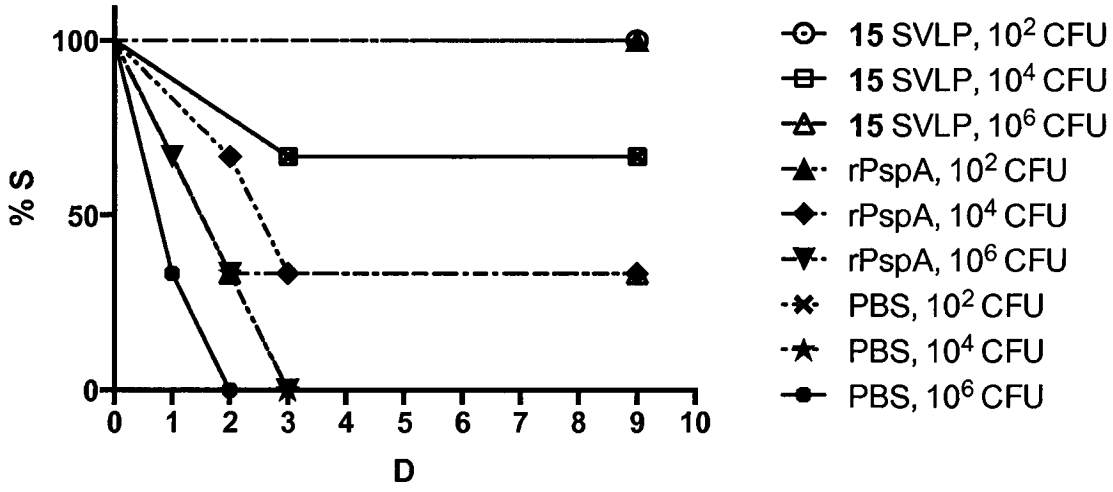

IgG EPT
10^6
10^5
10^4
10^3
10^2
10^1
rPspA
PR
rPspA
PR
rPspA
PR
15 SVLP
15 SVLP
rPspA + alum
rPspA + alum
NIS
NIS

Fig. 2


% S
100
50
0
0 1 2 3 4 5 6 7 8 9 10
D
15 SVLP, 10^2 CFU
15 SVLP, 10^4 CFU
15 SVLP, 10^6 CFU
rPspA, 10^2 CFU
rPspA, 10^4 CFU
rPspA, 10^6 CFU
PBS, 10^2 CFU
PBS, 10^4 CFU
PBS, 10^6 CFU

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/076313

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/315 A61K39/09 A61K39/085
ADD. A61K39/00

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)
C07K A61K C12N

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)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANGERMANN S ET AL: "PROTECTIVE HUMORAL RESPONSE AGAINST PNEUMOCOCCAL INFECTION IN MICE ELICITED BY RECOMBINANT BACILLE CALMETTE-GUERIN VACCINES EXPRESSING PNEUMOCOCCAL SURFACE PROTEIN A", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 180, no. 6, 1 December 1994 (1994-12-01), pages 2277-2286, XP000918247, ISSN: 0022-1007, DOI: 10.1084/JEM.180.6.2277	1-9
Y	the whole document	1-16

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2015	17/02/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Madruga, Jaime

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOTHER J ET AL: "STRUCTURAL PROPERTIES AND EVOLUTIONARY RELATIONSHIPS OF PSPA, A SURFACE PROTEIN OF STREPTOCOCCUS PNEUMONIAE, AS REVEALED BY SEQUENCE ANALYSIS", JOURNAL OF BACTERIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC; US, vol. 174, no. 2, 1 January 1992 (1992-01-01), pages 601-609, XP000600024, ISSN: 0021-9193 the whole document	1-16
X	WO 2012/100233 A1 (GENOCEA BIOSCIENCES INC [US]; CHILDRENS MEDICAL CENTER [US]; PATH VACC) 26 July 2012 (2012-07-26) cited in the application	1-9
Y	the whole document page 54, line 22 - page 55, line 15; claims; examples	1-16
X	WO 96/40290 A1 (CONNAUGHT LAB [US]) 19 December 1996 (1996-12-19)	1-9
Y	the whole document claims; examples 3,5	1-16
Y	WO 2008/068017 A1 (UNIV ZUERICH PROREKTORAT FORSC [CH]; UNIV ZUERICH [CH]; BOATO FRANCESC) 12 June 2008 (2008-06-12) cited in the application the whole document	1-16
Y	BOATO FRANCESCA ET AL: "Synthetic virus-like particles from self-assembling coiled-coil lipopeptides and their use in antigen display to the immune system", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, WILEY - V C H VERLAG GMBH & CO. KGAA, DE, vol. 46, no. 47, 12 October 2007 (2007-10-12), pages 9015-9018, XP002471327, ISSN: 1433-7851, DOI: 10.1002/ANIE.200702805 the whole document	1-16
Y	ARIN GHASPARIAN ET AL: "Engineered Synthetic Virus-Like Particles and Their Use in Vaccine Delivery", CHEMBIOCHEM, vol. 12, no. 1, 3 January 2011 (2011-01-03), pages 100-109, XP055115581, ISSN: 1439-4227, DOI: 10.1002/cbic.201000536 the whole document	1-16

2 -/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MERIT MELIN ET AL: "Development of cross-reactive antibodies to the proline-rich region of pneumococcal surface protein A in children", VACCINE, vol. 30, no. 50, 13 October 2012 (2012-10-13), - 1 November 2012 (2012-11-01), pages 7157-7160, XP055115762, ISSN: 0264-410X, DOI: 10.1016/j.vaccine.2012.10.004 the whole document	1-16
Y	C. C. DANIELS ET AL: "The Proline-Rich Region of Pneumococcal Surface Proteins A and C Contains Surface-Accessible Epitopes Common to All Pneumococci and Elicits Antibody-Mediated Protection against Sepsis", INFECTION AND IMMUNITY, vol. 78, no. 5, 1 March 2010 (2010-03-01), - 1 May 2010 (2010-05-01), pages 2163-2172, XP055115771, ISSN: 0019-9567, DOI: 10.1128/IAI.01199-09 cited in the application the whole document	1-16
A	BERNDT P ET AL: "SYNTHETIC LIPIDATION OF PEPTIDES AND AMINO ACIDS: MONOLAYER STRUCTURE AND PROPERTIES", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, ACS PUBLICATIONS, US, vol. 117, no. 37, 20 September 1995 (1995-09-20), pages 9515-9522, XP002030763, ISSN: 0002-7863, DOI: 10.1021/JA00142A019 the whole document	1-16
Y	RAJNI SHARMA ET AL: "Synthetic Virus-Like Particles Target Dendritic Cell Lipid Rafts for Rapid Endocytosis Primarily but Not Exclusively by Macropinocytosis", PLOS ONE, vol. 7, no. 8, 14 August 2012 (2012-08-14) , page e43248, XP55167520, DOI: 10.1371/journal.pone.0043248 the whole document	1-16

-/--

2

International application No
PCT/EP2014/076313

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TINA RIEDEL ET AL: "Synthetic Virus-Like Particles and Conformationally Constrained Peptidomimetics in Vaccine Design", CHEMBIOCHEM, vol. 12, no. 18, 11 November 2011 (2011-11-11), pages 2829-2836, XP55167514, ISSN: 1439-4227, DOI: 10.1002/cbic.201100586 the whole document -----	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/076313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012100233 A1	26-07-2012	AU 2012207088 A1	02-05-2013
		AU 2012207089 A1	02-05-2013
		CA 2861313 A1	26-07-2012
		CN 103501809 A	08-01-2014
		EP 2665490 A1	27-11-2013
		JP 2014503002 A	06-02-2014
		KR 20140017554 A	11-02-2014
		SG 192060 A1	30-08-2013
		US 2012189649 A1	26-07-2012
		WO 2012100233 A1	26-07-2012
		WO 2012100234 A1	26-07-2012
WO 9640290 A1	19-12-1996	AT 249844 T	15-10-2003
		AU 717890 B2	06-04-2000
		AU 6151996 A	30-12-1996
		CA 2223041 A1	19-12-1996
		DE 69630033 D1	23-10-2003
		DE 69630033 T2	01-04-2004
		EP 0831937 A1	01-04-1998
		FI 974423 A	04-02-1998
		IL 118579 A	03-12-2007
		JP H11510370 A	14-09-1999
		NO 975620 A	04-02-1998
		US 6251405 B1	26-06-2001
		US 6379675 B1	30-04-2002
		US 2002131983 A1	19-09-2002
		US 2006110408 A1	25-05-2006
		US 2008089911 A1	17-04-2008
		WO 9640290 A1	19-12-1996
		ZA 9604894 A	24-02-1997
WO 2008068017 A1	12-06-2008	AU 2007327829 A1	12-06-2008
		CA 2672052 A1	12-06-2008
		DK 2121732 T3	08-10-2012
		EP 2121732 A1	25-11-2009
		ES 2392080 T3	04-12-2012
		JP 5276598 B2	28-08-2013
		JP 2010512308 A	22-04-2010
		PT 2121732 E	02-10-2012
		SI 2121732 T1	31-12-2012
		US 2010015173 A1	21-01-2010
		WO 2008068017 A1	12-06-2008

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105793276 A
(43)申请公布日 2016.07.20

(21)申请号 201480066380.7
(22)申请日 2014.12.02
(30)优先权数据
13195402.6 2013.12.03 EP
(85)PCT国际申请进入国家阶段日
2016.06.03
(86)PCT国际申请的申请数据
PCT/EP2014/076313 2014.12.02
(87)PCT国际申请的公布数据
WO2015/082501 EN 2015.06.11
(71)申请人 维罗米蒂克斯股份公司
地址 瑞士施利伦
申请人 瑞士热带疾病与公共卫生研究所
巴塞尔大学 苏黎士大学
(72)发明人 A·加斯帕里安 A·祖尼加
N·盖伯 M·坦博里尼 M·尤德
G·普卢施克 A·马雷罗诺达尔斯
J·A·鲁滨逊
(74)专利代理机构 北京市中咨律师事务所
11247
代理人 史文静 黄革生
(51)Int.Cl.
C07K 14/315(2006.01)
A61K 39/09(2006.01)
A61K 39/085(2006.01)
A61K 39/00(2006.01)

权利要求书5页 说明书43页
序列表21页 附图1页

(54)发明名称

针对肺炎链球菌进行保护的富含脯氨酸的肽

(57)摘要

本发明涉及脂肽，其由肽链组成，所述肽链包含平行卷曲螺旋结构域、富含脯氨酸的肽抗原和脂质部分，它们均通过共价连接，聚集成合成病毒样颗粒。关注的富含脯氨酸的肽抗原包含带负电荷和带正电荷的氨基酸，且至少15%的氨基酸是脯氨酸。这类携带富含脯氨酸的来源于肺炎球菌蛋白的抗原的合成的病毒样颗粒用作抵抗由革兰氏阳性菌例如肺炎链球菌导致的感染性疾病。

CN 105793276 A

1.脂肽结构单元，由如下组成：

(1)包含平行卷曲螺旋结构域的肽链，其作为自立式的无脂质的肽，形成平行的二聚化、三聚化或更高级寡聚化螺旋束；

(2)包含至少一个带负电荷的氨基酸和至少一个带正电荷的氨基酸的富含脯氨酸的肽抗原，且其中至少15％的氨基酸是脯氨酸，其任选地连接至另外的抗原；和

(3)包含2或3个长烃基链的脂质部分，

其中肽链、富含脯氨酸的肽抗原和脂质部分直接或通过连接体共价连接。

2.权利要求1的脂肽结构单元，其中肽链包含21至200个氨基酸残基。

3.权利要求1或2的脂肽结构单元，其中肽链包含由3-8个七基序组成的卷曲螺旋结构域。

4.权利要求3的脂肽结构单元，其中在卷曲螺旋结构域中，每个七基序(abcdefg)中的位置a和d包含具有小至中等大小疏水性侧链和/或具有芳族或杂芳族侧链的α-氨基酸，在所有a和d位置的0、1或2个中具有极性不带电荷的残基的氨基酸，且在所有a和d位置的0或1个中具有极性阳离子残基的氨基酸或其酰化衍生物、或具有极性阴离子残基的氨基酸、或甘氨酸。

5.权利要求4的脂肽结构单元，其中

具有小至中等大小疏水性侧链的α-氨基酸是丙氨酸、异亮氨酸、亮氨酸、甲硫氨酸和缬氨酸；

具有芳族或杂芳族侧链的α-氨基酸是苯丙氨酸、酪氨酸、色氨酸和组氨酸；

具有极性不带电荷的残基的α-氨基酸是天冬酰胺、半胱氨酸、谷氨酰胺、丝氨酸和苏氨酸；

具有极性阳离子残基的α-氨基酸是精氨酸、赖氨酸和组氨酸；和

具有极性阴离子残基的α-氨基酸是天冬氨酸和谷氨酸。

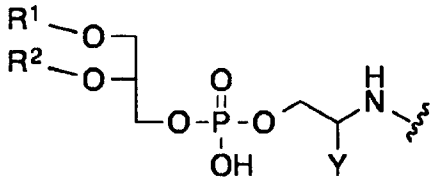
6.权利要求1-5中任一项的脂肽结构单元，其中富含脯氨酸的肽抗原包含至少一个谷氨酸残基和至少一个赖氨酸或精氨酸残基。

7.权利要求1-6中任一项的脂肽结构单元，其中富含脯氨酸的肽抗原来源于链球菌和/或葡萄球菌的蛋白质。

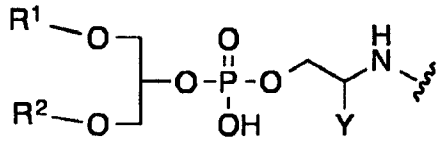
8.权利要求1-6中任一项的脂肽结构单元，其中富含脯氨酸的肽抗原来源于蛋白质PspA和/或PspC。

9.权利要求1-8中任一项的脂肽结构单元，其中富含脯氨酸的肽抗原包含SEQ ID NO:27-112的肽和这样的肽，其中1、2或3个氨基酸被另外的氨基酸替代。

10.权利要求1-9任一项的脂肽结构单元，其中脂质部分是类型Z^1-Z^8之一：

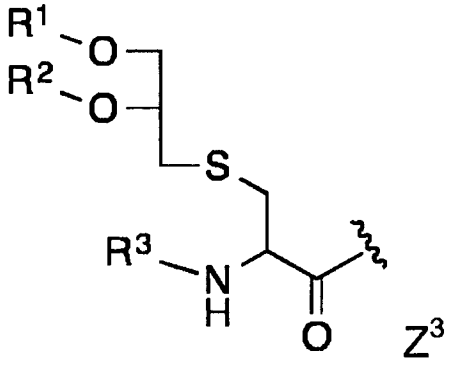


Z^1



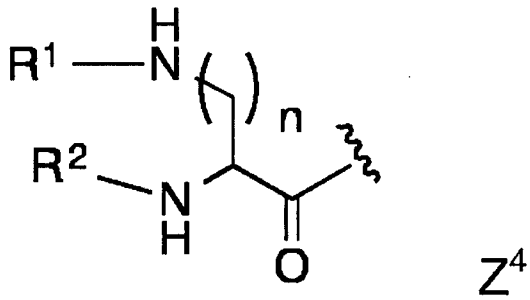
Z^2

其中R^1和R^2是长烃基或是长烃基-C=O,且Y是H或COOH,

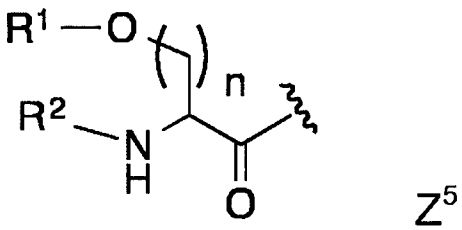


Z^3

其中R^1、R^2和R^3是长烃基或是长烃基-C=O或R^1和R^2是长烃基或是长烃基-C=O,且R^3是H或乙酰基或低级烷基-C=O,

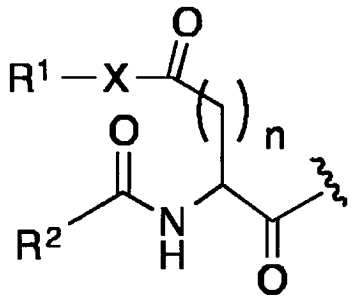


Z^4



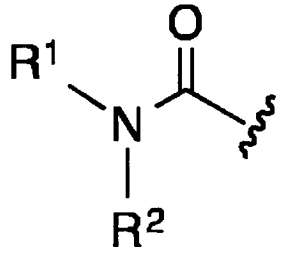
Z^5

其中R^1和R^2是长烃基或是长烃基-C=O,且n是1、2、3或4,

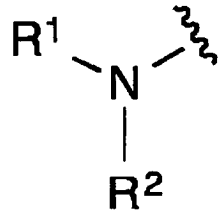


Z^6

其中R^1和R^2是长烃基，X是O或NH，且n是1、2、3或4；或



Z^7



Z^8

其中R^1和R^2是长烃基，

且其中长烃基是由8-25个碳原子和任选地1、2或3个链中的双键组成的直链或支链烷基或烯基。

11.权利要求10的脂肽结构单元，其中脂质部分是式Z^3的二-棕榈酰基-S-甘油基半胱氨酰基，其中R^1和R^2是棕榈酰基，且R^3是H或乙酰基。

12.权利要求1-11中任一项的脂肽结构单元，其中包含平行卷曲螺旋的肽链在一个末端上连接至PR肽抗原并且在另一个末端上连接至脂质部分。

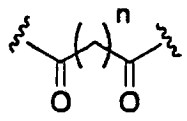
13.权利要求12的脂肽结构单元，其中肽链PC与脂质部分LM在该肽链的一个末端或接近一个末端上直接共价连接为：

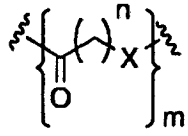
LM-PC (1)

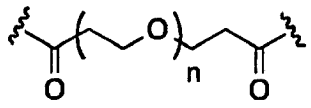
或通过连接体(L)共价连接为：

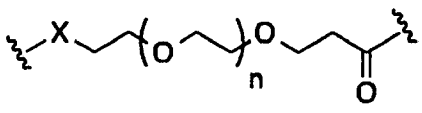
LM-L-PC (2)

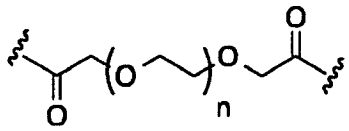
其中连接体L选自：

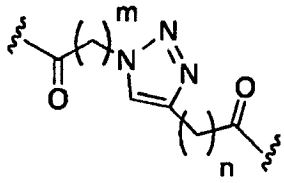

L1

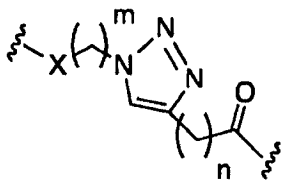

L2

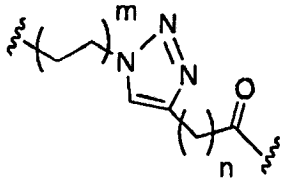

L3

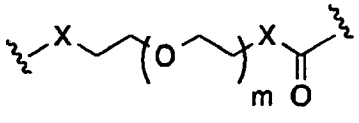

L4

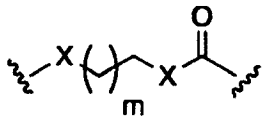

L5

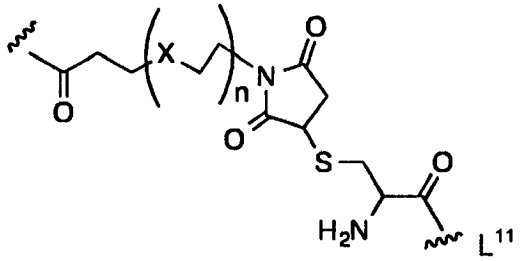

L6

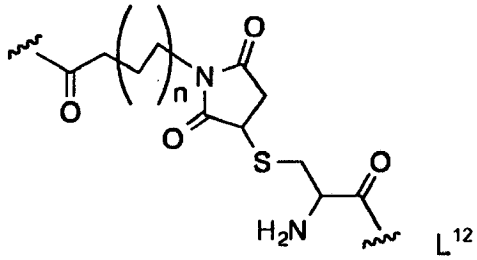

L7

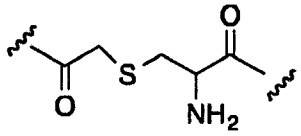

L8

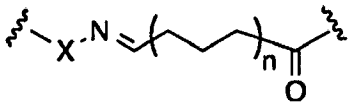

L9

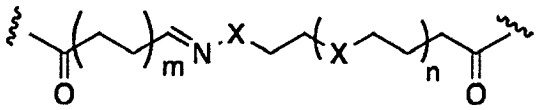

L10

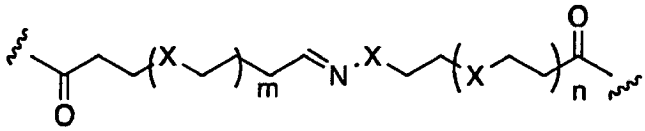

L11


L12


L13


L14


L15


L16

其中X是O或NH，m是1至45，且n是1至45。

14.合成病毒样颗粒，由螺旋脂肽束组成，该螺旋脂肽束包含2、3、4、5、6或7个根据权利要求1-13中任一项的脂肽结构单元。

15.包含权利要求14的合成病毒样颗粒的疫苗。

16.抵抗由革兰氏阴性菌导致的疾病的接种方法，其中对有此需要的患者施用免疫原性有效量的根据权利要求14的合成病毒样颗粒。

针对肺炎链球菌进行保护的富含脯氨酸的肽

发明领域

[0001] 本发明涉及由肽链组成的聚集成合成病毒样颗粒的多聚化脂肽，所述肽链包含平行卷曲螺旋结构域、富含脯氨酸的肽抗原和脂质部分，它们均通过共价连接。这些携带富含脯氨酸抗原的合成病毒样颗粒用作抵抗由革兰氏阳性菌例如肺炎链球菌(Streptococcus pneumoniae)导致的感染性疾病的疫苗。

[0002] 发明背景

[0003] 革兰氏阳性细菌(包括链球菌(Streptococci)或葡萄球菌(Staphylococci))是众多严重性疾病的病原体和病原，所述疾病包括肺炎、脓毒症、脑膜炎、伤口感染、心内膜炎、急性风湿热、新生儿脓毒症或中毒性休克综合征。因此，对于开发抵抗这些病原体的疫苗存在需求。已经得到用于一些肺炎链球菌血清型的疫苗；它们存在缺陷，例如高度复杂性的生产过程。

[0004] 肺炎链球菌是高度不同的多糖包裹的甲型溶血性链球菌，其通常定居于人鼻咽并且可以导致非侵害性肺炎球菌性疾病，例如中耳炎、窦炎和非菌血症性肺炎，和更严重性的侵害性疾病，例如菌血症/脓毒症、脑膜炎和菌血症性肺炎，主要是在年轻的儿童和中老年人中。

[0005] 多糖荚膜是侵害过程中主要的毒力决定因素并且防止C3b调理素作用和嗜中性粒细胞的非调理素杀伤作用。目前得到批准的疫苗包含单独地配制在肺炎球菌多糖菌苗(PPSV)中的荚膜多糖抗原类或在肺炎球菌缀合物疫苗(PCV)中与载体蛋白例如修饰的白喉毒素CRM 197缀合的荚膜多糖抗原类。存在40种血清群中的90种以上的不同荚膜血清型。

[0006] 多糖肺炎球菌菌苗可以提供血清型-特异性保护作用，但交叉保护作用甚至在同一血清群中较低。在2000年，在美国引入缀合疫苗**Prevnar®7**后已经观察到了血清型的替代品。在新出现的血清型中还有多重耐药荚膜-切换变体。因此，对于下一代靶向非荚膜的其它抗原的肺炎球菌疫苗存在需求。

[0007] 用于包含入下一代肺炎球菌疫苗的一种潜在的抗原是肺炎球菌表面蛋白A(PspA)。PspA是单体多态胆碱(cholin)-结合蛋白并且包含N-末端α-螺旋部分，其与自身形成反向平行的卷曲螺旋，即富含脯氨酸的区，其中有时分散有相对保守的非脯氨酸区段和包含多个胆碱结合结构域重复的C-末端区。

[0008] PspA的N-末端区包含免疫显性表位。包含该区的重组蛋白和表达该区的细菌载体已经在各种模型中显示保护潜能。例如，Langermann等人已经制备了表达PspA的重组卡介苗(Bacille Calmette-Guérin)(rBCG)载体。为了能够锚定细菌膜中的PspA，使PspA-衍生的基因片段与Mtb19脂蛋白融合(参见Langermann S.等人，J.Exp.Med.1994,180,2277-2286)。存在于应用PspA作为疫苗抗原相关的安全性担忧，因为N-末端区可以模拟人肌球蛋白，且由此免疫接种包括该区的免疫原可能导致组织交叉反应性抗体。因此，近期进行尝试使用PspA的其它区作为抗原。可以适合于包含入下一代肺炎球菌疫苗的另一个PspA区是富含脯氨酸的区。尽管PspA的富含脯氨酸的区(PRR)是多态的，但是它包含几个保守的基序，包括短氨基酸基序，如PKP、PAPAP、PEKP和存在于一些PspA分子中的高度保守的非脯氨酸区

段(NPB)(Brooks-Walter,A.等人,Infect Immun1999,67,6533-6542;Hollingshead,S.K.等人,Infect Immun,2000,68,5889-5900;Daniels,C.C.等人,Infect Immun,2010,78,2163-2172.)。尽管PRR不含免疫显性表位,但是使用酶联免疫测定法(EIA)与硫氧还蛋白(Trx)融合蛋白作为抗原在儿童中检测到了针对该PspA部分的抗体(Melin,M.等人,Vaccine2012,30,7157-7160)。因为NPB是高度保守的,所以作者推定针对PRR的抗体可以与大部分菌株通过其NPB表位识别发生交叉反应。

[0009] PspA的PRR具有小尺寸(至多约100个氨基酸)且由此在单独地用作抗原时可能不足以是免疫原性的。已经生产了大肠杆菌(Escherichia coli)Trx融合蛋白,且已经在静脉内感染小鼠模型中证实了其保护潜能(WO 2007/089866和Daniels,C.C.等人,Infect Immun,2010,78,2163-2172)。然而,Trx融合蛋白作为疫苗可能不适合于人体应用,这归因于诱导对非保护性Trx表位的免疫应答的潜能和天然PR表位的差的结构代表。此外,NPB或富含脯氨酸(PR)的序列还可以包含非保护性表位,且由此对于集中对保护性表位的免疫应答用于功效是关键。

[0010] 可以在另外的肺炎球菌蛋白中发现了类似的PR序列,包括表面蛋白PspC(也称作CbpA或Hic)和PhtX蛋白PhtA、PhtB、PhtD和PhtE,且衍生自这类另外的肺炎球菌蛋白的富含脯氨酸的区可以适合于包含入下一代肺炎球菌疫苗,如来自PspA的富含脯氨酸的序列。

[0011] 来自其它革兰氏阳性菌的几种免疫原性细菌表面蛋白包含富含脯氨酸的序列,其同样可以被抵抗这些病原体的疫苗靶向。这些蛋白质包括来自其它链球菌的表面蛋白,例如酿脓链球菌(S.pyogenes)的M6、SclA和SclB蛋白;无乳链球菌(S.agalactiae)的CBeta(bac)和BibA;或变异链球菌(S.mutans)的P1粘附素;或来自金黄色葡萄球菌(S.aureus)的蛋白质。

[0012] 合成的细菌脂肽类似物在疫苗研究中因其佐剂效应和作为肽抗原的载体已经受到广泛关注(Ghielmetti M.等人,Immunobiology 2005,210,211-215)。已经报道了许多脂肽构建体,其中已经使具有已知佐剂效应的脂质与肽偶合,生成自我-佐剂性疫苗候选物。特别充分的研究在于三棕榈酰基-S-甘油基半胱氨酸(N-棕榈酰基-S-(2,3-双-(O-棕榈酰氧基)-丙基)-半胱氨酰基-或Pam3Cys)和二棕榈酰基-S-甘油基半胱氨酸(2,3-双-(O-棕榈酰氧基)-丙基)-半胱氨酰基-或Pam2Cys)。在革兰氏阴性菌的内膜和外膜的脂蛋白成分中发现了这些脂质部分。专利申请WO 98/07752描述了脂肽类在药物靶向目的中的用途,其中肽部分可以是能够诱导三重螺旋结构的胶原蛋白-样序列。专利申请WO 2008/068017描述了合成病毒样颗粒,其包含螺旋脂肽束并且具有带有脂质芯和肽外表面的球形或球状结构。脂肽类的肽链包含卷曲螺旋结构域。脂肽结构单元的肽链中的卷曲螺旋结构域的特性决定组合成合成病毒样颗粒的结构单元数量。

[0013] 发明概述

[0014] 本发明涉及由如下组成的脂肽结构单元:(1)包含平行卷曲螺旋结构域的肽链,作为自立式(self-standing)的无脂质肽,其形成平行的二聚化、三聚化或更高级寡聚化螺旋束;(2)富含脯氨酸的(PR)肽抗原,其包含至少一个带负电荷的和至少一个带正电荷的氨基酸,且其中至少15%的氨基酸是脯氨酸,所述肽抗原任选地连接至另外的抗原;和(3)包含2个或3个长烃基链的脂质部分;其中所述肽链、所述PR肽抗原和所述脂质部分直接或通过连接体共价连接。优选地,包含平行的卷曲螺旋的肽链在一端上连接至所述PR肽抗原,而在另

一端上连接至所述脂质部分。

[0015] 这些脂肽结构单元聚集成螺旋脂肽束和合成病毒样颗粒(SVLP)。所述PR抗原呈递在SVLP表面上强化了对PR表位的免疫应答。

[0016] 优选包含来源于链球菌和/或葡萄球菌、更优选来源于肺炎链球菌(Streptococcus pneumoniae)的PR肽抗原的脂肽结构单元。

[0017] 本发明还涉及生产脂肽结构单元、螺旋脂肽束和合成病毒样颗粒的方法;携带PR肽抗原的脂肽结构单元、螺旋脂肽束和合成病毒样颗粒在制备疫苗中的用途;和使用这类疫苗的接种的方法。本发明同样涉及包含携带PR抗原的合成病毒样颗粒的药物制品。

[0018] 本发明包含来源于链球菌和/或葡萄球菌、特别是来源于肺炎链球菌的PR肽抗原的组合物用于诱导对肺炎链球菌或其它革兰氏阳性菌的免疫应答,并且用于预防或治疗感染性疾病,例如由肺炎链球菌导致的肺炎球菌性疾病。

[0019] 附图简述

[0020] 图1:来自用脂肽15(封闭型圆圈和正方形)或白矾佐剂化的重组PspA(rPspA,封闭型三角形)免疫两次的BALB/c小鼠的血清和来自未免疫的对照组的血清(开放符号)中的IgG ELISA终点滴度。符号表示来自个体小鼠的终点滴度血清,直线表示中间值。对表示富含脯氨酸的区的肽(PR肽)和包含完整N-末端α-螺旋部分、富含脯氨酸的区和非脯氨酸区段(rPspA)的重组PspA蛋白测定滴度。

[0021] 图2:使用递增剂量的肺炎链球菌血清型1免疫和未免疫的BALB/c小鼠的攻击后以天数(D)计的存活时间。存活百分比(%S)显示在y-轴上,且免疫原和攻击剂量(以CFU计)显示在右侧。给小鼠免疫2次脂肽15或rPspA+白矾,然后通过静脉内攻击。

[0022] 发明详述

[0023] 本发明涉及脂肽结构单元,其由如下组成:

[0024] (1)包含平行卷曲螺旋结构域的肽链,作为自立式的无脂质肽,其形成平行的二聚化、三聚化或更高级寡聚化螺旋束;

[0025] (2)富含脯氨酸的(PR)肽抗原,其包含至少一个带负电荷的和至少一个带正电荷的氨基酸,且其中至少15%的氨基酸是脯氨酸,所述肽抗原任选地连接至另外的抗原;和

[0026] (3)包含3个或优选2个长烃基链的脂质部分(LM);其中所述肽链、所述PR肽抗原和所述脂质部分直接或通过连接体、特别是2个不同的连接体共价连接。

[0027] 优选地,包含平行的卷曲螺旋的肽链在一端上连接至所述PR肽抗原,而在另一端上连接至所述脂质部分。

[0028] 肽链(PC)包含平行的卷曲螺旋结构域。这类卷曲螺旋结构域结合成确定的螺旋束,例如结合成二聚化、三聚化、四聚化、五聚化、六聚化或七聚化束。平行的卷曲螺旋结构域不同于反向平行的卷曲螺旋,其中单体肽链回环从而通过以反向平行排列的方式排列2个(或多个)单体肽的部分结构域以形成螺旋子结构。所述平行卷曲螺旋结构域可以包含12－120个氨基酸残基,优选21－80个氨基酸残基。卷曲螺旋结构域包含2个或更多个连续重复的模式(通常为七个重复单元,其中7个结构位置标记为a-g,其中a和d表示疏水性残基),其作为自立式的无脂质肽类具有自我组装成平行卷曲螺旋螺旋束的特性(Lupas A.N.,Gruber M.;The structure of alpha-helicalcoiled coils,Adv.Protein Chem..2005,70,37-78)。所述肽链必须多聚化成确定寡聚化状态的平行的卷曲螺旋螺旋束

(例如二聚体、三聚体、四聚体、五聚体、六聚体或七聚体,特别是二聚体、三聚体、四聚体或五聚体)。优选的肽序列是非人的序列以避免在应用于人体接种时的自体免疫疾病风险。

[0029] 所述脂肽结构单元还包含富含脯氨酸的(PR)肽抗原,其包含至少一个带负电荷的和至少一个带正电荷的氨基酸。本文考量的带电荷的氨基酸是带有侧链的在生理pH下带正电荷或带负电荷的氨基酸。在天然存在的氨基酸中,本文考量的最常见的带正电荷的氨基酸是赖氨酸、精氨酸和组氨酸;最常见的带负电荷的氨基酸是谷氨酸和天冬氨酸。如果至少15%的氨基酸是脯氨酸,则肽被视为"富含脯氨酸"的。优选富含脯氨酸的肽类,其包含至少一个谷氨酸残基和至少一个赖氨酸或精氨酸残基。

[0030] 优选的PR肽抗原来源于链球菌和/或葡萄球菌,例如来源于革兰氏阳性菌,其选自酿脓链球菌、无乳链球菌、变异链球菌、猪链球菌(Streptococcus suis)、马链球菌(Streptococcus equi)、缺乳链球菌(Streptococcus dysgalactiae)、大消化链球菌(Peptostreptococcus magnus)和金黄色葡萄球菌。

[0031] 优选地,这种PR肽抗原来源于蛋白质PspA和/或PspC或其它针对肺炎球菌感染起保护的蛋白质,包括PhtX蛋白。

[0032] "来源的"是指所述肽抗原的氨基酸序列或氨基酸序列的主要部分(即50%或以上)来源于一种或多种天然存在的蛋白质,而0%至最多50%的氨基酸序列重新设计。还包括PR肽抗原,其包含来自不同PspA/PspC分子和/或来自其它包含富含脯氨酸的片段的蛋白质的富含脯氨酸的序列的组合。"富含脯案的片段"是指该片段中包含的至少15%的氨基酸是脯氨酸。

[0033] 特别关注的富含脯氨酸的(PR)肽抗原来源于肺炎链球菌且在PspA或PspC的螺旋区的C-末端后和非脯氨酸区段(如果PspA或PspC序列包含非脯氨酸区段)或重复区前紧邻定位。或者,PR肽抗原位于肺炎球菌多组氨酸三联蛋白质(PhtX)的中心区。或者,所述PR肽抗原位于革兰氏阳性菌的PR蛋白质区内,所述革兰氏阳性菌选自酿脓链球菌、无乳链球菌、变异链球菌、猪链球菌、马链球菌、缺乳链球菌、大消化链球菌和金黄色葡萄球菌。例如变异链球菌的P1粘附素的中心区。

[0034] 保护性PR肽抗原通过下列步骤鉴定:测序PspA和/或PspC和/或其它来自临床隔离群的基因,选择部分PR区,合成PR肽,并且使其与并入或为脂肽部分的肽链(PC)缀合。

[0035] 通过施用脂肽缀合物和自其获得的合成病毒样颗粒并且观察在肺炎球菌性脓毒症或其它由链球菌感染导致的疾病的动物模型中的活性和功效测试PR肽抗原的功效。

[0036] PR肽抗原任选地与另外的抗原连接。考量的另外的抗原是其它的肺炎球菌肽类或多糖类,特别是具有氨基酸序列SEQ ID NO:113至119的肽类;和下述其它抗原。

[0037] 所述PR肽抗原直接或通过连接体在该PR肽抗原的N-或C-末端上缀合,并且连接至包含卷曲螺旋结构域的肽链(PC)N-或C-末端或任选地连接至氨基酸侧链。或者,所述PR肽抗原通过该PR肽抗原的侧链残基例如末端或内部天冬氨酸、谷氨酸、赖氨酸、鸟氨酸或半胱氨酸侧链与包含卷曲螺旋结构域的肽链(PC)缀合。所考量的连接体是2—20个氨基酸的短肽;羟基烷基-或氨基烷基-羧酸;取代或未取代的聚烯氧基二醇类,优选包含1至12个C_2和/或C_3烯氧基单元;聚烯氧基二醇嵌段共聚物(例如普流罗尼克);单糖、二糖和寡糖类,其可以在一个或多个位置上包含乙酰基、甘油-磷酸或其它取代基;多糖类,例如聚(唾液酸)及其衍生物(例如肽缀合物)、蛋白原(proteinogenic)或非蛋白原氨基酸;和C_1-C_8饱和或不饱

和烃类，且可以包含如下官能团的一个或多个：二硫键、胺、酰胺、乙缩醛、酯、醚、硫醚、腙、酰肼、亚胺、肟、脲、硫脲、碳酸酯、亚氨基碳酸酯、脒、酰胺、二酰亚胺、烷基琥珀酰亚胺(其也可以水解成酰胺)、磺酰胺、砜或包含一个或多个选自氮和氧的原子的杂环，优选三唑。另外考量的是上述的连接体的组合，包括实施例中使用的那些。

[0038] 用于缀合肽类或其它抗原与抗原递送系统例如载体蛋白、聚合物、树状聚体、纳米粒或病毒样颗粒的任意方法可以用于使PR肽抗原与包含平行卷曲螺旋结构域的肽链(PC)缀合。这类方法是本领域技术人员众所周知的，参见，例如Hermanson,G.T,Bioconjugate Techniques,第2版,Academic Press,2008。

[0039] PR肽抗原由5-200个氨基酸、优选8-80个氨基酸组成。多个PR肽缀合物可以与疫苗制剂联用。PR肽抗原也可以一起融合，以便产生长的人工PR肽。PR缀合物还可以与包含另外的肺炎球菌肽类或多糖类的缀合物组合。包含官能团(例如氨基-、卤代-、肼基-、羟基氨基-或硫氢基基团)的氨基酸及其衍生物可以并入PR肽，以便有利于PR肽缀合并且增强稳定性。

[0040] 所述肽链(PC)还可以包含氨基酸序列，其包括一个或多个T-辅助细胞表位和/或促进脂肽结构单元在水中的溶解性的极性残基串。

[0041] 可以并入肽链(PC)的T-辅助表位包括下表1中列出的那些及其变体，其中1、2或3个氨基酸被另外的氨基酸替代或被缺失。

[0042] 表1

[0043]

T-辅助表位	SEQ ID NO:	序列 [a)]
TT830-843	1	QYIKANSKFIGITE
TT1064-1079	2	IREDNNITLKLDRCNN
TT1084-1099	3	VSIDKFRIFCKANPK
TT947-968	4	FNNFTVSFWLRVPKVSASHLET
TT1174-1189	5	LKFIIKRYTPNNEIDS
DTD271-290	6	PVFAGANYAAWAVNVAQVID
DTD321-340	7	VHHNTEEIVAQSIALSSLMV
DTD331-350	8	QSIALSSLMVAQAIPLVGEL
DTD351-370	9	VDIGFAAYNFVESIINLFQV
DTD411-430	10	QGESGHDIKITAENTPLPIA
DTD431-450	11	GVLLPTIPGKLDVNKSKTHI
TT632-651	12	TIDKISDVSTIVPYIGPALN
CTMOMP36-60	13	ALNIWDRFDVFCTLGATTGYLKGNS
TraT1	14	GLQGKIADAVKAKG
TraT2	15	GLAAGLVGMAADAMVEDVN
TraT3	16	STETGNQHHYQTRVVSNANK
HbcAg50-69	17	PHHTALRQAILCWGELMTLA
HbSAg19-33	18	FFLLTRILTIPQSLD
HA307-319	19	PKYVKQNTLKLAT
MA17-31	20	YSGPLKAEIAQRLEDV
MVF258-277	21	GILESRGIKARITHVDTESY
MVF288-302	22	LSEIKGVIVHRLEGV
CS.T3*	23	IEKKIAKMEKASSVFNVVNS
SM Th	24	KWFKTNAPNGVDEKIRI
PADRE1 [b)]	25	aKFVAAWTLKAAa
PADRE2 [b)]	26	aK-Chx-VAAWTLKAAa

[a)] 参考文献：SEQID NO：1-5和17-20：Eur. J. Immunol. 2001,31, 3816 -3824；SEQID NO：6-12：JID 2000,181, 1001-1009；SEQID NO：13-16, 21-22和24：US 5,759,551；SEQID NO：23：Nature1988, 336, 778-780；SEQID NO：25-26：Immunity1994, 1, 751 -761.

[b)] „a“表示D-Ala且„Chx“表示丙氨酸环己酯。

[0044] 所述肽链(PC)的总长度优选为21至200个氨基酸残基，更优选21至120个氨基酸残基。

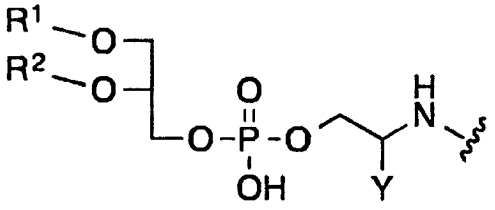
[0045] 所述脂质部分(LM)包含脂质锚，其带有2或3个、优选2个长烃基链和组合这些烃基链并且使其直接或通过连接体连接至肽链(PC)的结构。该脂质部分还可以再次连接至PR肽，由此与包含平行卷曲螺旋的肽链缀合，然而，优选所述脂质部分连接至所述肽链。优选

的脂质部分是包含2或3个、优选2个延长的烃基链的脂质。

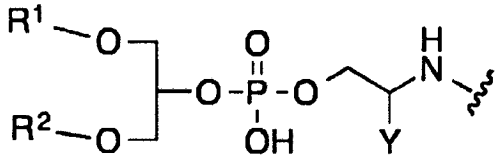
[0046] "长烃基"是指至少7个碳原子的直链烷基或烯基，例如8至50个C原子、优选8至25个C原子组成的直链烷基或烯基。烯基优选在链上具有1、2或3个双键，其各自具有E或Z几何形状，正如通常在天然脂肪酸和脂肪醇中发现的。在"长烃基"的定义中还包括支链烷基或烯基，例如在从链末端开始计数为第二或第三个碳原子上带有甲基或乙基取代基的烷基，例如如在2-乙基-己基中。

[0047] "低级烷基"是指1至7个碳原子(C_1-C_7)、优选1至4个碳原子(C_1-C_4)的烷基，例如甲基、乙基、正丙基、异丙基、正丁基、仲丁基、异丁基或叔丁基。

[0048] 本发明特别优选的脂质部分是式Z^1至Z^8的那些，

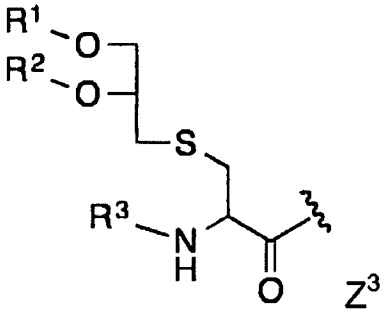
[0049] 

Z^1



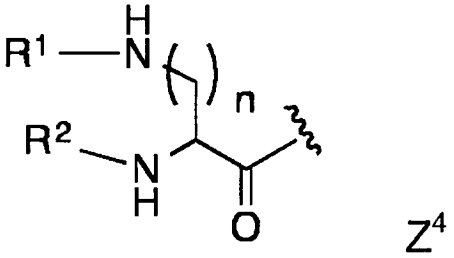
Z^2

[0050] 其中R^1和R^2是长烃基或长烃基-C=O，且Y是H或COOH，

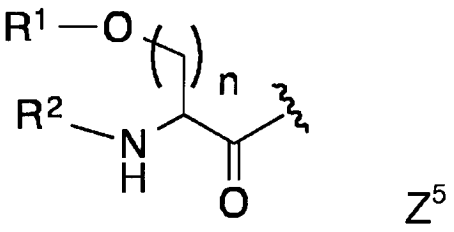
[0051] 

Z^3

[0052] 其中R^1、R^2和R^3是长烃基或长烃基-C=O或R^1和R^2是长烃基或长烃基-C=O，且R^3是H或乙酰基或低级烷基-C=O，

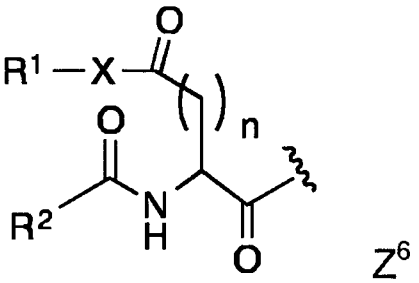
[0053] 

Z^4

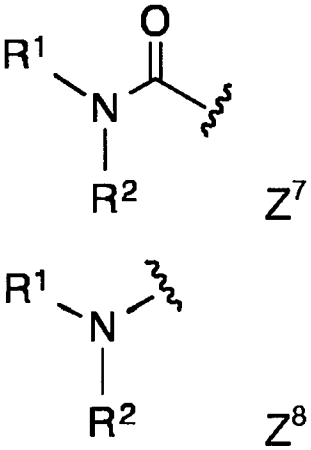


Z^5

[0054] 其中R^1和R^2是长烃基或长烃基-C=O，且n是1、2、3或4，

[0055] 

[0056] 其中R^1和R^2是长烃基，X是O或NH，且n是1、2、3或4，或

[0057] 

[0058] 其中R^1和R^2是长烃基。

[0059] 所述脂质部分包含至少2个长烃基链，例如在脂肪酸中发现的，例如如在Z^1—Z^8中。一个优选的脂质部分是不同类型的磷脂，例如式Z^1或Z^2的磷脂，其具有酯-或醚-连接的延长的烷基或烯基链，例如1,2-二棕榈酰基-sn-甘油-3-磷酸乙醇胺的对映体或非手性类似物，例如1,3-二棕榈酰基-甘油-2-磷酸乙醇胺。优选的脂质部分是三-或二-棕榈酰基-S-甘油基半胱氨酰基残基(类型Z^3)或类型Z^4至Z^8的脂质部分。最优选实施例中所述的脂质部分。

[0060] 所述肽链(PC)在一端或接近一端上、即N末端或C末端、优选N末端共价连接至脂质部分(LM)。所述脂质部分可以如下所述中被直接连接，

[0061] LM-PC (1)

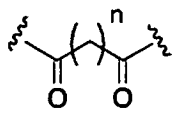
[0062] 或通过连接体(L)连接，如下所示，

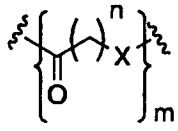
[0063] LM-L-PC (2)。

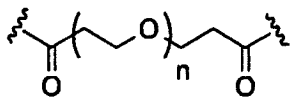
[0064] 如果所述肽链(PC)和所述脂质部分(LM)直接连接，这优选通过肽链(PC)的脂质部分羰基官能团与氨基官能团(例如N末端氨基官能团)之间的酰胺键来实现。具体的脂质部分Z^1、Z^2和Z^8优选通过其肽链(PC)的胺官能团与羧基官能团(例如C末端羧基官能团)之间的酰胺键连接。

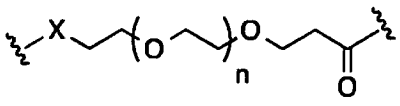
[0065] 本领域普通技术人员显而易见，存在各种适合的连接体和偶合策略，包括、但不限于基于二羧酸衍生物的连接体、包含一个或多个乙二醇单元、氨基酸残基(包括α-、β-、γ-、δ-氨基酸)或糖(碳水化合物)单元或包含杂环的连接体。所考量的具体连接体是连接体L^1至L^{16}，其中n是1至45，且m是1至45，例如，其中n是1至20，且m是1至20，如连接官能团C=O和/或X所示，其中X是O或NH：

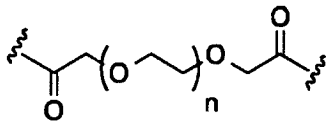
[0066]

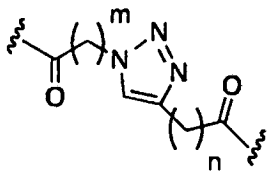

L1

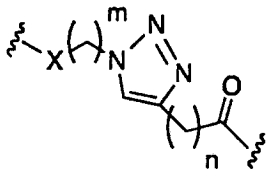

L2

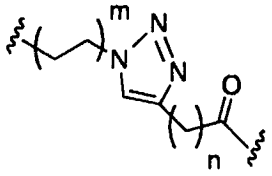

L3

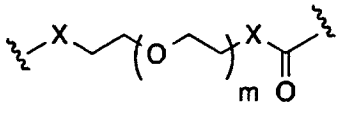

L4

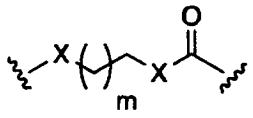

L5

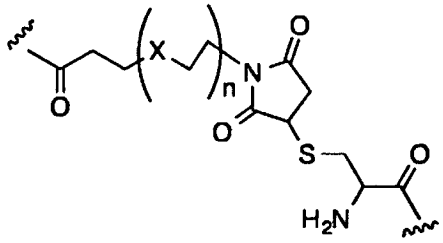

L6

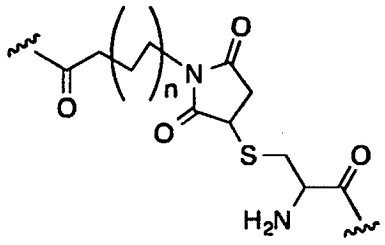

L7

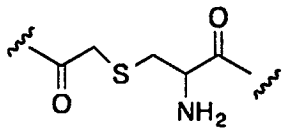

L8

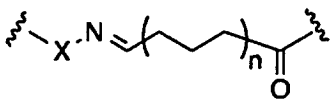

L9

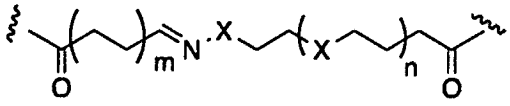

L10

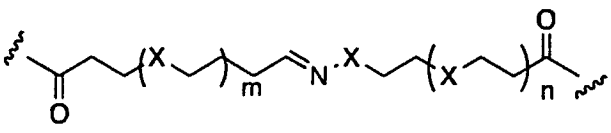

L11


L12


L13


L14


L15


L16

[0067] 最优选实施例中所述的连接体。

[0068] 具体的连接体L^1至L^{16}如下被连接至LM和PC：

[0069] 对L^1至L^{16}所示的羰基官能团可以通过酰胺键连接至适合的脂质部分(LM)的氨基官能团和/或肽链(PC)的氨基官能团，例如N末端氨基官能团。或者，对L^1至L^{16}所示的羰基官能团可以通过替代相应的羰基官能团、特别是脂质部分Z^3-Z^7连接至脂质部分(LM)。

[0070] 对L^1－L^{16}所示的官能团X(具有含义为NH或O)可以通过酰胺键(X＝NH)或通过酯键(X＝O)连接至适合的脂质部分(LM)的羰基官能团和/或肽链(PC)的羧基官能团,例如C末端羧基官能团。

[0071] L^8的末端CH_2基团可以连接至适合的脂质部分(LM)的氨基官能团、肽链(PC)的氨基官能团例如N末端氨基官能团或适合的脂质部分(LM)的羰基官能团。

[0072] 作为在这种方面中理解的"接近一端/接近一末端"是指所述脂质部分或所述连接体分别键合至所述肽的从N末端或C末端计算的第一、第二、第三、第四或第五位氨基酸。所述脂质部分可以直接或通过连接体连接至所述肽结构的主链或连接至接近该末端的这些氨基酸之一的侧链。

[0073] "卷曲螺旋结构域"通过谨慎地选择适合的氨基酸序列设计,这些适合的氨基酸序列通过自主自我结合形成螺旋的热力学稳定的、α-螺旋平行束。

[0074] 卷曲螺旋结构域包括基于规范串连七重序列重复单元的肽类,所述规范串连七重序列重复单元形成右旋两亲性α-螺旋,其然后与左旋超螺旋装配形成螺旋束。还包括来自基于不规范的、非七重的重复单元构成的肽,所述基于不规范的、非七重的重复单元形成不一定为左旋乃至有规则的超螺旋的卷曲螺旋。

[0075] 规范的卷曲螺旋广泛地出现在天然存在的生物活性肽类和蛋白质中,并且也已经重新设计。阐明了一组规则,其用于设计采用确定的寡聚化状态、拓扑学和稳定性的螺旋束的卷曲螺旋肽类(例如二聚体、三聚体、四聚体、五聚体、六聚体或七聚体)。这些规则允许设计者构建与指定目标结构相容的肽序列。最重要地,规范的卷曲螺旋肽类的序列包含特征性七-残基基序,其典型的重复3-10次。一个七基序内的位置在传统上称作abcdefg,其中大部分(但不是全部)是出现在a和d位置上且在另外位置上一般为极性的、有利于螺旋的残基的疏水性残基。沿肽链的串连七基序具有a－d残基之间的平均分离,使得它们落在α-螺旋的一面。当两个或多个螺旋共同填充入卷曲螺旋束时,该螺旋的疏水性表面结合并且彼此卷绕,以便疏水性表面之间的接触最大化。可以出现在七个重复单元内的每个位置上的残基类型影响螺旋束的稳定性和寡聚化状态。一般地,大部分疏水性残基(Ala、Ile、Leu、Met、Val)或芳族疏水性侧链(Phe、Trp和Tyr)用于a和d位置上。其余的b、c、e、f和g位置倾向于比a和d位置更具有许可性,不过,极性和有利于螺旋的残基(Ala、Glu、Lys和Gln)是有利的。a和d位置上的残基的选择可以影响卷曲螺旋的寡聚化状态(即二聚体vs.三聚体)。因此,当非-β-支链残基(例如Leu)出现在d位置上时,二聚体是有利的;在这些位置上,β-支链残基(Val和Ile)不利于二聚体。另一方面,在二聚体中,β-支链残基(Ile、Val)优选在a位置上。另一个规则在于a＝d＝Ile或Leu有利于三聚体,其用于设计特异性地形成平行三聚体的卷曲螺旋。这些和其它设计规则更详细地在Woolfson,D.N.,Adv.Prot.Chem.2005,70,79-112中讨论。

[0076] 七基序编码通过其疏水性表面寡聚化的两亲性α-螺旋。卷曲螺旋结构域包括至少3个串连七重复单元基序。每个链上七重复单元的上限数将影响螺旋束的稳定性。它主要受长肽类化学合成的可行性的限制,但包含多于3个的七重复单元(例如4、5、6、7和8个七重复单元)的序列是优选的。下文讨论的实施例形成三聚化α-螺旋卷曲螺旋,但本发明同样涉及二聚化、四聚化、五聚化、六聚化和七聚化卷曲螺旋结构域。

[0077] 本发明的卷曲螺旋结构域可以具有较长的重复单元,例如11-残基重复单元和15-

残基重复单元，例如存在于天然存在的卷曲螺旋上。因此，当使用具有除七之外的周期性的卷曲螺旋基序时，形成聚集物结构所需的螺旋束也可以产生。具有非常规的周期性的卷曲螺旋也是可能的。在许多天然存在的卷曲螺旋中，未打断的七重复单元模式可以包含各种不连续性。两个常见的不连续性是一个残基插入七重复单元模式，以及插入3或4个残基。例如，在流感血凝素的三聚化卷曲螺旋中观察到一个残基插入。其它天然存在的卷曲螺旋展示出不是七的周期性，例如，在海葡萄嗜热菌(Staphylothermus marinus)的表面层蛋白质tetrabrachion中发现的11个残基的有规则的周期性(称作十一体(hendecads))。

[0078] 在病毒壳蛋白质类中天然存在的卷曲螺旋肽序列的其它实例是在HIV-1的gp41外被蛋白和RSV的F-糖蛋白中形成三聚化螺旋束的卷曲螺旋基序。这些卷曲螺旋结构域包括在本发明的卷曲螺旋结构域的定义中。

[0079] 优选的卷曲螺旋肽类应当包含3-8个串连连接的七基序。卷曲螺旋内的七基序口可以具有相同的序列，或它们可以各自具有不同的序列。在所有情况下，一个七基序内的7个氨基酸的7个位置使用字母设计：a b c d e f g。因此，卷曲螺旋肽包含具有位置$(abcdefg)_{3-8}$的氨基酸序列。

[0080] 优选包含3-8个串连连接的七基序的卷曲螺旋肽序列，其中每个七基序(abcdefg)中的位置a和d包含属于下文定义的组1和/或组2的α-氨基酸。此外，所有a和d位置的不超过2个可以被属于组3的任意氨基酸残基占据，而所有a和d位置的不超过1个可以被属于组4或组5的任意氨基酸残基或甘氨酸占据。此外，在位置b、c、e、f和g上，属于组3、4和5的α-氨基酸是优选的，但属于组1和组2的氨基酸是允许的，外加在任意一个七基序内的这些位置中不超过1个可以是甘氨酸，但都不是脯氨酸。

[0081] 组1包含具有小至中等大小疏水性侧链的α-氨基酸残基。疏水性残基是指在生理pH下不带电荷的并且被水溶液排斥的氨基酸侧链。这些侧链一般不含氢键供体基团，例如伯和仲酰胺类、伯和仲胺类及其相应的质子化盐、硫醇类、醇类、脲类或硫脲类。然而。它们可以包含氢键接受体基团，例如醚类、硫醚类、酯类、叔酰胺类或叔胺类。该组中遗传编码的氨基酸包括丙氨酸、异亮氨酸、亮氨酸、甲硫氨酸和缬氨酸。

[0082] 组2包含具有芳族或杂芳族侧链的氨基酸残基。芳族氨基酸残基是指疏水性氨基酸，其具有包含至少一个环的侧链，该环具有共轭的芳香π(pi)-电子系统。此外，它可以包含另外的疏水性基团，例如低级烷基、芳基或卤素、氢键供体基团例如伯和仲胺类及其相应的质子化盐、伯和仲胺类、醇类和氢键接受体基团，例如醚类、硫醚类、酯类、叔酰胺类或叔胺类。遗传编码的芳族氨基酸包括苯丙氨酸和酪氨酸。杂芳族氨基酸残基是指疏水性氨基酸，其具有包含至少一个环的侧链，该环具有并入了至少一个杂原子例如O、S和N的共轭芳香π-系统。此外，这类残基可以包含氢键供体基团，例如伯和仲酰胺类、伯和仲胺类及其相应的质子化盐、醇类和氢键接受体基团，例如醚类、硫醚类、酯类、叔酰胺类或叔胺类。遗传编码的杂芳族氨基酸包括色氨酸和组氨酸。

[0083] 组3包含含有带有极性不带电荷的残基的侧链的氨基酸。极性不带电荷的残基是指亲水性侧链，其在生理pH下不带电荷，但不被水溶液排斥。这类侧链典型地包含氢键供体基团，例如伯和仲酰胺类、伯和仲胺类、硫醇类和醇类。这些基团可以与水分子形成氢键网状构造。此外，它们还可以包含氢键受体基团，例如醚类、硫醚类、酯类、叔酰胺类或叔胺类。遗传编码的极性不带电荷的氨基酸包括天冬酰胺、半胱氨酸、谷氨酰胺、丝氨酸和苏氨酸。

[0084] 组4包含含有具有极性阳离子残基及其酰化衍生物的侧链的氨基酸，所述酰化衍生物例如酰氨基-衍生的残基和脲-衍生的残基。极性阳离子侧链是指碱性侧链，其在生理pH下质子化。遗传编码的极性阳离子氨基酸包括精氨酸、赖氨酸和组氨酸。瓜氨酸是脲-衍生的氨基酸残基的实例。

[0085] 组5包含含有具有极性阴离子残基的侧链的氨基酸。极性阴离子是指酸性侧链，其在生理pH下脱质子化。遗传编码的极性阴离子氨基酸包括天冬氨酸和谷氨酸。具体的极性阳离子残基是-$(CH_2)_aCOOH$，其中a是1至4。

[0086] 更优选包含3－8个串连连接的七基序的卷曲螺旋肽序列，其中每个七基序(abcdefg)可以具有如下序列的任意一种：

[0087] 1xx1xxx(分别是指位置abcdefg)；

[0088] 1xx2xxx(分别是指位置abcdefg)；

[0089] 2xx1xxx(分别是指位置abcdefg)；或

[0090] 2xx2xxx(分别是指位置abcdefg)；

[0091] 其中1是来自组1的遗传编码的氨基酸，2是来自组2的遗传编码的氨基酸，且其中x是来自组1、2、3、4或5的遗传编码的氨基酸或甘氨酸。

[0092] 同样优选在天然存在的肽类和蛋白质中鉴定的卷曲螺旋肽序列，但不包括人体来源的那些。例如，它们是在病毒和细菌蛋白中鉴定的卷曲螺旋。

[0093] 本发明还涉及携带PR肽抗原的合成病毒样颗粒和制备这类合成病毒样颗粒的方法，包括将所述脂肽结构单元溶于适合的载体，优选水性缓冲系统(例如缓冲盐水或未缓冲的盐水)。在制备合成病毒样颗粒后，例如，通过冻干或喷雾干燥除去溶剂。

[0094] 本发明还涉及引起免疫应答的方法，其中将免疫原性有效量的携带如本文所述的PR肽抗原的合成病毒样颗粒施用于动物。尽管温血动物、尤其是人类在本文中被视为最优先考量的，但是可以使用任意的动物。

[0095] 本发明还涉及疫苗(或同样涉及任意的其它药物制品或药物)，其包含一种或多种携带PR肽抗原的合成病毒样颗粒作为主要或另外的活性成分所述颗粒是单独的或与药学上可接受的载体的组合。

[0096] 所述疫苗还可以包含一种或多种佐剂，例如无机盐(例如氢氧化铝、磷酸铝、硫酸铝、磷酸钙)、单磷酸类脂A(MPL)、包含皂苷类的植物提取物(例如QS-21)、咪唑并喹啉类(例如咪喹莫特)、胞壁酰二肽类和三肽类、脂肽类、水包油型乳剂(例如Montanide ISA 720)、细胞因子(例如IL-2或GM-CSF)、分支杆菌和细菌衍生物(例如弗氏完全佐剂)、BCG、核酸衍生物(例如聚C)和本领域技术人员已知的其它佐剂。

[0097] 疫苗的一些成分还可以被包封在可生物降解的聚合物中或与之连接，例如，所述可生物降解的聚合物可以用于控释，例如聚乳酸、聚-ε-己内酯、聚羟基丁酸、聚原酸酯类、聚乙缩醛类、聚二氢吡喃类、聚氰基丙烯酸酯类和交联或水凝胶的两亲性嵌段共聚物；或疫苗的一些成分可以被配制在脂质体中。

[0098] 按照本身已知的方式制备疫苗，例如，通过常规的溶解和冻干方法，和/或该疫苗可以包含赋形剂，例如防腐剂、稳定剂、湿润剂、张度调节剂和/或乳化剂、增溶剂、用于调节渗透压的盐和/或用于稳定pH的缓冲物质。

[0099] 所述疫苗可以是液体形式或固体(例如冻干)形式，并且可以通过常规的众所周知

的灭菌技术灭菌或进行无菌过滤。可以将得到的水溶液包装照此使用，或冻干、喷雾干燥或按照另一种方式除去溶剂。固体形式可以在施用前与无菌稀释剂(例如水)组合，或可以将其照此施用。同样，所述疫苗可以包含乳剂、分散液或混悬液或适合于预期的施用途经的任意其它形式。

[0100] 所述疫苗可以通过任意适合的肠或胃肠外途经施用，例如鼻内、口服、舌下、肌内、真皮内、透皮和皮下或经皮途经。还可以使用本领域公知的其它途经。

[0101] 装置可以用于施用，例如常用的针头和注射器、显微针、用于施用固体的弹果装置(ballistic devices)(例如在WO 99/27961中)、贴片(例如在WO98/20734中)、无针头注射系统(例如在WO01/054539中)、喷雾装置等，视剂型和施用途经的不同而定。可以给装置预填充或涂布疫苗。

[0102] 所述疫苗在适合的剂型中包含约0.05%－约50%的活性成分。用于胃肠外施用的单位剂型是，例如安瓿、预装注射器或小瓶，例如包含在约0.25ml－1.5ml的剂量体积中的约0.0001mg－约0.75g活性成分的小瓶。

[0103] 活性成分的剂量取决于预期的接受者(例如物种)、其年龄、体重和个体情况和施用途经。用于特定活性成分和特定目标群体的最佳剂量可以通过标准研究确定，包括观察在受试者中的适当的免疫应答。

[0104] 足以赋予致病性肺炎球菌或其它革兰氏阳性菌免疫性的疫苗用量通过本领域技术人员众所周知的方法确定。该用量基于疫苗接受者的特征和对肺炎球菌或其它革兰氏阳性菌感染导致的疾病的想要的免疫性水平来确定。

[0105] 将所述疫苗作为单剂量或两次或多次剂量在足够的间隔时间点施用。该疫苗还可以与另外的疫苗一起施用。例如，该疫苗可以与另外的疫苗在最初的加强方案中联用。

[0106] 所述疫苗用于预防或治疗目的或它们两者，用于预防和/或治疗菌血症和其它由肺炎链球菌感染导致的疾病，例如肺炎、急性窦炎、中耳炎、脑膜炎、菌血症、败血病、骨髓炎、脓毒性关节炎、心内膜炎、腹膜炎、心包炎、蜂窝织炎或脑脓肿或carriage。该疫苗同样用于预防或治疗目的或它们两者，用于预防和/或治疗由A型链球菌或变异链球菌导致的疾病，例如咽头炎、脓皮病、风湿热、肾小球肾炎或骨疽。

[0107] 例如，所述疫苗针对菌血症、肺炎和脑膜炎进行保护或针对侵害性肺炎球菌性疾病(IPD)；感染，其中肺炎链球菌可以分离自血液或另一种正常的无菌地点进行保护；或针对菌血症、肺炎和中耳炎进行保护。或者，所述疫苗针对咽头炎、脓皮病、风湿热、肾小球肾炎或骨疽进行保护。

[0108] 所述疫苗可以施用于不同的目标群体，包括首次接种或感染或不能对感染或接种预先应答的群体；适当年龄的老年人(例如超过65、75或85岁)；处于风险升高的成年人，例如在健康机构中的人；具有风险因素的年轻的成年人；免疫受损的人或儿科人群。

[0109] 本发明还涉及制备疫苗的方法，包括混合携带PR肽抗原的合成病毒样颗粒与携带另外的抗原的合成病毒样颗粒或不由合成病毒样颗粒携带的抗原。

[0110] 本发明还涉及针对携带保护性PR肽抗原的合成病毒样颗粒的抗体，尤其是针对PR肽抗原的抗体。针对PR肽抗原的抗体可以与广泛种类的遗传差异的菌株和不同的荚膜血清型发生交叉反应并且延长被动转移模型中的平均存活时间。

[0111] 针对PR肽抗原的保护性抗体可以具有调理吞噬(opsonophagocytic)活性(OPA)。

使用OPA的抗体可以通过适合的测定法例如调理吞噬杀伤测定法(OPKA)、使用来自供体的细胞系或全血在体外检测。保护性抗体还可以介导由其它不牵涉OPA的机制的保护作用。

[0112] 本发明还涉及针对如本文所述的PR肽类的保护性抗体在制备用于治疗和/或预防目的药物制品或药物以及还在制备诊断试剂盒中的用途。

[0113] 并非所有的PR肽类都可以等同地作为疫苗抗原具有保护作用。保护性PR肽类是优选的。可以使用众所周知的方法鉴定它们，例如在适合的链球菌性疾病的动物模型中的攻击研究。例如，在肺炎球菌性脓毒症模型中，可以用携带如本文所述的PR肽抗原的合成病毒样颗粒免疫小鼠，且随后用致死剂量的肺炎链球菌通过静脉内攻击。在这种模型中，用携带保护性PR肽抗原的合成病毒样颗粒免疫的小鼠随后具有明显比免疫的对照组小鼠更长的存活时间。

[0114] PR肽抗原还可以来自其它致病性链球菌，例如酿脓链球菌、无乳链球菌、变异链球菌、马链球菌、猪链球菌、缺乳链球菌、大消化链球菌或其它致病性革兰氏阳性菌，例如金黄色葡萄球菌，且携带这些PR肽抗原的合成病毒样颗粒同样用于针对这些细菌的疫苗。

[0115] 将优选的来自肺炎链球菌PspA和PspC的PR肽抗原序列采集在下表2中。肽抗原P3是组合2个序列的合成构建体。

[0116] 表2

名称	SEQ ID NO:	序列
P1	27	PAPKPEQPAEQPKPAPAPQPAPAPKPEKT
P2	28	PKPEQPAPAPKPEQPAKPEKPA
P3	29	PAPKPEQPAEQPKPEQPAPAPKPEQPAKPEKP
P4	30	PAPKPEQPAEQPKPA
P5	31	PAPQPAPAPKPEKT
P6	32	QPAEQPKPAPAPQPAP
P7	33	PAPAPKPEQPAEQPKP
P8	34	PAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P9	35	PAEQPKPAPAPQPAPAPKPEKPAEQ
P10	36	PKPAPAPQPAPAPKPEKPAEQPKPEKT
P11	37	KAEKPAPAPQPEQPAPAPKT
P12	38	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPEQPTPA
P13	39	PAPAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPEQPTPAPKT
P14	40	PEQPAPAPKPEQPAPAPKPEQPTPAPKPEQPTPAPKT
P15	41	PKPEQPTPAPKPEQPTPAPKT
P16	42	PKPEQPAEQPKPAPAPQ
P17	43	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPATPKT
P18	44	PKPEQPAKPEKPAEEPTQPEKPATPKT
P19	45	PAPAPQPAPAPKPAPAPQPEKPAEQPKAEKPA
P20	46	PETPAPAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKSA
P21	47	PAPAPKPEQPAPAPKPEKSA
P22	48	PKPEQPAPAPKPEKSA
P23	49	KAEKPAPAPKPEQPVPAPKT
P24	50	PAPAPKPAPAPQPEKPAPAPAPKPEKSA
P25	51	PAPEQPTEPTQPEKPAEETPAPKPEKPAEQPKAEKT
P26	52	PAPKPEKPAEQPKAEKT
P27	53	PAPAPKPEQPAEQPKPAPAPQPEKPAEEPENPAPAP
P28	54	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P29	55	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P30	56	PAPEQPTEPTQPEKPAEETPAPKPEKPAEQPKAEKT
P31	57	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P32	58	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P33	59	APAPKPETPAPAPEAPAPAPAPKPEQPAPAPKPEKS
P34	60	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKPEKT
P35	61	APAPETPAPEAPAEQPKPAPAPQPAPAPKPEKPAEQPKAEKPA

[0117]

P36	62	PQPEQPAPAPKPEQPAPAPKPEQPTPAPKPEHP
P37	63	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPE
P38	64	PAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPP
P39	65	PAPAPAPKPEQPAPAPAPKPEQPAPAPAPKPEQPA
P40	66	PAPAPKPEQPAPAPAPKPEQPAPAPAPKPEQPT
P41	67	PAPAPQPEQPAPAPKPEQPAPAPKPEQPTPAPKPE
P42	68	PAPAPKPEQPAEQPKPAPAPQPAPAPKPEKQ
P43	69	PAPAPQPEQPAPAPQPEQPAPAPKPEQPAPAPKPA
P44	70	PKPEQPTPAPKPEQPTPAPKPEQPTPAPKPEQPT
P45	71	PEKPAPAPEKPAPAPEKPAPA
P46	72	PAPKPAPAPKPAPAPAPKPEKPA
P47	73	PAPAPTPEAPAPAPKP
P48	74	PKPEQPAKPEKPAEEPTQPEKPA
P49	75	PAKPEKPAEEPTQPEKPA
P50	76	PAPAPKPEQPAKPEKPAEEPTQPEKPA
P51	77	PKPEQPAPAPNPEQPAKPEKPAEEPTQPEKPA
P52	78	PKPEQPAPAPAPKPEQPAPAPAPKPEQPA
P53	79	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPA
P54	80	PKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPA
P55	81	PAPAPQPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPA
P56	82	PAPAPKPEQPTPAPKPEQPTPAPKPEQPAPAPKPEQPAPAPKP
P57	83	PARALQPEQPAPAPKPEQPTPAPKPEQPTPAPKPEQPAPAPKP

[0118] 在SEQ ID NO:27－83的优选的PR肽中，1、2或3个氨基酸可以被其它的氨基酸替代。

[0119] 将可选的来自肺炎链球菌和来自酿脓链球菌、无乳链球菌、变异链球菌、猪链球菌、马链球菌、缺乳链球菌、大消化链球菌和金黄色葡萄球菌蛋白质的PR肽抗原序列采集在下表3中。

[0120] 表3

[0121]

名称	SEQ ID NO:	序列 a)
P58	84	SRLEQPSLQPTPEPSPGPQPAPN
P59	85	RPEEPSPQPTPEPSPSPQPAPSNP
P60	86	HWVPDSRPEQPSPQSTPEPSPSPQPAPNPQPAPSNP
P61	87	PKSNQIGQPTLPNNSLATPSPSLPINPGTSHE
P62	88	PEVTPTPETPEQPGEKAPEKSPEVTPTPETPEQP
P63	89	PEVTPTPETPEQPGEKAPEK
P64	90	PEKSPEVTPTPETPEQP
P65	91	KAPEKSPEVTPTPEMP
P66	92	PGKPAPKTPEVPQKPDTAPHTPKTP
P67	93	KPSAPKAPEKAPAPKAPK
P68	94	PAPKAPKASEQSSNPKAPAPKSAP
P69	95	PGPAGPRGLQGPQGPRGDKGET
P70	96	PQAPSTPEKQPEVPESP
P71	97	PETPDAPSTPKDEPQAP
P72	98	PAPVEPSYEAEPTPPTRTPDQAEPNKPT
P73	99	PTYETEKPLEPAPVEPSYEAEPT
P74	100	KPTAPTKPTYETEKPLKPAPVAPNYEKEPT
P75	101	KPVVPEQPDEPGEIEPIP
P76	102	PEVPSEPETPTPPTPEVPAEPGKPVPPAK
P78	103	KYTPKKPNKPIYPEKPKDKTPPTKPDHS
P79	104	PEKPVEPSEPST
P80	105	KPVEPSEPSTPDVPSNPSNPSTPDVPSTPDVPSNPSTPEVPSNP
P81	106	PQVEPNVPDTPQEKPLT
P82	107	KPLTPLAPSEPSQPSIPETPLIPSEPSVPET
P83	108	PEVKPDVKPEAKPEAKPA
P84	109	KPEAKPEAKPA
P85	110	PDVKPEAKPEAKPDVKPEAK
P86	111	PETPDTPKIPELPQ
P87	112	PDTPQAPDTPHVPESPKTPE

a)来自肺炎链球菌的蛋白质的SEQ ID NO: 84-87; 酿脓链球菌: 88-92, 95; 马链球菌: 93-94, 猪链球菌: 96-97; 变异链球菌: 98-100; 金黄色葡萄球菌: 101-103; 大消化链球菌: 104; 缺乳链球菌: 105-107; 无乳链球菌: 108-112。

[0122] 在优选的SEQ ID NO:84-112的PR肽类中,1、2或3个氨基酸可以被其它氨基酸替代。

[0123] 在单独施用时提供有限的保护潜能的另外的序列可以与PR肽抗原、特别是来源于PspA或PspC的区的序列或其它蛋白质缀合,它们在每一第3或第4位上不含脯氨酸和/或包含小于15%的脯氨酸,特别是如下序列:

[0124] QQAEEDYARRSEEEYNRLPQQQPPKAEKP(非脯氨酸区段)(SEQ ID NO:113)

[0125] 和

[0126] AEDQKEEDRRNYPTNTYKTLELEIAESDVEV(来自PspC的螺旋肽)(SEQ ID NO:114)。

[0127] 可以与PR肽抗原组合的另外的序列包括来源于细菌表面蛋白的序列,所述细菌表

面蛋白不含富含脯氨酸的区，包括：

[0128] 来自StkP的序列，优选C-末端79-82个氨基酸，

[0129] SVAMPSYIGSSLEFTKNNLIQIVGIKEANIEVVEVTTAPAGSAEGMVVEQSPRAGEKVDLNKTRVKISIYKPKTTSATP(SEQ ID NO:115)，

[0130] 及其片段；

[0131] 来自PsaA的序列，优选氨基酸250-309：

[0132] SLFVESSVDDRPMKTVSQDTNIPIYAQIFTDSIAEQGKEGDSYYSMMKYNLDKIAEGLAK(SEQ ID NO:116)；

[0133] 来自胆固醇依赖性溶细胞素的序列，例如Ply的第4个结构域，氨基酸360-471：

[0134] NGDLLLDHSGAYVAQYYITWNELSYDHQGKEVLTPKAWDRNGQDLTAHFTTSIPLKGNVRNLSVKIRECTGLAWEWWRTVYEKTDLPLVRKRTISIWGTTLYPQVEDKVEND(SEQ ID NO:117)，

[0135] 及其片段；

[0136] 链球菌聚组氨酸三联蛋白质，例如来自肺炎链球菌PhtD的C-末端一半的片段，例如氨基酸680-770：

[0137] VEHPNERPHSDNGFGNASDHVRKNKVDQDSKPDEDKEHDEVSEPTHPESDEKENHAGLNPSADNLYKPSTDTEETEEEAEDTTDEAEIPQV(SEQ ID NO:118)，

[0138] 或氨基酸771-839：

[0139] ENSVINAKIADAEALLEKVTDPSIRQNAMETLTGLKSSLLLGTKDNNTISAEVDSLLALLKESQPAPIQ(SEQ ID NO:119)，

[0140] 或其片段。

[0141] 在优选的序列SEQID NO:113-119中，1、2或3个氨基酸可以被另其它的氨基酸替代。

[0142] 另外的序列包括PCT/US2012/022127或US 2005/0020813 A1中所述的序列。可选序列包括EP 0280576A2中所述的序列。

[0143] 另外的抗原可以与携带PR肽抗原的合成病毒样颗粒组合。例如，如下蛋白质可以与携带肺炎球菌性PR肽抗原的合成病毒样颗粒组合：WO 98/18931、WO 98/18930、US 6,699,703、US 6,800,744、WO 97/43303和WO 97/37026中鉴定的肺炎链球菌蛋白质；Lyt家族(LytX)、Pht家族(PhtX)、Sp128、1型或2型菌毛蛋白质；其它链球菌抗原，例如WO1993/005155、WO 2002/034771、WO 2002/083859、WO 2002/34771、WO 2003/093306、WO 2004/041157或WO 2005/002619中鉴定的那些；或其它抗原，例如Sp101、Sp130、Sp125或Sp133。

[0144] 糖抗原也可以与携带PR肽抗原的合成病毒样颗粒组合，例如肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、14、18C、19A、19F、22F、23F或33F的荚膜糖。或者，其它糖类可以与PR肽抗原组合，例如来源于其它肺炎链球菌血清型的糖类和/或来自其它革兰氏阳性菌的糖类(例如来源于无乳链球菌、酿脓链球菌和/或金黄色葡萄球菌的糖类)。

[0145] 同样，来自其它革兰氏阳性菌的蛋白质可以与携带PR肽抗原的合成病毒样颗粒组合，例如一种或多种来自酿脓链球菌的蛋白质，包括M蛋白、纤连蛋白结合蛋白(Sfbl)、链球

菌血红素结合蛋白(Shp)或链球菌溶血素S中鉴定的蛋白质(SagA);和/或一种或多种来自金黄色葡萄球菌的蛋白质,例如α-毒素、聚集因子A(ClfA)、胶原结合蛋白(CNA)、纤连蛋白结合蛋白A(FbA)、胞外纤维蛋白原结合蛋白(Efb)、离子调节的表面决定子(Isd)蛋白、青霉素结合蛋白2a(PBP2a)、丝氨酸天冬氨酸重复蛋白(Sdr)和/或IgG结合子(Sbi)。同样,来源于这类蛋白质的肽抗原也可以与携带PR肽抗原的合成病毒样颗粒组合。

实施例

[0146] 缩写::

[0147] Boc,叔丁氧基羰基;

[0148] BSA,牛血清白蛋白;

[0149] DIEA,二异丙基乙胺;

[0150] DMF,N,N-二甲基甲酰胺;

[0151] EDT,乙二硫醇;

[0152] Fmoc,9-芴基甲氧羰基;

[0153] HATU,2-(1H-9-氮杂苯并三唑-1-基)-1,1,3,3-四甲基脲鎓六氟磷酸盐;

[0154] HBTU,2-[1H-苯并三唑-1-基]-1,1,3,3-四甲基脲鎓六氟磷酸盐;

[0155] HOBt,N-羟基苯并三唑;

[0156] Pbf,2,2,4,6,7-五甲基二氢苯并呋喃-5-磺酰基;

[0157] NMP,N-甲基吡咯烷酮;

[0158] MBHA,甲基二苯甲胺;

[0159] OD,光密度;

[0160] iPr_2O,二异丙基醚;

[0161] PCR,聚合酶链反应

[0162] PyBOP,(苯并三唑-1-基氧基)-三吡咯烷子基鏻-六氟磷酸盐;

[0163] PEO6,N-Fmoc-21-氨基-4,7,10,13,16,19-六氧杂二十一烷酸;

[0164] r.t.,室温;

[0165] RP-HPLC,反相高效液相色谱法;

[0166] TA,茴香硫醚;

[0167] TIS,三异丙基硅烷;

[0168] Trt,三苯甲基;

[0169] TFA,三氟乙酸;

[0170] TFE,2,2,2-三氟乙醇

[0171] t_R,保留时间

[0172] SD,标准偏差

[0173] 实施例1:PR肽类的设计和合成

[0174] 使用两条引物(LSM13和SKH2)、根据Hollingshead,Becker等人,Infect Immun,2000,68,5889-5900扩增来自高毒力血清型1临床隔离群SP1577(Leimkugel等人,JID,2005,192,192-199)的PspA的富含脯氨酸的区并且测序。

[0175] LSM13:5`-GCAAGCTTATGATATAGAAATTTGTAAC-3`(SEQ ID NO:120)

[0176] SKH2:5`-CCACATACCGTTTTCTTGTTTCCAGCC-3`(SEQ ID NO:121)

[0177] 通过PCR,使用引物LSM13和SKH2和GoTaq聚合酶(PCR条件:退火48℃ 1min,延伸72℃ 3min,30个循环)扩增富含脯氨酸的区。使用引物LSM13和SKH2从PCR反应中分离大小约为1.2kb的得到的片段并且测序。可以读取pspA基因的约1'100个碱基。翻译的核苷酸序列如下所示。包括非脯氨酸区段的富含脯氨酸的区用斜体字表示。

[0178] XXLGAGFVXX XPTXXXXXEA PVASQXKAEK DXDAXKRDAE NXKKALEEAKXXQKKYEDDQ KKTEEKXKKE KEASKEEQAA NLKYQQELVK YASEKDSVKKAKILKEVEEA EKEHKKKRAE FEKVRSEVIP SAEELKKTRQ KAEEAKAKEAELIKKVEEAE KKVTEAKQKL DAERAKEVAL QAKIAELENE VYRLETELKGIDESDSEDYV KEGLRAPLQS ELDAKRTKLS TLEELSDKID ELDAEIAKLEKNVEYFKKTD AEQTEQYLAA AEKDLADKKA ELEKTEADLK KAVNEPEKPAEET

PAPAPKP EQPAEQPKPA PAPQPAPAPK PEKTDDQQAE EDYARRSEEE YNRLPQQQPP KAEKPAPAPK PEQPVPAPKT GWKQENGMWC R(SEQ ID NO:122)

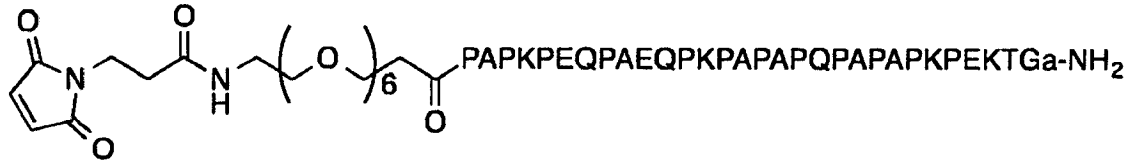
[0179] 从该序列中,选择P1PR序列

[0180] (PAPKPEQPAEQPKPAPAPQPAPAPKPEKT,SEQ ID NO:27)。P1位于SP1577PspA的螺旋/卷曲螺旋区与非脯氨酸区段之间。

[0181] 为了能够与SVLP脂肽缀合,设计并且合成下列马来酰亚氨基肽:

[0182] 马来酰亚氨基肽1:

[0183]



(1)

[0184] 在马来酰亚氨基肽1中,使3-马来酰亚氨基丙酸与P1(SEQ ID NO:27)中的N-末端通过21-氨基-3,6,9,12,15,18-六氧杂二十一烷-21-酸连接体偶合,并且将甘氨酸添加到C-末端P1,后面是作为酰胺("NH_2")的D-丙氨酸残基("a"),以便赋予针对外蛋白酶类的稳定性。

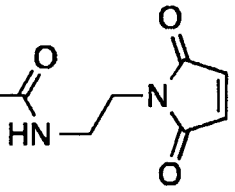
[0185] 使用如下Fmoc固相肽合成(SPPS)方法合成马来酰亚氨基肽1:

[0186] 在ABI 433A肽合成仪上使用Rink酰胺MBHA树脂(载量:0.69mml/g)(362mg,0.25mmol)和标准Fmoc-SPPS方案组装肽PAPKPEQPAEQPKPAPAPQPAPAPKPEKTGa(由甘氨酸-D-丙氨酸延伸的SEQ ID NO:27)。使用如下氨基酸(以正确的次序):Fmoc-Ala-OH、Fmoc-D-Ala-OH、Fmoc-Glu(OtBu)-OH、Fmoc-Gln(Trt)-OH、Fmoc-Gly-OH、Fmoc-Lys(Boc)-OH、Fmoc-Pro-OH和Fmoc-Thr(tBu)-OH。在组装和除去N-末端Fmoc保护基后,用N-甲基-2-吡咯烷酮(NMP)和CH_2Cl_2洗涤树脂。为了偶合马来酰亚胺,用DMF洗涤部分树脂(约0.1mmol),制备Fmoc-PEO6-OH(115mg,0.2mmol)PyBOP(104mg,0.2mmol)、HOBt(27mg,0.2mmol)和DIEA(66μl,0.4mmol)在4.5ml DMF中的溶液,混合30秒,在氩气气氛中加入到树脂中。将该混合物振摇16h。过滤树脂,用DMF洗涤4x。然后通过用20%哌啶的DMF溶液(6x 2min.)处理除去Fmoc基团。然后再用DMF洗涤树脂,制备3-马来酰亚氨基丙酸(34mg,0.2mmol)、PyBOP(104mg,0.2mmol)、HOBt(27mg,0.2mmol)和DIEA(66μl,0.4mmol)在DMF中的溶液,在氩气下加入到树

脂中。将树脂振摇3h，过滤，依次用DMF、CH_2Cl_2和MeOH洗涤4次，用KOH颗粒真空干燥过夜。为了从树脂上裂解肽并且除去侧链保护基，制备TFA/TIS/TA/苯酚85:5:5:5(10ml)，在氩气气氛下加入到干燥树脂中。将树脂振摇3h，过滤，用iPr_2O沉淀马来酰亚氨基肽1，预冷却至-20℃(50ml)。然后用iPr_2O洗涤肽4次，风干过夜，通过使用制备型C18柱的RP-HPLC(Agilent Zorbax SB300PrepHT,250x 21.5mm)和线性梯度10-40%MeCN的H_2O溶液(+0.1%TFA)纯化，历时16min，冻干，得到1，为白色粉末。通过使用Agilent XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100%MeCN的H_2O溶液(+0.1%TFA)分析肽，25min：纯度>97%；t_R=8.53min。

[0187] ESI-MS：针对$C_{163}H_{259}N_{41}O_{51}$计算的MW：3609.1Da；MW测定值：3609.7(±0.02%)。

[0188] 马来酰亚氨基肽2：

[0189] Ac-PAPKPEQPAEQPKPAPAPQPAPAPKPEKTG- 

(2)

[0190] 在这一马来酰亚氨基肽中，将甘氨酸加入到P1(SEQ ID NO:27)的C-末端上，使马来酰亚与甘氨酸P1通过氨基乙基间隔区偶合。N-末端是乙酰化的。

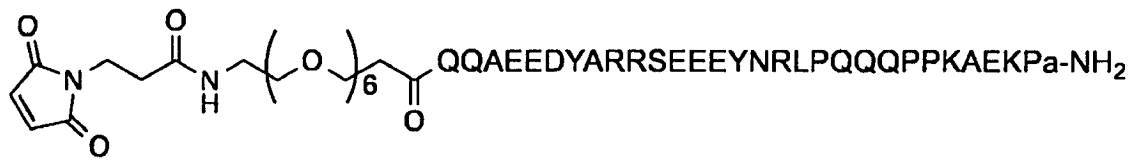
[0191] 使用Fmoc SPPS在ABI 433A上如对1所述组装马来酰亚氨基肽2上的肽链，除了使用向2-氯三苯甲基树脂上预先加载Fmoc-Gly-OH至树脂取代水平为0.6mmol/g(416mg，0.25mmol)，而不是使用Rink酰胺MBHA树脂作为固相支持物。在组装和除去N-末端Fmoc保护基后，通过用0.5M Ac_2O、0.05M HOBt和0.136M DIEA在NMP(10ml)中的溶液处理、同时振摇30min使树脂乙酰化。然后用DMF将树脂洗涤4次，用CH_2Cl_2洗涤4次，并用TFE/CH_2Cl_2 2:8(10ml)处理，同时在氩气中振摇4h，以便从树脂上释放完全侧链保护的肽。过滤树脂，用10ml TFE/CH_2Cl_2 2:8洗涤2次，浓缩滤液，用4℃冷Et_2O沉淀被保护的肽，并用Et_2O洗涤4次。然后真空干燥被保护的肽过夜，储存在-20℃下。

[0192] 为了使马来酰亚胺偶合，将部分粗的侧链被保护的肽(100mg)、HATU(15mg，39μmol)、HOAt(5mg，39μmol)溶于DMF(0.8ml)，加入DIEA(23μl，142μmol)，将该混合物搅拌1min。加入N-(2-氨基乙基)马来酰亚胺TFA盐(150mg，60μmol)在DMF(0.2ml)中的溶液，将该混合物在氩气气氛中搅拌3h。然后减压除去DMF。将侧链被保护的肽混悬于0.3ml CH_2Cl_2，用4℃冷Et_2O沉淀，用Et_2O洗涤4次，并真空干燥过夜。

[0193] 然后除去侧链保护基，沉淀肽，如上述对1所述纯化，通过使用Agilent XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100%MeCN的H_2O溶液(+0.1%TFA)分析终产物2，25min：纯度>97%；t_R=7.06min。MALDI-TOF MS：对$C_{146}H_{227}N_{39}O_{43}$计算的MW：3216.6Da；MW测定值：3215.7Da(±0.05%)。

[0194] 马来酰亚氨基肽3：

[0195]



(3)

[0196] 在马来酰亚氨基肽3中，使3-马来酰亚氨基丙酸与SEQ-ID NO:113的N-末端通过21-氨基-3,6,9,12,15,18-六氧杂二十一烷-21-酸连接体偶合，用D-丙氨酸(“a”)将C-末端封端，酰胺化。SEQ ID NO:113对应于P1577PspA的非脯氨酸区段。

[0197] 如上述对马来酰亚氨基肽1所述合成和纯化马来酰亚氨基肽3，通过使用XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100％MeCN的H_2O溶液(+0.1％TFA)分析，25min:纯度>97％；t_R=5.31min。MALDI-TOF MS:对$C_{174}H_{272}N_{50}O_{63}$计算的MW:4072.3Da;MW测定值:4071.0Da(±0.05％)。

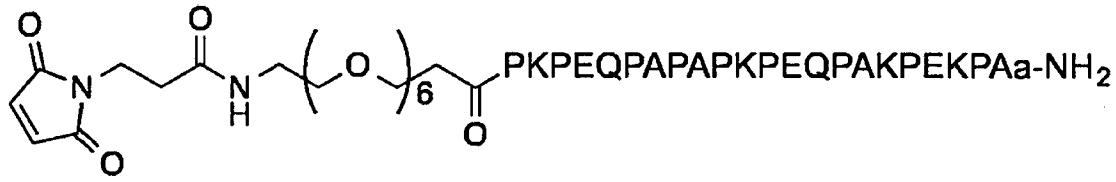
[0198] 通过对pspA或pspC基因测序得到其它PR序列，或者，可以在公共数据库中得到该序列，例如UniProtKB。例如，血清型19A隔离群TCH8431的PspA序列(UniProtKB登录号D6ZPW2)为：

[0199] MNKKKMILTS LASVAILGAG FVTSQPTVVR AEESPVASQS KAEKDYDAAVKKSEAAKKHY EEAKKKAEDA QKKYDEDQKK TEAKAEKERK ASEKIAEATKEVQQAYLAYL QASN ESQRKE ADKKIKEATQ RKDEAEAAFA TIRTTIVVPEPSELAETKKK AEEAKAEEKV AKRKYDYATL KLALAKKEVE AKELEIEKLQYEISTLEQEV ATAQHQVDNL KKLLAGADPD DGTEVIEAKL KKGEAELNAKQAELAKKQTE LEKLLDSLDP EGKTQDELDK EAEEAELDKK ADELQNKVADLEKEISNLEI LLGGADPEDD TAALQNKLAA KKAELAKKQT ELEKLLDSLDPEGKTQDELD KEAEEAELDK KADELQNKVA DLEKEISNLE ILLGGADSEDDTAALQNKLA TKKAELEKTQ KELDAALNEL GPDGDEEET***P APAPQPEQPA PAPKPEQPAP APKPEQPAPA PKPEQPAPAP KPEQPAKPEK PAEEPTQPEK PATPKT***GWKQ ENGMWYFYNT DGSMATGWLQ NNGSWYYLNA NGSMATGWVKDGDTWYYLEA SGAMKASQWF KVSDKWYYVN SNGAMA TGWL QYNGSWYYLNANGDMATGWL QYNGSWYYLN ANGDMATGWA KVNGSWYYLN ANGAMATGWAKVNGSWYYLN ANGSMATGWV KDGDTWYYLE ASGAMKASQW FKVSDKWYYVNGLGALAVNT TVDGYKVNAN GEW(SEQ ID NO:123)

[0200] 从该序列中，选择P2序列(PKPEQPAPAPKPEQPAKPEKPA,SEQ ID NO:28)。P2紧接位于TCH8431 PspA的螺旋/卷曲螺旋区之后。为了有利于使SVLP脂肽缀合，设计并且合成下列马来酰亚氨基肽类：

[0201] 马来酰亚氨基肽4

[0202]



(4)

[0203] 这一马来酰亚氨基肽包含通过21-氨基-3,6,9,12,15,18-六氧杂二十一烷-21-酸连接体与P2(SEQ ID NO:28)N-末端偶合的3-马来酰亚氨基丙酸。“a”表示D-丙氨酸。C-末端被酰胺化。

[0204] 如上述对马来酰亚氨基肽1所述合成和纯化马来酰亚氨基肽4，通过使用XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100％MeCN的H_2O溶液(+0.1％TFA)分析，25min:纯度＞97％；t_R=5.21min。MALDI-TOF MS:对$C_{131}H_{210}N_{32}O_4$计算的MW:2889.3Da,MW测定值:2888.8Da(±0.05％)。

[0205] 马来酰亚氨基肽5

[0206] 

(5)

[0207] 在这一马来酰亚氨基肽中，甘氨酸被添加到P2(SEQ ID NO:28)C-末端，并且使马来酰亚胺与甘氨酸通过氨基乙基间隔区偶合。N-末端是乙酰化的。

[0208] 如上述对马来酰亚氨基肽2所述合成和纯化马来酰亚氨基肽5，通过使用XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100%MeCN的H_2O溶液(+0.1%TFA)分析，25min:纯度>97%；t_R=6.31min。ESI MS:对$C_{113}H_{178}N_{30}O_{33}$计算的MW:2484.8Da;MW测定值:2483.2Da(±0.02%)。

[0209] 马来酰亚氨基肽6

[0210] 通过融合来自两种或多种不同PspA蛋白质的短PR序列生成人工PR序列。例如，通过融合P1中的N-末端残基PAPKPEQPAEQ(SEQ ID NO:124)至P2(SEQ ID NO:28)并且用Gly残基替代P2中的C-末端Ala设计序列P3(SEQ ID NO:29)。为了能够缀合，设计并且合成下列马来酰亚氨基肽：

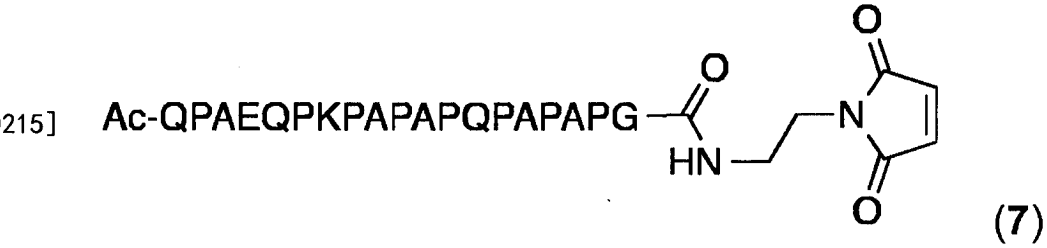
[0211] 

6
(6)

[0212] 如上述对马来酰亚氨基肽2所述合成和纯化马来酰亚氨基肽6，通过使用XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度10-100%MeCN的H_2O溶液(+0.1%TFA)分析，25min:纯度>97%；t_R=10.11min。MALDI-TOF MS:对$C_{164}H_{255}N_{45}O_{50}$计算的MW:3657.1Da;MW测定值:3654.9Da(±0.05%)。

[0213] PR肽抗原的另外的实例如下所述：

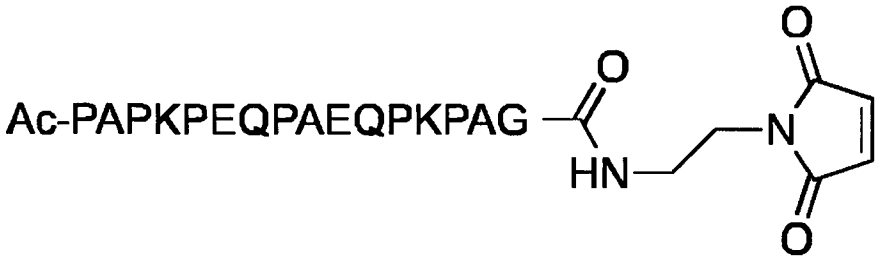
[0214] 马来酰亚氨基肽7

[0215] 

(7)

[0216] 如上述对马来酰亚氨基肽2所述合成和纯化马来酰亚氨基肽7。ESI MS:对$C_{91}H_{137}N_{25}O_{27}$计算的MW:2012.0Da;MW测定值:2012.4Da(±0.05%)。

[0217] 马来酰亚氨基肽8

[0218] 

(8)

[0219] 如上述对马来酰亚氨基肽2所述合成和纯化马来酰亚氨基肽8。ESI MS:对$C_{81}H_{124}N_{22}O_{25}$计算的MW:1804.9Da;MW测定值:1805.4Da(±0.05%)。

[0220] 马来酰亚氨基肽9

[0221] 

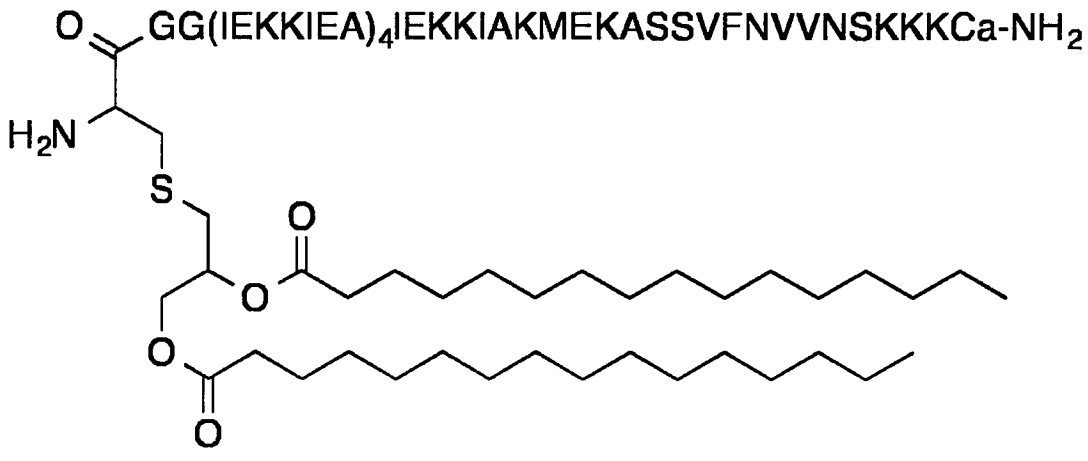
(9)

[0222] 这一马来酰亚氨基肽来源于PhtD(P60,SEQID NO:86)的PR肽。N-末端是乙酰化的。如上述对马来酰亚氨基肽2所述合成和纯化马来酰亚氨基肽,通过使用XDB-C18柱(250x 4.6mm)的分析型RP-HPLC和线性梯度20-100%MeCN的H_2O溶液(+0.1%TFA)分析,25min:纯度>97%;t_R=3.41min。ESI-MS MS:对$C_{136}H_{201}N_{37}O_{48}$计算的MW:3120.4Da;MW测定值:3120.6Da(±0.05%)。

[0223] 实施例2:PR肽抗原与脂肽的缀合

[0224] 为了制备用于免疫的脂肽缀合物,合成下列4种脂肽结构单元。

[0225] 脂肽结构单元10

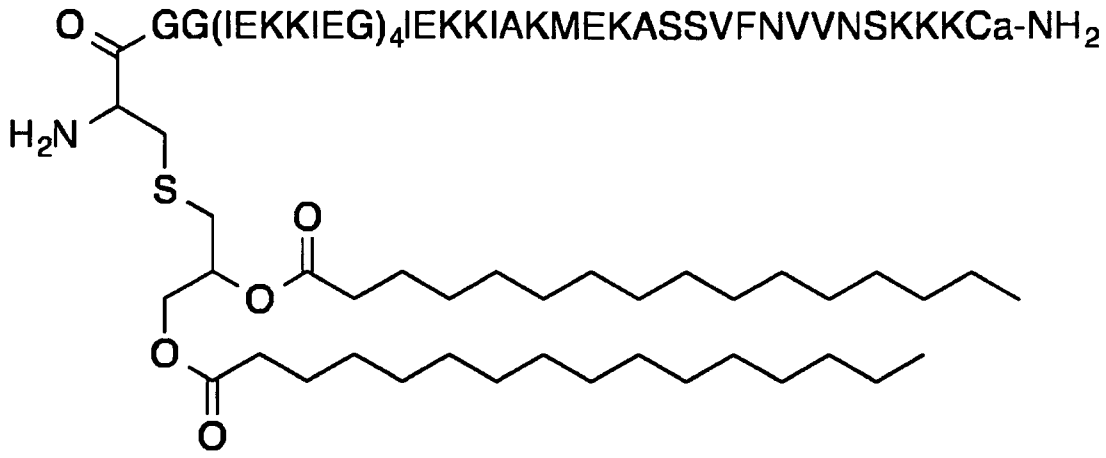
[0226] 

(10)

[0227] 这种脂肽对应于WO 2008/068017的实施例13。如WO 2008/068017和Ghasparian,Riedel等人,Chembiochem,2011,12,100-109中所述进行合成并且表征产物。分析型RP-HPLC(Interchrom UP5WC4-25QS,25－100%MeCN的H_2O溶液(+0.1%TFA),历时25min.):纯度>96%,t_R=22.71min。MALDI-TOF:对$C_{312}H_{552}N_{74}O_{85}S_3$计算的MW:6796.4Da;MW测定值:6798.2Da(±0.05%)。

[0228] 脂肽结构单元11

[0229]



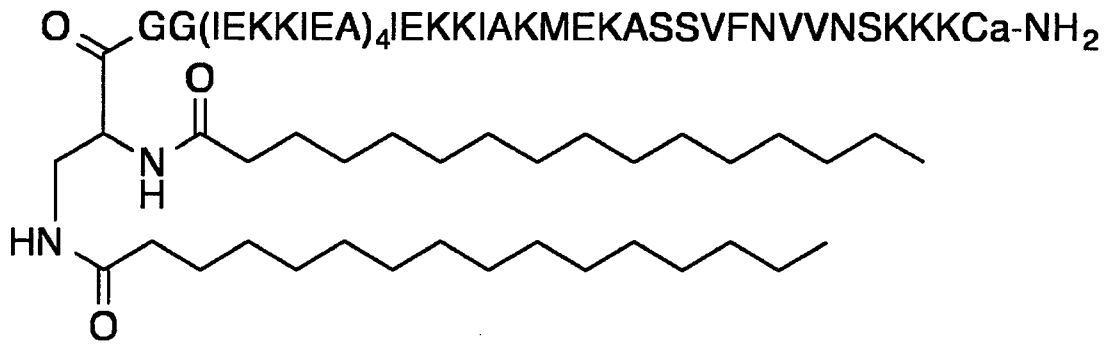
(11)

[0230] 这种脂肽结构单元包含修饰的卷曲螺旋结构域，其在七重复单元“defgabc” IEKKIEG(SEQ ID NO:125)的“c”位置上具有Gly。

[0231] 如WO 2008/068017中所述合成并且纯化修饰的脂肽结构单元。分析型RP-HPLC (Interchrom UP5WC4-25QS,25－100%MeCN的H_2O溶液(+0.1%TFA)，历时25min.)：纯度>98%，t_R=21.41min。ESI-MS：对$C_{308}H_{544}N_{74}O_{85}S_3$计算的MW 6740.3Da；测定值6741.7Da。

[0232] 脂肽结构单元12

[0233]



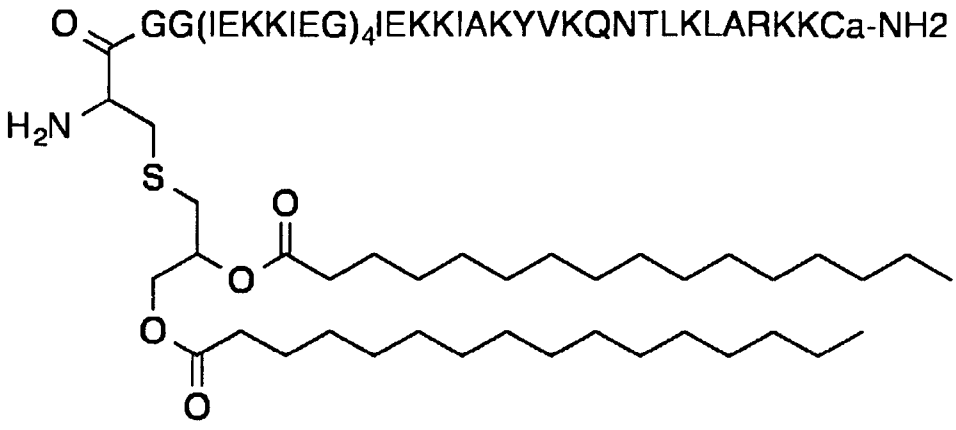
(12)

[0234] 这种脂肽结构单元包含修饰的脂质N,N'-二棕榈酰基.-2,3-二氨基丙酰胺(“Pam_2Dap”)。“a”表示D-丙氨酸。

[0235] 如WO 2008/068017中所述合成并且纯化脂肽结构单元，除了在合成结束时并入Pam2Dap而不是Pam2Cys。通过分析型HPLC和MS分析脂肽。分析型RP-HPLC(C4柱，A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20－100%B,25min.)：纯度：>95%。t_R=22.1min。ESI-MS：MW计算值9594.5Da，测定值9596.17Da。

[0236] 脂肽结构单元13

[0237]



(13)

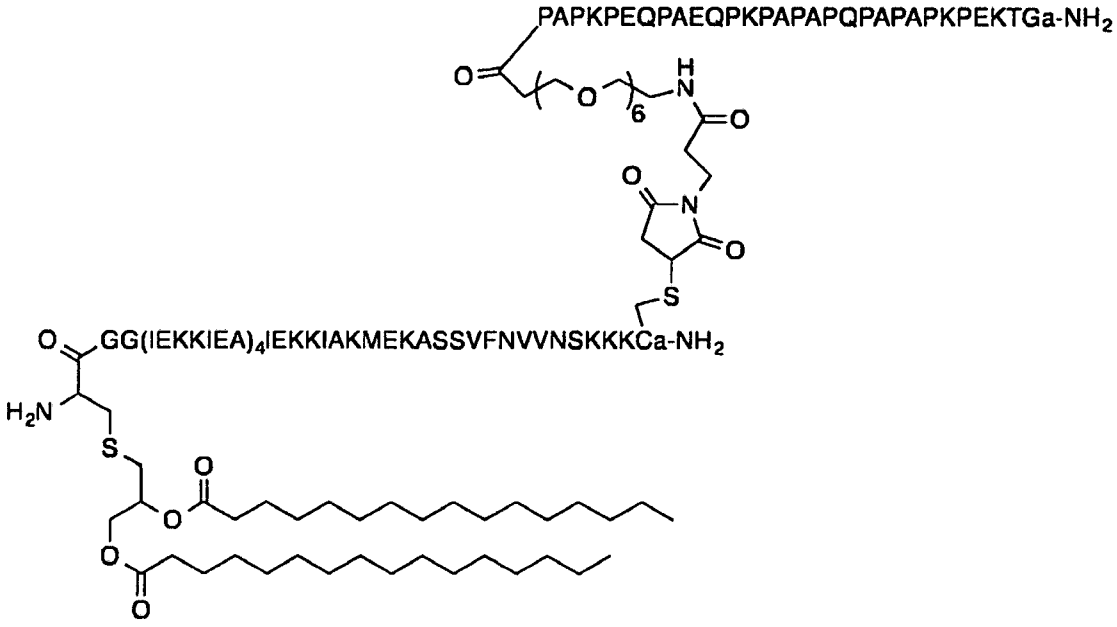
[0238] 这种脂肽结构单元包含泛宿主性T-辅助表位(KYVKQNTLKLARK,SEQID NO:126),其来源于来自流感血凝素残基307-309(SEQID NO:19)的HLA-DRB 101限制表位(Stern,LJ.等人Nature1994,368,215)。"a"表示D-丙氨酸。

[0239] 基本上如WO 2008/068017中所述合成并且纯化脂肽结构单元,并且通过分析型HPLC和MS分析。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20-100%B,25min.):纯度:>95%。t_R=22.35min。ESI-MS:对$C_{302}H_{538}N_{74}O_{79}S_2$计算的MW:6530.0Da;测定值6530.4Da(±0.05%)。

[0240] 合成如下缀合物:

[0241] 缀合物14(马来酰亚氨基肽1+脂肽10)

[0242]



(14)

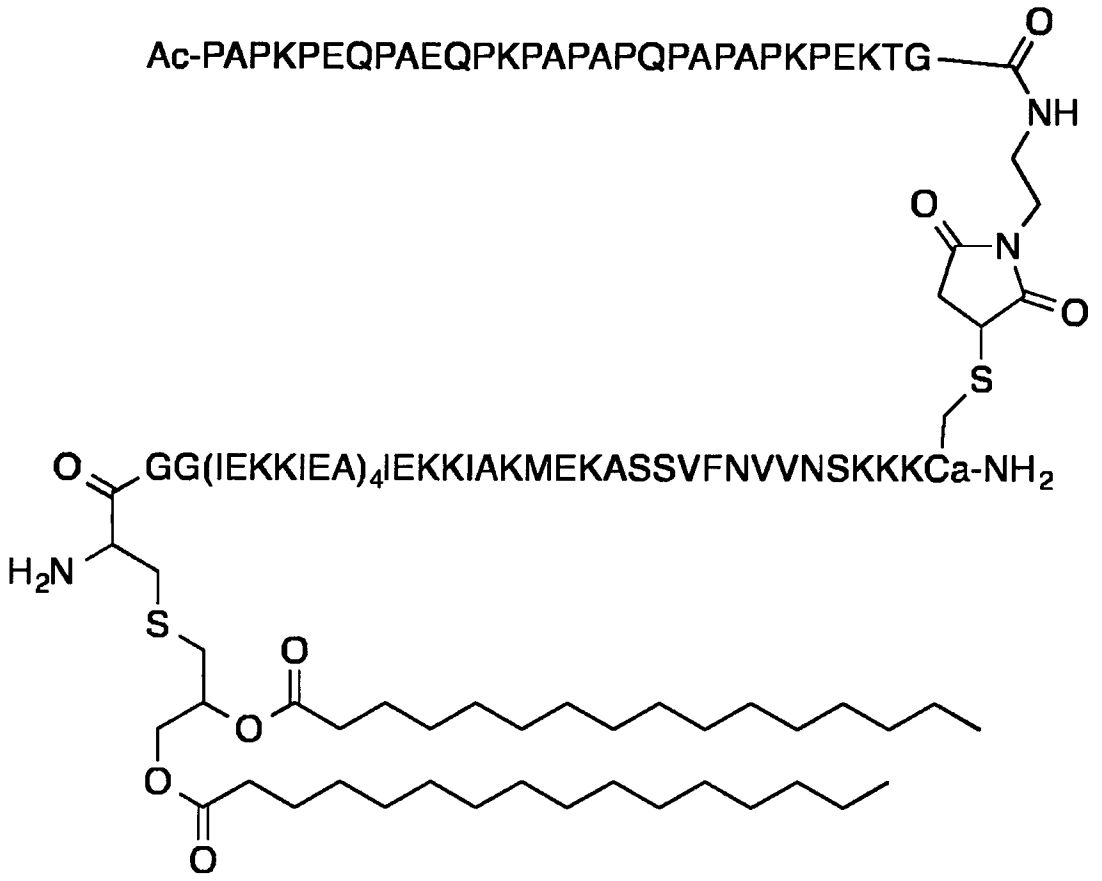
[0243] 1与10缀合基本上如WO 2008/068017中所述进行。向10(6.0mg,0.9μmol)在H_2O/MeCN 1:1(3ml)中的溶液中加入1(4.8mg,1.3μmol)在H_2O/MeCN 1:1(2.4ml)中的溶液。谨慎地调节pH,使用0.1NaOH维持在pH 6.5-7.0,将该混合物在室温搅拌2h。然后通过使用C4制备型柱(Interchrom UP5WC4-25M,250x 10mm)的RP-HPLC和梯度50-100%MeCN的H_2O溶液(+0.1%TFA)纯化缀合物,历时17min。通过使用Interchrom UP5WC4-25QS柱(250x 4.6mm)的分析型RP-HPLC和梯度20-100%MeCN的H_2O溶液(+0.1%TFA)分析缀合物14,25min:纯度

>97%;t_R=22.34min。MALDI-TOF MS:对$C_{475}H_{811}N_{115}O_{136}S_3$计算的MW:10406.6Da;测定值10407.8Da(±0.1%)。

[0244] 将该缀合物混悬于PBS中,平衡30min.,稀释至0.5mg/ml,通过使用Wyatt DynaPro Titan仪器的动态光散射(DLS)在4℃、25℃和37℃、应用激光强度400'000计数/s和10s获取时间分析。通过规律化分析的大小分布是单模态的,且大小分散性很小。平均流体动力学半径(R_h)为12.0nm,且%Pd值在25℃为12.3%。R_h和%Pd的类似值在其它温度下得到。

[0245] 缀合物15(马来酰亚氨基肽2+脂肽10)

[0246]



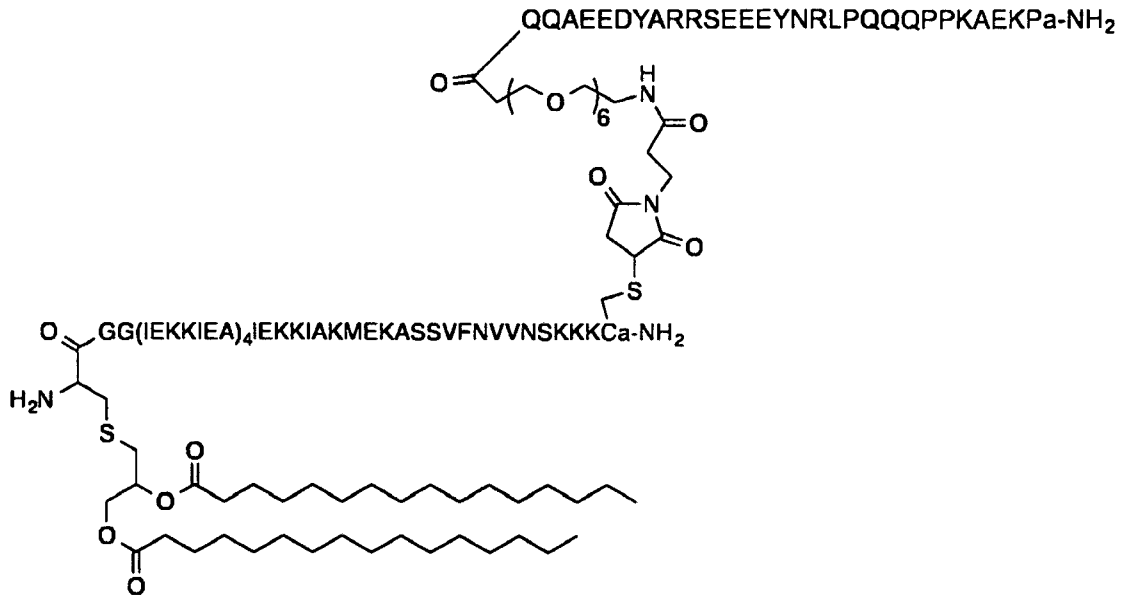
(15)

[0247] 2与10缀合和缀合物的纯化基本上如上述对缀合物14中所述进行。通过使用Interchrom UP5WC4-25QS柱(250x 4.6mm)的分析型RP-HPLC和梯度20—100%MeCN的H_2O溶液(+0.1%TFA)分析产物15,25min:纯度>97%;t_R=22.41min。ESI-MS:对$C_{458}H_{779}N_{113}O_{128}S_3$计算的MW:10012.9Da;测定值10011.1Da(±0.1%)。

[0248] 制备缀合物12在PBS中的混悬液,使用DLS如上述对14所述分析。R_h值在13.2-14.2nm的范围,且%Pd值在25℃下在12.6-18.0%的范围。

[0249] 缀合物16(马来酰亚氨基肽3+脂肽10)

[0250]



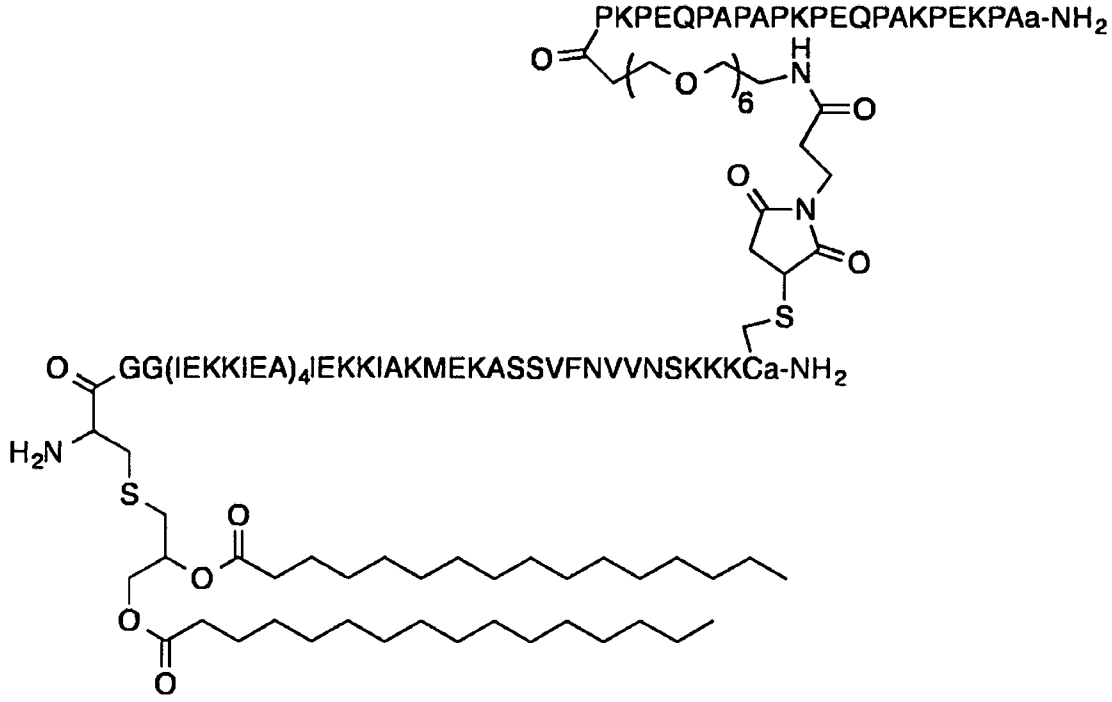
(16)

[0251] 3与10缀合和缀合物的纯化基本上如上述对缀合物14中所述进行。通过使用Interchrom UP5WC4-25QS柱(250x 4.6mm)的分析型RP-HPLC和梯度20－100%MeCN的H_2O溶液(+0.1%TFA)分析产物16,25min:纯度>97%;t_R=22.0min。MALDI-TOF MS:对$C_{475}H_{811}N_{115}O_{136}S_3$计算的MW:10869.8Da;测定值10'872.3Da(±0.1%)。

[0252] 制备缀合物13在PBS中的混悬液,使用DLS如上述对14所述分析。R_h值在14.0-15.0nm的范围,且%Pd值在13.0-13.7%的范围。缀合物14和缀合物16颗粒的混合物的DLS分析产生了12.3-13.3nm的R_h和约25-26%的%Pd值,表明混合颗粒不会改变总大小分布。

[0253] 缀合物17(马来酰亚氨基肽4+脂肽14)

[0254]



(17)

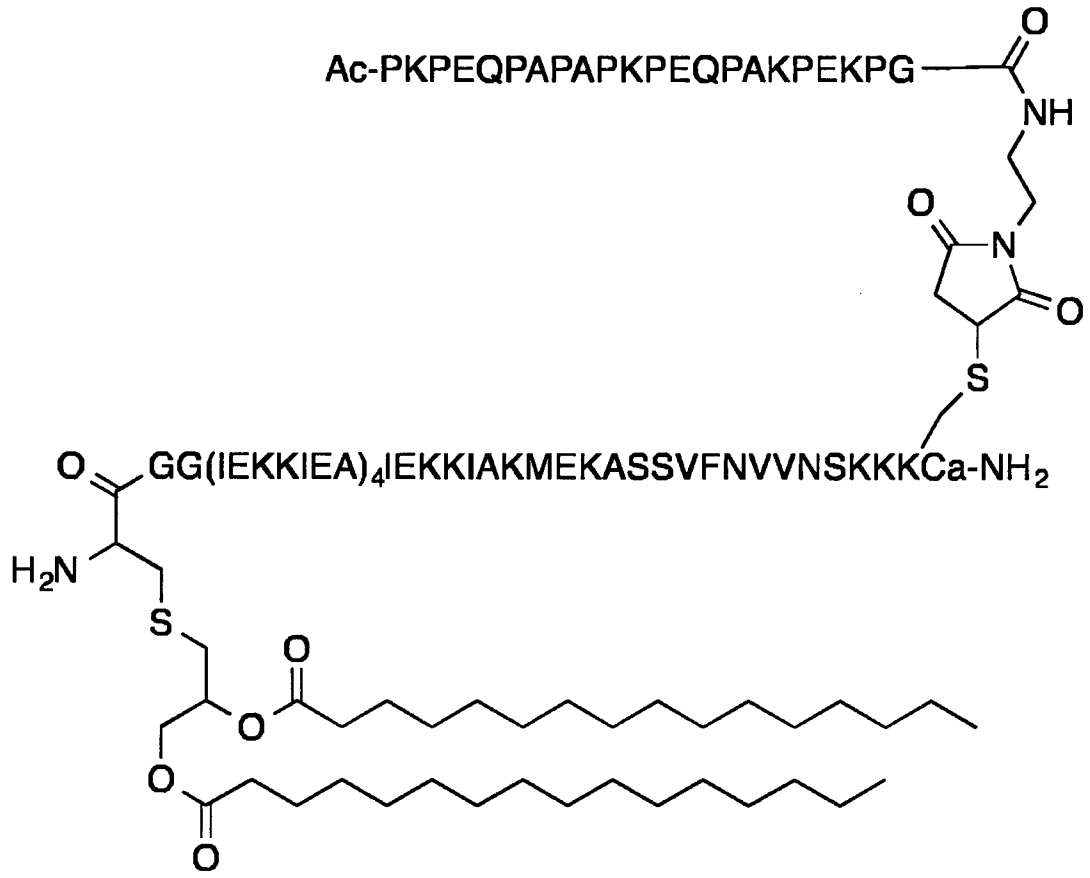
[0255] 4与10缀合和缀合物的纯化基本上如上述对缀合物17中所述进行。通过C4分析型

柱(Interchrom,UP5WC4-25QS,4.6mm x 250mm,**300 Å**)的反相HPLC和MALDI-MS分析产物17。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20－100%B,25min.):纯度:>95%。t_R=18.8min。MALDI-TOF MS:对$C_{443}H_{762}N_{106}O_{126}S_3$计算的MW:9685.6Da;测定值9686.2Da(±0.1%)。

[0256] 制备缀合物17在PBS中的混悬液,使用DLS如上述对14所述分析。R_h值在11.1-11.8nm的范围,且%Pd值约为13%。

[0257] 缀合物18(马来酰亚氨基肽5+脂肽10)

[0258]



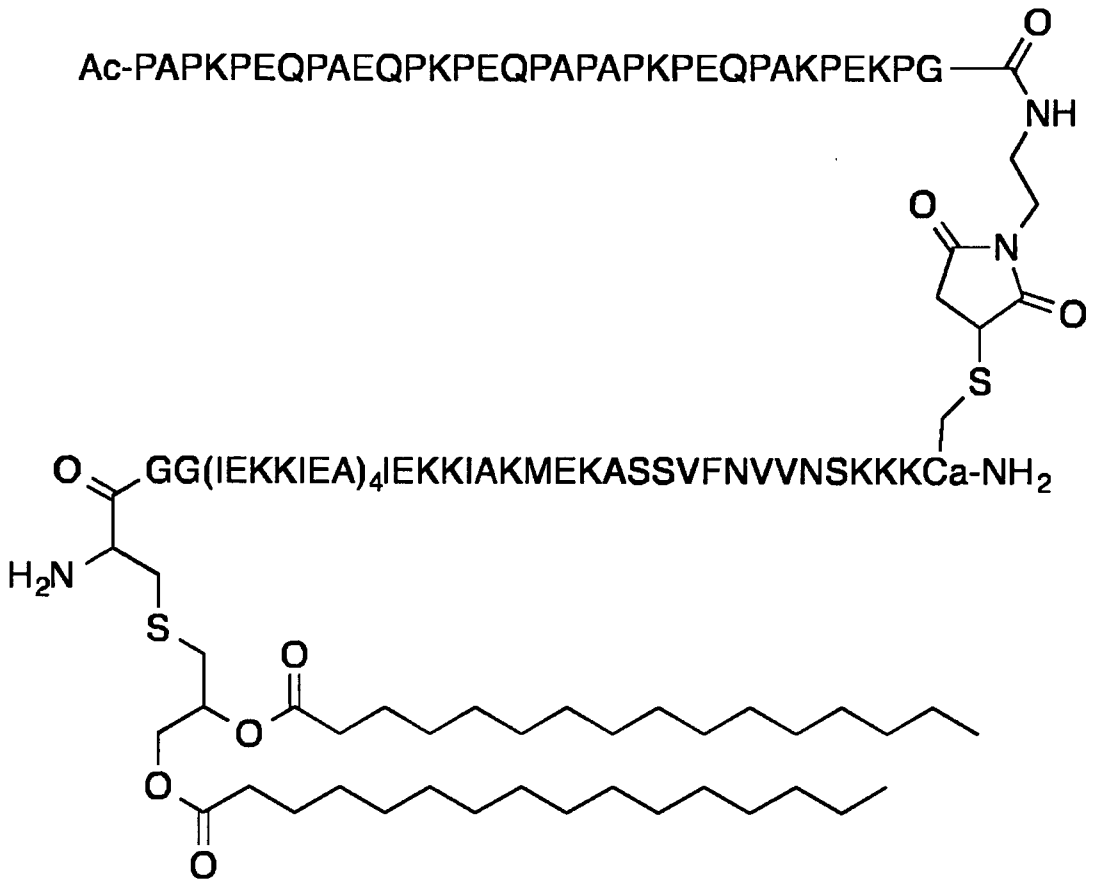
(18)

[0259] 5与10缀合和缀合物的纯化基本上如上述对缀合物18中所述进行。通过使用C4分析型柱(Interchrom,UP5WC4-25QS,4.6mm x 250mm,**300Å**)的反相HPLC和MALDI-MS分析产物18。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20－100%B,25min.):纯度:>96%。t_R=22.55min。MALDI-TOF MS:对$C_{425}H_{728}N_{104}O_{118}S_3$计算的MW:9279.2Da;测定值9280.2Da(±0.1%)。

[0260] 制备缀合物18在PBS中的混悬液,使用DLS如上述对14所述分析。R_h值在10.0-10.5nm的范围,且%Pd值约为16%。

[0261] 缀合物19(马来酰亚氨基肽6+脂肽10)

[0262]



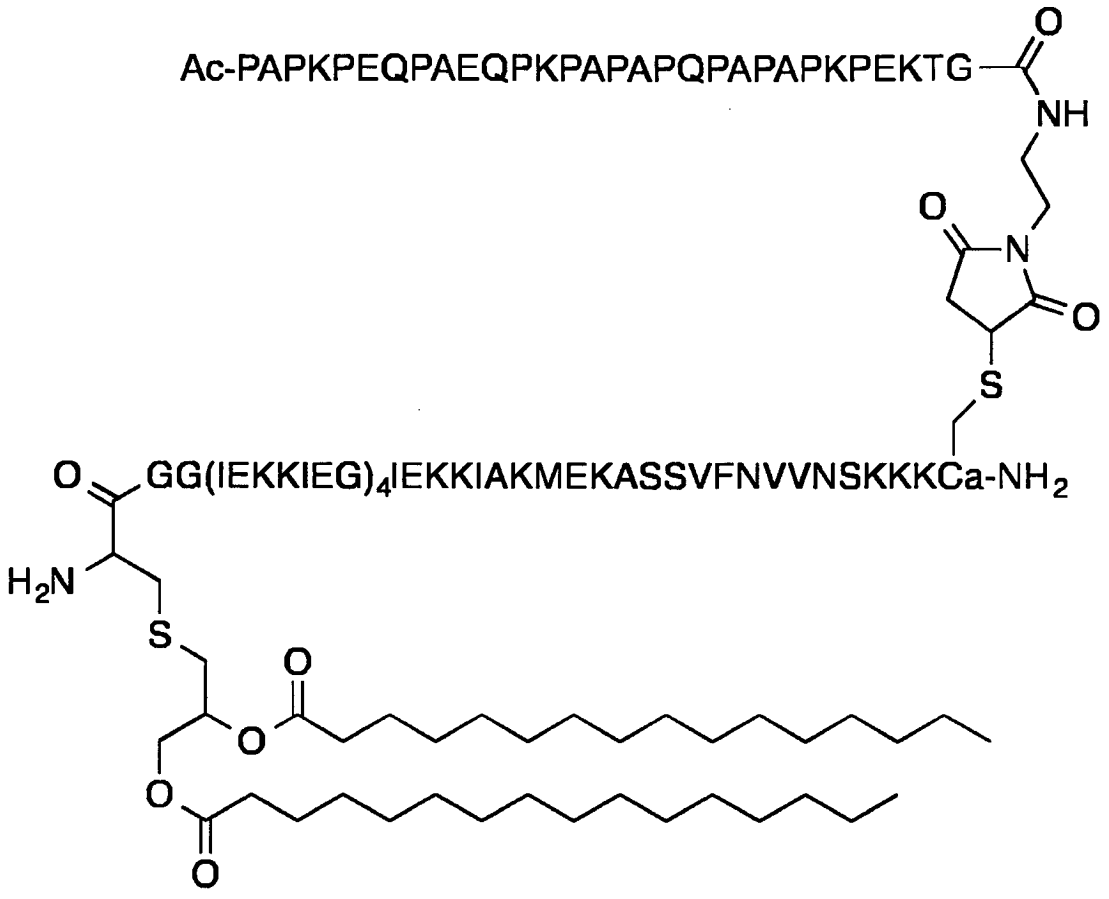
(19)

[0263] 6与10缀合和缀合物的纯化基本上如上述对缀合物14中所述进行。通过使用C4分析型柱(Interchrom,UP5WC4-25QS,4.6mm x 250mm,**300 Å**)的反相HPLC和MALDI-MS分析产物19。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20-100%B,25min.):纯度:>96%。t_R=22.34min。MALDI-TOF MS:对$C_{476}H_{807}N_{119}O_{135}S_3$计算的MW:10453.4Da;测定值10452.7Da(±0.1%)。

[0264] 制备缀合物17在PBS中的混悬液,使用DLS如上述对19所述分析。R_h值在12.1-14.5nm的范围,且%Pd值为12-20%。

[0265] 缀合物20(马来酰亚氨基肽6+脂肽11)

[0266]



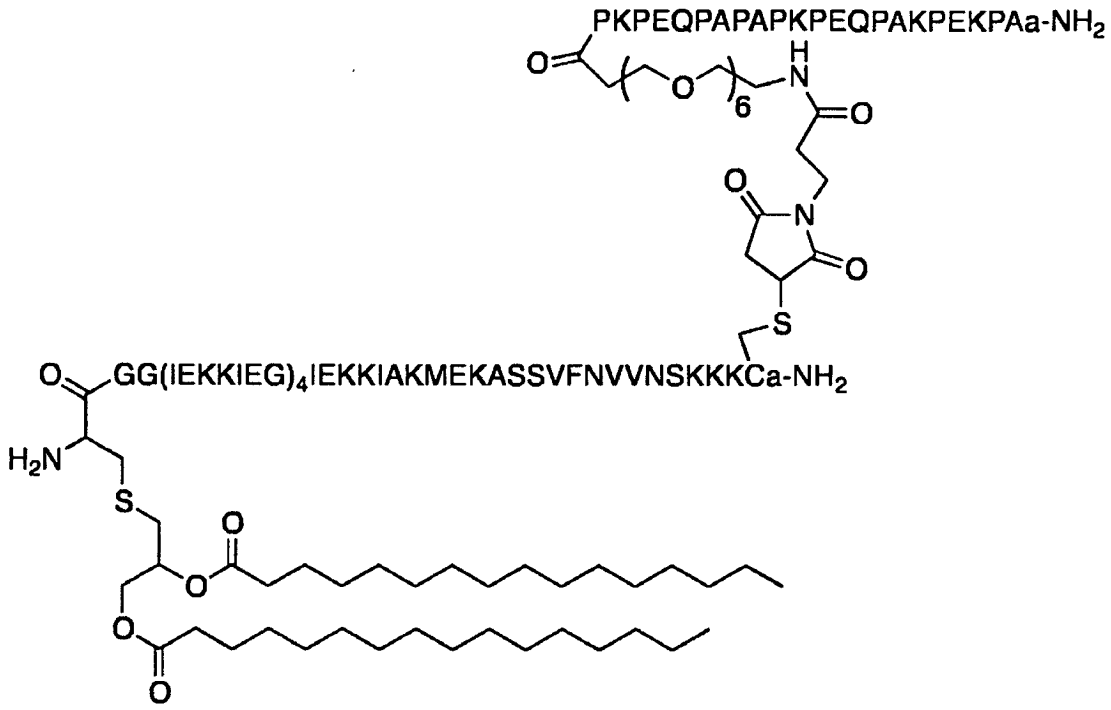
(20)

[0267] 6与11缀合和缀合物的纯化基本上如上述对缀合物14中所述进行。通过使用C4分析型柱(Interchrom,UP5WC4-25QS,4.6mm x 250mm,**300 Å**)的反相HPLC和MALDI-MS分析产物20。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20-100%B,25min.):纯度:>98%。t_R=22.34min。ESI-MS:对$C_{454}H_{773}N_{113}O_{129}S_3$(琥珀酰亚胺环水解)计算的MW:9975.02Da;测定值9974.7Da(±0.01%)。

[0268] 制备缀合物20在PBS中的混悬液,使用DLS如上述对14所述分析。R_h值在11.7-12.3nm的范围,且%Pd值约为20%。

[0269] 缀合物21(马来酰亚氨基肽4+脂肽11)

[0270]



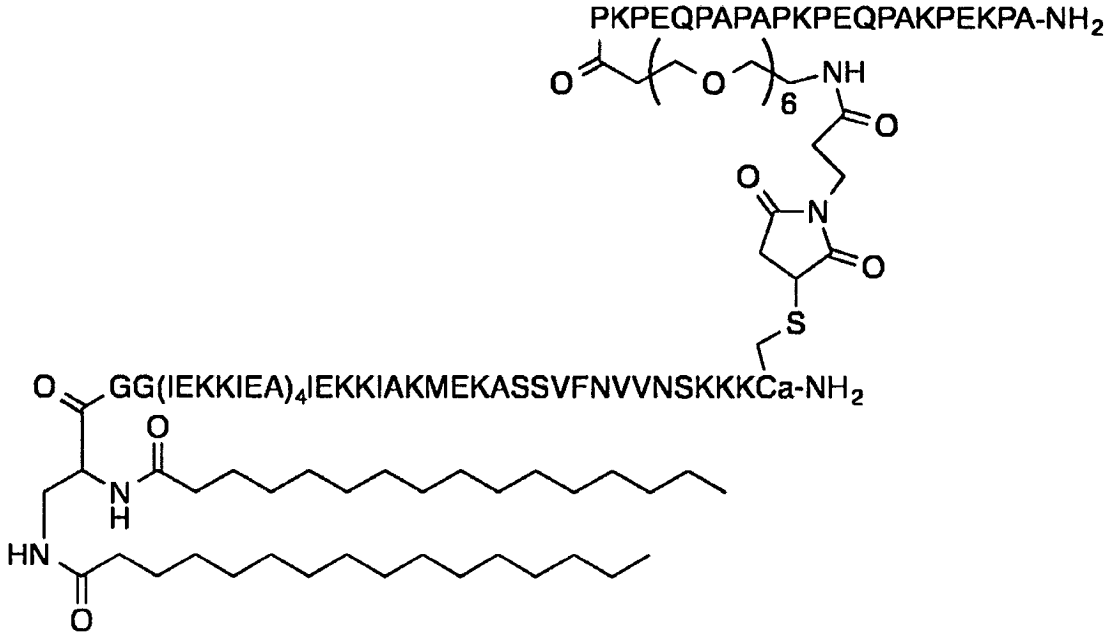
(21)

[0271] 使马来酰亚氨基肽4与11缀合，且如上述对缀合物14所述纯化该缀合物。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20－100%B,25min.):纯度:>95%.t_R=17.78min。对$C_{439}H_{753}N_{105}O_{127}S_3$计算的MW:9630.5Da;测定值9631.2Da.

[0272] DLS(0.5mg/ml,在PBS中,25℃):R_h=10.7nm;%Pd=12-13%。

[0273] 缀合物22(马来酰亚氨基肽6+脂肽12)

[0274]



(22)

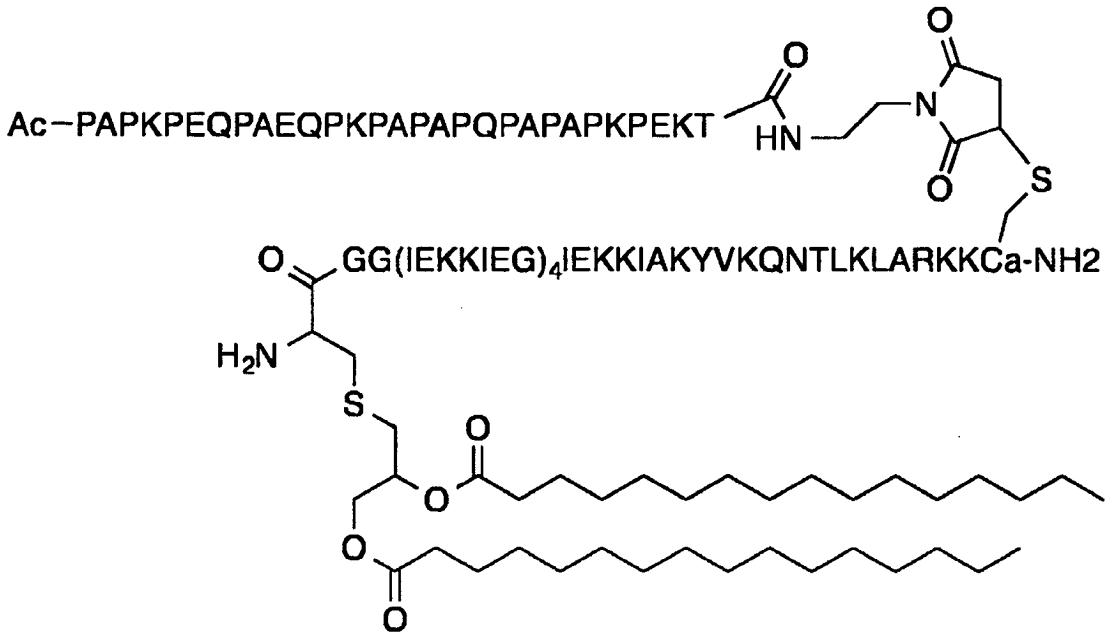
[0275] 使马来酰亚氨基肽4与12缀合，且如上述对缀合物14所述纯化该缀合物。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20-100%B,25min.):纯度:>95%。t_R=18.37min。MALDI-TOF:对于$C_{440}H_{757}N_{107}O_{124}S_2$计算的MW:9594.5Da,测定值9595.1(±

0.1%)Da。

[0276] DLS测量(在PBS中0.5mg,25°)得到R_h为10.4-11.1nm且%Pd值为10-12%。

[0277] 缀合物23(马来酰亚氨基肽6+脂肽13)

[0278]



(23)

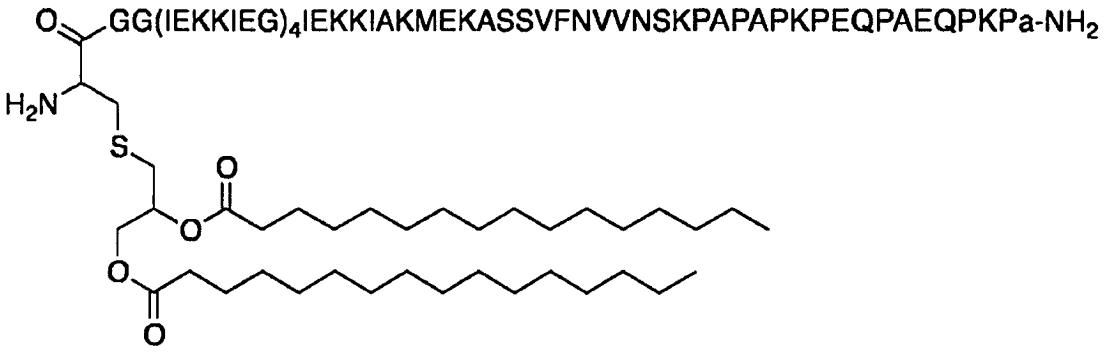
[0279] 使马来酰亚氨基肽6与13缀合,且如上述对缀合物14所述纯化该缀合物。分析型RP-HPLC(C4柱,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20—100%B,25min.):纯度:>95%。t_R=18.25min。MALDI-TOF:对$C_{448}H_{765}N_{113}O_{123}S_2$(琥珀酰亚胺环水解)计算的MW:9768.7Da,测定值9767.0Da(±0.1%)。

[0280] DLS测量(在PBS中0.5mg,25°)得到R_h为9.9-10.2nm且%Pd值为15-18%。

[0281] 通过融合PR肽抗原的N-末端与脂肽结构单元的C-末端制备另外的脂肽类。制备了下列融合脂肽。

[0282] 脂肽(24)

[0283]



(24)

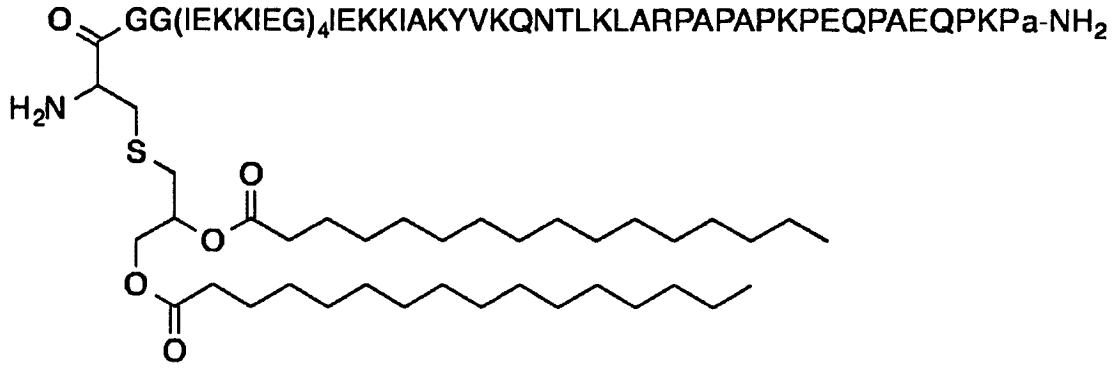
[0284] 在本实施例中,使PR序列P7,PAPAPKPEQPAEQPKP(SEQ ID NO:33)直接与GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSK(SEQ ID NO:127)的C-末端融合,得到序列GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSKPAPAPKPEQPAEQPK P(SEQ ID NO:128)。通过添加Pam2Cys使N-末端脂化,并且向脂肽24中的C-末端添加D-丙氨酸(“a”)。

[0285] 使用常规固相态合成方法合成融合脂肽24(W.C.Chan,P.D.White,Fmoc Solid Phase Peptide Synthesis:A Practical Approach,Oxford University Press,Oxford,UK,2000),且如上述对脂肽10所述纯化。MALDI-TOF MS:对于$C_{369}H_{633}N_{89}O_{104}S_2$计算的MW:8044.6Da;测定值:8044.6Da(±0.1%)。分析型RP-HPLC(Interchrom UP5WC4-25QS,25—100%MeCN在H_2O中的溶液(+0.1%TFA),历时25min.):纯度>98%,t_R=20.48min。

[0286] DLS(在PBS中0.5mg/ml,25℃):R_h=10.7nm;%Pd=12-13%。

[0287] 脂肽(25)

[0288]



(25)

[0289] 在本实施例中,使PR序列P7,PAPAPKPEQPAEQPKP(SEQ ID NO:33)直接与GG(IEKKIEG)$_4$IEKKIAKYVKQNTLKLAR(SEQ ID NO:129)的C-末端融合,得到序列GG(IEKKIEG)$_4$IEKKIAKMEKASSVFNVVNSKPAPAPKPEQPAEQPKP(SEQ ID NO:130)。通过添加Pam2Cys使N-末端脂化,并且向脂肽25中的C-末端添加D-丙氨酸("a")。

[0290] 如上述对24所述合成并且纯化融合脂肽25。ESI-MS:对于$C_{360}H_{639}N_{91}O_{99}S$计算MW:7966.6Da;测定值:7967.0Da(±0.1%)。分析型RP-HPLC(Interchrom UP5WC4-25QS,25—100%MeCN的H_2O溶液(+0.1%TFA),历时25min.):纯度>98%,t_R=21.41min。

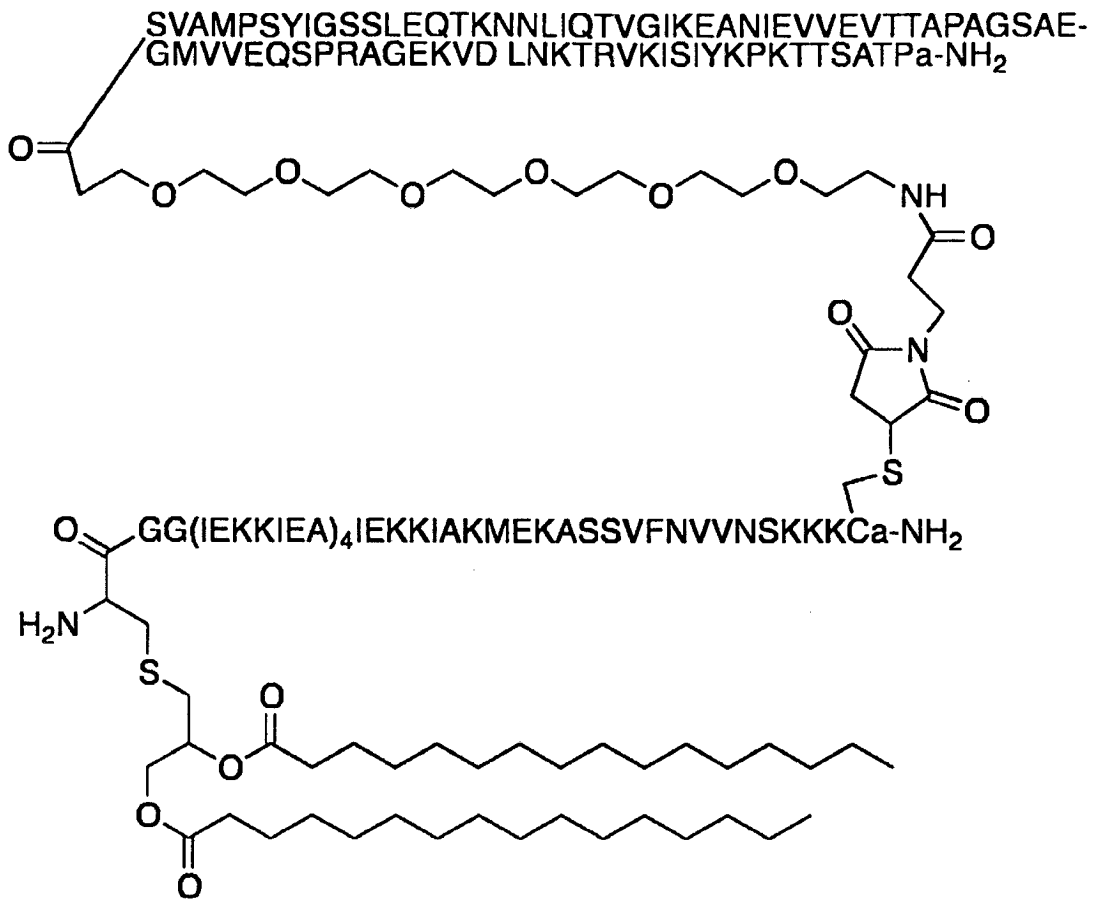
[0291] DLS(在PBS中0.5mg/ml,25℃):R_h=12.2-13.7nm;%Pd=10-15%。

[0292] 实施例3:对照品的制备

[0293] 制备下列对照化合物用于免疫接种和攻击实验。

[0294] 缀合物26(非PR缀合物)

[0295]



(26)

[0296] 这种缀合物包含StkP(SEQ ID NO:115)的C-末端部分(StkP-C;PASTA+C-末端),除了将2个突变(F594Q和I602T)并入StkP序列以除去表面暴露的疏水性残基,得到序列

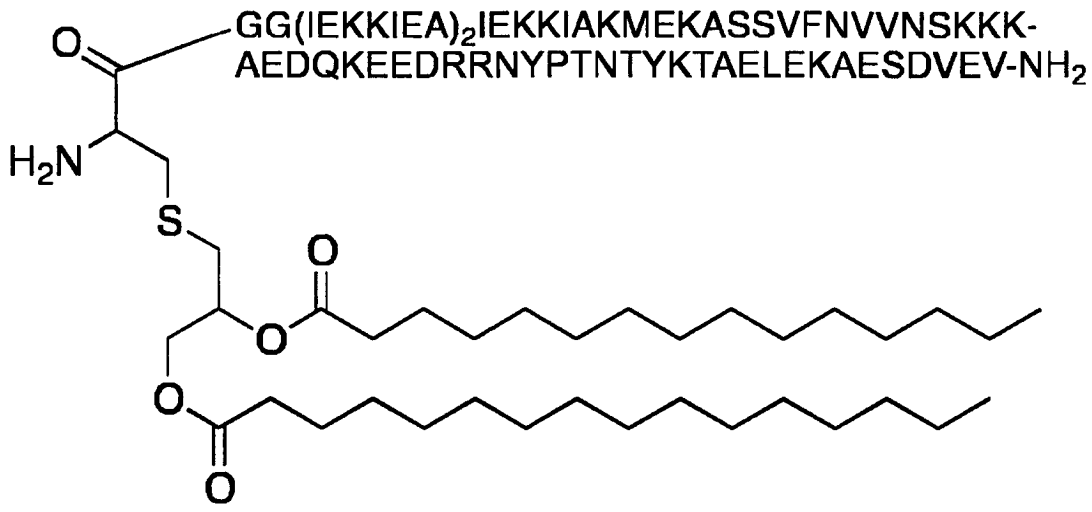
[0297]

SVAMPSYIGSSLEQTKNNLIQTVGIKEANIEVVEVTTAPAGSAEGMVVEQSPRAGEKVDLNKTRVKISIYKPKTTSATP(SEQ ID NO:131)。用a-NH2阻断C-末端,其中"a"表示D-丙氨酸。通过NMR发现,缀合物中的这种StkP肽采用有规则的青霉素结合蛋白和Ser/Thr激酶结合(PASTA)结构域-结构。

[0298] 合成相应的马来酰亚氨基肽27(3-马来酰亚氨基丙酰基)-21-氨基-4,7,10,13,16,19-六氧杂二十一烷酰-(SEQ ID NO:131)-a-NH_2)并且如上述对14所述与10缀合。通过HPLC、MALDI-MS和DLS分析缀合物26。分析型RP-HPLC(Interchrom UP5WC4-25QS,250x4.6mm,A=H_2O+0.1%TFA,B=MeCN+0.1%TFA,20-100%B,25min.):纯度:>95%。t_R=18.41min。MALDI-TOF:对$C_{700}H_{1201}N_{177}O_{216}S_5$计算的MW:15713.41Da;测定值15715.4Da(±0.1%)。DLS(在PBS中0.5mg/ml,25℃):R_h=16.3nm,%Pd=16.9%。

[0299] 脂肽28

[0300]



(28)

[0301] 在这种脂肽中，C末端上的31个氨基酸对应于来自TIGR4的PspC(SEQ ID NO:116)的氨基酸344－377，除了2个突变被并入抗原以改善溶解性(L366A，I370K；TIGR4PspC编号)，得到肽AEDQKEEDRRNYPTNTYKTAELEKAESDVEV(SEQ ID NO:132)。用r-NH2阻断C-末端，其中“r”表示D-精氨酸。这种肽进一步通过短连接体(KKK)与通用T-辅助细胞表位CST.3＊(SEQ ID NO:23)偶合。

[0302] 如WO 2008/068017中所述合成并且纯化脂肽28，并且通过HPLC、MALDI-MS和DLS分析。分析型RP-HPLC(Interchrom UP5WC4-25QS，250x 4.6mm，A＝H_2O+0.1%TFA，B＝MeCN+0.1%TFA，20-100%B，25min.)：纯度：＞95%．t_R＝18.41min。MALDI-TOF：对$C_{386}H_{654}N_{100}O_{121}S_2$计算的MW：8696.1Da；测定值8696.0Da。DLS(在PBS中0.5mg/ml，25℃)：R_h＝7.9nm；%Pd＝29%。

[0303] 重组PspA蛋白(rPspA)

[0304] 克隆来自肺炎链球菌菌株SP1577的PspA的富含脯氨酸的区，并且将其表达为重组Trx融合蛋白(rPspA)。克隆和表达来自SP1577菌株的重组PR如WO 2007/089866中所述进行。通过SDS-PAGE、使用抗-PspA抗体的斑点印迹和质谱法证实纯度和身份。该蛋白质的序列为：

[0305] MSDKIIHLTD DSFDTDVLKA DGAILVDFWA EWCGPCKMIA PILDEIADEYQGKLTVAKLN IDQNPGTAPK YGIRGIPTLL LFKNGEVAAT KVGALSKGQLKEFLDANLAG SGSGHMHHHH HHSSGLVPRG SGMKETAAAK FERQHMDSPDLGTDDDDKAM ADLKKAVN EP EKPAEET

PAP APKPEQPAEQ PKPAPAPQPA

PAPKPEKTDD QQAEEDYARR SEEEYNRLPQ QQPPKAEKPA PAPKPEQPVP

*APKPEQPVPA PKT*GWKQE(SEQ ID NO:133)

[0306] 包括非脯氨酸区段的PspA富含脯氨酸的区如斜体字所示。

[0307] 实施例4：小鼠免疫研究

[0308] 在小鼠中测试缀合物对肺炎链球菌的免疫原性。全部实验根据Swiss规则和动物权利保护条例进行并且经过责任当局批准。

[0309] 为了分析抗体应答，给远系杂交种的6-8周龄雌性NMRI远系杂交种小鼠(10只/组)经皮下免疫2次表4中所示的0.1ml制剂，在第0天和第21天，间隔3周进行。给对照组动物免

疫PBS或rPspA+白矾的盐水溶液。在第一次免疫前和第二次免疫后10天采集血液，并且使用ELISA分析血清，以便测定IgG抗体针对富含脯氨酸的肽的滴度；使用Western Blot以便测定IgG与内源性蛋白质结合，并且使用流式细胞术以便测定IgG与完整肺炎球菌的表面结合。

[0310] 表4.用于小鼠免疫的制剂

[0311]

制剂	佐剂	浓度(mg/ml)[a)]
在 PBS 中的 14	无	0.2
在 PBS 中的 15	无	0.2
在 PBS 中的 16	无	0.2
在 PBS 中的 17	无	0.2
在 PBS 中的 18	无	0.2
在 PBS 中的 19	无	0.2
在 PBS 中的 16 + 14	无	0.2 + 0.2
在盐水中的 rPspA	白矾	0.2
PBS	无	-
a) 显示总脂肽缀合物/蛋白质浓度。		

[0312] 为了进行ELISA，在4℃，用5μl/ml PR肽类或rPspA在PBS中的溶液(pH 7.2，50μl/孔)将MaxiSorp 96-孔微量滴定板(Nunc，Fischer Scientific)包被过夜。基本上如WO 2008/068017中所述，使用用于IgG检测的山羊抗-小鼠IgG(γ-链-特异性)抗体(Sigma，St.Louis，MO)和1mg/ml磷酸对-硝基苯酯(Sigma)进行ELISA。将终点滴度定义为测试血清OD大于PBS平均OD+两个SD的最高血清稀释度。

[0313] 为了对免疫血清进行Western Blot，在37℃、5％CO_2在血液琼脂平板上培养SP1577并且制备总细菌裂解物。通过SDS-PAGE在还原条件下分离裂解物并且印迹在硝酸纤维素膜上。将印迹用免疫或免疫前血清温育(在PBS中1:500)，并且使用ECL系统展开。将PspA-特异性单克隆抗体用作阳性对照。

[0314] 为了进行流式细胞术分析(FACS)，如上所述培养SP1577，用福尔马林使其失活30min.，用5mg/ml在PBS中的无脂肪酸的BSA封闭，并且将约7x 10^5CFU与免疫或免疫前血清(在PBS中1:100)一起在室温下温育1h。使用Alexafluor 488-缀合的二抗检测表面结合的IgG。

[0315] 将ELISA几何平均值终点滴度(GMT)±平均值的一个标准偏差(SEM)和来自Western Blot和FACS分析(作为阳性vs.阴性的反应性)的结果总结在表5中。

[0316] 表5:2次免疫后小鼠中的IgG应答

[0317]

免疫原	ELISA (GMT ± SEM)[a)]	Western Blot (阳性/阴性)	FACS (阳性/阴性)
14	26013 ± 30255	9/1	8/2
15	271227 ± 82014	10/0	10/0
16	65606 ± 13592	10/0	10/0
17	82066 ± 36672	8/2	6/4
18	10354 ± 6760	7/3	5/5
19	263069 ± 62452	7/3	7/3
14+ 16[a)]	9027 ± 6150	10/0	10/0
14 + 16[b)]	65606 ± 13592	10/0	10/0
rPspA + 白矾	212977 ± 65684	10/0	10/0
PBS	< 100	0/10	0/10
[a)]免疫前血清显示在 ELISA、Western Blot 和 FACS 中无显著反应性。 [b)]在 ELISA 中测定的对 14 的 IgG 应答。 [c)]在 ELISA 中测定的对 16 的 IgG 应答。			

[0318] 尽管在远系杂交种小鼠中抗体应答可变，但是全部小鼠如ELISA中测定的均出现抗原-特异性IgG的高滴度，且大部分免疫血清中的IgG结合内源性PspA并且结合完整SP1577细胞。在免疫前血清和来自PBS-免疫小鼠的血清中未检测到显著水平的抗原-特异性IgG。

[0319] 使用Western Blot和FACS和一组有遗传差异的肺炎球菌菌株评价引起的IgG的交叉反应性，所述一组有遗传差异的肺炎球菌菌株代表属于来自PspA家族1-3的不同进化枝的不同PspA，它们定义在Hollingshead,Becker等人,Infect Immun,2000,68,5889-5900中；和不同的肺炎球菌荚膜血清型，包括目前批准的肺炎球菌缀合疫苗未涵盖的血清型。

[0320] 培养细菌并且使用来自免疫小鼠的血清如上述对SP1577所述进行Western Blot和FACS分析。将结果总结在下表6中。

[0321] 表6:小鼠血清中的IgG与遗传多样的肺炎球菌的交叉反应性

[0322]

免疫原	试验 a)	菌株/血清型						
		SP1577/1	SP920/8	SP4408/19A	SP1272/2	SP1388/4	SP1260/4	SP716/5
14	WBA	++	+	+	++	+	+	++
	FACS	++	+	++	++	+	++	++
15	WBA	++	++	++	++	+	++	++
	FACS	++	+	++	+	+	++	++
16	WBA	++	++	+	++	++	++	++
	FACS	++	++	++	++	++	++	++
17	WBA	++	-	-	+	++	-	-
	FACS	++	++	++	++	++	-	-
18	WBA	++	-	-	+	++	-	-
	FACS	++	++	++	++	++	-	-
19	WBA	+	-	-	+	++	-	+

[0323]

	FACS	+	+	++	++	++	++	++
14 + 16	WBA	++	++	++	++	++	++	++
	FACS	++	++	++	++	++	++	++
rPspA + 白矾	WBA	++	++	++	++	++	++	++
	FACS	++	++	++	++	++	++	++
a)使用两次免疫后的血清。免疫前血清在测定中未显示反应性。++=强带/信号；+= 弱带/信号；-= 未检测到结合。								

[0324] 使用遗传多样的组得到的结果表明，用PR肽抗原免疫引起广泛的交叉反应性IgG，不过，一些PR肽抗原构建体引起超过其它的更广泛的交叉反应性IgG。

[0325] 为了进行攻击性研究，给NMRI远系杂交种小鼠用如上所述的不同制剂免疫。为了比较免疫原性，给另外的动物用rPspA+白矾、缀合物44或脂肽46免疫。在最终免疫后10天给小鼠取血，如上所述通过ELISA分析血清，以便测定血清转换。然后对小鼠经尾静脉用100倍预先测定的致死剂量(100x LD_{100})肺炎球菌通过静脉内(iv)攻击，在攻击前所述肺炎球菌通过在小鼠中传代被赋予高度毒力。通过腹膜内(ip)或静脉内(iv)注射使细菌传代。攻击后，检测小鼠的健康状态，历时14天。对濒临死亡的动物实施安乐死，并且记录达到濒临死

亡的时间。将用脂肽类或蛋白质免疫的动物的存活和达到濒临死亡的时间与单独用PBS免疫的动物的存活和达到濒临死亡的时间比较。

[0326] 将使用菌株SP1577得到的结果总结在下表7中。SP1577菌株在小鼠中是具有高度毒力的。未保护的小鼠死亡或在攻击后的前12-24h变成濒临死亡。因此，保护显示了高功效。

[0327] 表7:针对菌株SP1577致死性攻击保护NMRI小鼠

[0328]

制剂	传代	到濒临死亡的天数		存活的 P-值[b]
		测试	对照(PBS)	
在 PBS 中的 14	ip	1, 2, 3 x > 14	5 x 1	0.0143
在 PBS 中的 14	iv	2 x 1, 5, 2 x > 14	5 x 1	0.0495
在 PBS 中的 15	ip	1, 2, 3, 2 x > 14	5 x 1	0.0143
在 PBS 中的 15	iv	3 x 2, 2 x > 14	5 x 1	0.0027
在 PBS 中的 16 + 14	ip	1, 1.5, 4, 2 x > 14	5 x 1	0.0143
在 PBS 中的 16 + 14	iv	3 x 2, 6, 14	5 x 1	0.0027
在 PBS 中的 18	ip	1, 1.5, 3 x > 14	5 x 1	0.0143
在 PBS 中的 19	ip	2 x 2, 3 x > 14	5 x 1	0.0027
在 PBS 中的 17+26+28	ip	2 x 1, 3 x > 14	5 x 1	0.0495
rPspA + 白矾	ip	3 x 1, 2 x >14	5 x 1	NS
rPspA + 白矾	iv	3 x 1, 5, >14	5 x 1	NS
在 PBS 中的 16	iv	5 x 1	5 x 1	NS
在 PBS 中的 26	ip	5 x 1	5 x 1	NS
在 PBS 中的 28	ip	4 x 1, 1 x 3	5 x 1	NS
[b] 通过时序检验显示与用 PBS 免疫的小鼠分布比较的存活分布的 P-值。NS = 无显著性(P > 0.05)。				

[0329] 用与SVLPs缀合的PR肽抗原免疫的小鼠部分被保护，且平均存活时间比PBS免疫的小鼠和rPspA免疫的小鼠延长。通过时序检验将对于用SVLPs或rPspA免疫的小鼠得到的存活分布与用单独的PBS(阴性对照)免疫的小鼠的存活分布比较。用单独的PR缀合物或与其它抗原的组合的PR缀合物免疫的小鼠结果显著不同于PBS免疫的小鼠的那些结果。P-值范围在0.0027-0.0143。尽管一些小鼠得到保护，但是用rPspA免疫的小鼠的结果与用PBS免疫的小鼠相比无显著性差异(P=0.1336)。该差异在较大的组中更为显著。StkP和PspC-衍生的缀合物的结果与PBS没有显著不同，表明这些抗原在该模型中无保护作用。

[0330] 为了进行被动免疫/攻击实验，以便测定抗体是否介导保护作用，在小鼠中产生单克隆抗体。在一个实验中，给BALB/c小鼠免疫3次缀合物17。从1只小鼠的脾细胞中生成产生抗原特异性单克隆IgG抗体(mAbs)的7个B细胞杂交瘤品系，并且通过FACS分析测试其与不同肺炎球菌的交叉反应性。1个mAb(5H8)结合广泛的菌株(临床隔离群)，其代表不同PspA分化体和荚膜血清型。在ELISA中，其它的mAbs结合PR抗原，但不结合完整的细菌。表位作图显示mAb 5H8识别在出现在广泛种类的不同PspA序列中的PR肽的C-末端部分上的表位，而另一种6mAbs识别另外的表位，它们通常较少在不同PspA序列中发现。为了进行攻击，将0.1－0.5mg纯化的5H8或另外的mAbs通过iv注射施用于5NMRI小鼠组。用StkP-衍生的mAb(1A7)被动免疫的动物和首次用于实验的动物用作对照组。在足够的平衡时间后，用传导的肺炎球菌iv攻击小鼠，并且如上所述监测健康状态和达到濒临死亡的时间。使用菌株SP1577得到的结果如下表8中所示。

[0331] 表8:NMRI小鼠对使用菌株SP1577致死性攻击的被动保护

[0332]

剂量 (mg/ml)	mAb	达到濒临死亡的天数		存活的 P-值 [a)]
		测试	对照	
0.5	5H8	1.5, 2, 3, 2 x >14	5 x 1	0.0027
0.1	5H8	2 x 1, 1 x 1.5, > 14	5 x 1	0.0495
0.5	3H5	5 x 1	5 x 1	NS
0.5	1H9	5 x 1	5 x 1	NS
0.5	1A7	5 x 1	5 x 1	NS
[a)] 通过时序检验显示存活分布与首次用于实验的小鼠的分布对比的 P-值。NS = 无显著性(P > 0.05)。				

[0333] 在被动免疫后仅mAb 5H8与首次用于实验的小鼠相比得到显著性差异结果。保护作用是剂量依赖性的。MAbs 3H5和1H9在这种被动保护模型中未显示显著作用。测序揭示出菌株SP1577的PspA仅包含5H8表位，但不含另外2个PR-衍生的抗体的表位。在该模型中对于StkP-衍生的mAb1A7未观察到保护作用。

[0334] 结果显示，当单独施用或与另外的抗原组合施用，不施用佐剂时，用形成SVLP的携带富含脯氨酸的肽抗原的脂肽免疫在小鼠中引起高度肺炎链球菌交叉反应性抗体。

[0335] 实施例5:兔免疫研究

[0336] 为了在非啮齿动物中表征抗体应答，给新西兰白色兔在第0、28和56天时sc免疫3次在0.4ml PBS中不同浓度的缀合物17、18或19，不使用或使用佐剂(R848)(表9)。在第0、14、38和66天时采血样用于测定血清转换。

[0337] 表9:用于兔免疫的制剂

[0338]

组	制剂	佐剂	浓度(mg/ml)[a)]
1	在 PBS 中的 17	无	0.35
2	在 PBS 中的 22	无	0.35
3	在 PBS 中的 21	无	0.1
4	在 PBS 中的 21	无	0.025
5	在 PBS 中的 21	R848	0.025
a) 显示脂肽浓度。			

[0339] 如上述对小鼠所述通过Western Blot和FACS、使用兔-IgG-特异性二抗和不同肺炎球菌隔离群分析免疫前和第3次免疫后的血清。基本上如上所述，还通过ELISA、使用用于IgG检测的单克隆抗-兔IgG(γ-链特异性)碱性磷酸酶抗体与4-硝基苯基-磷酸酯分析IgG应答的发生。结果如表10中所示。所有免疫的兔均出现结合内源性表达的PspA和在完整肺炎球菌上表达的PspA的IgG来应答免疫。免疫前血清显示在这些测定中无显著反应性。在来自免疫的兔的免疫血清中，甚至在不使用佐剂时施用低剂量的缀合物后，检测到高滴度的PR肽-特异性IgG抗体。

[0340] 表10:NZW兔中IgG应答的发生(N=3)

[0341]

组	ELISA (GMT ± SEM)[a)]			Western Blot (阳性/阴性)[b)]	FACS (阳性/阴性)[b)]
	1st imm.	2nd imm.	3rdimm.		
1	4.01 ± 0.22	4.92 ± 0.15	4.66 ± 0.16	3/0	3/0
2	2.96 ± 0.05	4.13 ± 0.19	4.15 ± 0.08	3/0	3/0
3	< 2.3	4.47 ± 0.05	4.67 ± 0.16	3/0	3/0
4	< 2.3	2.84 ± 0.25	3.79 ± 0.34	3/0	3/0
5	< 2.3	3.70 ± 0.38	4.56 ± 0.08	3/0	3/0
a) 显示 1(1st imm.)、2(2nd imm.)或 3(3rd imm.)免疫后的 log10 几何平均滴度(GMT) ± 一个平均值的标准偏差(SEM)。 b)显示了菌株 SP1577 的结果。					

[0342] 结果显示，同样在当不使用佐剂施用时，用形成SVLP的携带富含脯氨酸的肽抗原的脂肽免疫在非啮齿动物中引起广泛的交叉反应性抗体。

[0343] 实施例6:BALB/c小鼠中抗原特异性抗体应答的比较

[0344] 将缀合物15SVLPs引起的抗原-特异性抗体应答与重组PspA蛋白引起的抗原-特异性抗体应答对比。给6－8周龄雌性BALB/c小鼠(18只/组)在第0和第21天时间隔3周经皮下用在PBS中的0.1ml缀合物15或如上述实施例5中所述制备的在盐水中的rPspA+白矾免疫2次。在第二次免疫后10天采血。

[0345] 为了测定抗体应答的抗原特异性，如实施例5中所述进行ELISA，其中使用PR肽SEQ ID NO:27用作用于测量PR-特异性抗体的包被抗原，并且将rPspA用作用于测量总抗-PspA抗体(即针对N-末端表位、NPB和PR的抗体)的包被抗原。结果如图1中所示。在ELISA中，来自

非免疫接的小鼠的血清未显示与任一抗原的交叉反应性。

[0346] 正如预期的，rPspA为高度免疫原性的(抗-PspA IgG GMT±SEM＝81'969±28'068)，但不能引起显著水平的抗-PR抗体(抗-PspA IgG GMT±SEM＝213±218)。这不能归因于针对结合PR肽的rPspA的抗体增加失败，因为在ELISA中，针对缀合物15SVLPs增加的抗体识别PR肽(抗-PspA IgG GMT±SEM＝27'583±6'204)和rPspA抗原(20'919±3'444)。因此，可能的情况是，大部分rPspA-引起的抗体结合N-末端α螺旋部分中的表位。

[0347] 为了测定缺乏N-末端α螺旋的PspA是否可以引起更多的PR-特异性抗体，给小鼠免疫截短的重组PspA-Trx融合蛋白(rPspA-δ-N-末端；包含PR和NPB)。令人惊奇地，截短的蛋白质也不能引起显著水平的抗-PR抗体：对于该蛋白质，GMT±SEM为128'496±28'481，而对于PR抗原为213±1'094。这显著低于缀合物15SVLP-免疫的对照组动物(对于该蛋白质，GMT±SEM为32'776±6974，而对于PR肽为47'679±23'383)。这些结果共同表明SVLPs比重组PspA引起明显更高水平的PR-特异性抗体。

[0348] 实施例7：对增加的攻击剂量的保护

[0349] 为了测定免疫的BALB/c小鼠对于增加的攻击剂量的保护作用，给小鼠免疫缀合物15或如上述实施例6中所述的rPspA，通过静脉内以递增剂量血清型1细菌(范围从10^2—10^6CFU)攻击，并且监测存活。如上述Aaberge I.S.等人，Microbial Pathogenesis，1995，18，141-152中所述制备标准化肺炎球菌血清型1细菌接种物。通过给BALB/c小鼠静脉内注射递增剂量的细菌并且监测存活验证未免疫小鼠中的LD_{50}。

[0350] 免疫小鼠的保护作用依赖于细菌攻击剂量和免疫原类型。在低攻击剂量下，保护作用比得上用rPspA或脂肽结构单元15SVLP免疫的动物(参见图2)。在较高攻击剂量(100-10'000x LD_{50})下，用缀合物15SVLPs免疫的小鼠的保护作用优于用rPspA免疫的那些小鼠的保护作用。这些结果共同表明通过SVLPs引起的抗-PR抗体比针对rPspA提起的抗体在更宽的细菌攻击剂量范围内有保护作用。

序列表

<110> 维罗米蒂克斯股份公司(Virometix AG)
瑞士热带疾病与公共卫生研究所 (Swiss Tropical and Public Health Institute)
巴塞尔大学 (Universitaet Basel)
苏黎士大学 (Universitaet Zuerich)

<120> 针对肺炎链球菌进行保护的富含脯氨酸的肽类

<130> P3101PC00

<150> EP13195402.6
<151> 2013-12-03

<160> 133

<170> PatentIn 版本 3.5

<210> 1
<211> 14
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 1

Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu
1 5 10

<210> 2
<211> 16
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 2

Ile Arg Glu Asp Asn Asn Ile Thr Leu Lys Leu Asp Arg Cys Asn Asn
1 5 10 15

<210> 3
<211> 15
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 3

[0001]

Val Ser Ile Asp Lys Phe Arg Ile Phe Cys Lys Ala Asn Pro Lys
1 5 10 15

<210> 4
<211> 22
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 4

Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser
1 5 10 15

Ala Ser His Leu Glu Thr
20

<210> 5
<211> 16
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 5

Leu Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn Glu Ile Asp Ser
1 5 10 15

<210> 6
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 6

Pro Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala
1 5 10 15

Gln Val Ile Asp
20

<210> 7
<211> 20
<212> PRT
<213> 人工

<220>

<223> 合成构建体

<400> 7

Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser
1 5 10 15

Ser Leu Met Val
20

<210> 8
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 8

Gln Ser Ile Ala Leu Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu
1 5 10 15

Val Gly Glu Leu
20

<210> 9
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 9

Val Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn
1 5 10 15

Leu Phe Gln Val
20

<210> 10
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 10

Gln Gly Glu Ser Gly His Asp Ile Lys Ile Thr Ala Glu Asn Thr Pro
1 5 10 15

[0002]

Leu Pro Ile Ala
20

<210> 11
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 11

Gly Val Leu Leu Pro Thr Ile Pro Gly Lys Leu Asp Val Asn Lys Ser
1 5 10 15

Lys Thr His Ile
20

<210> 12
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 12

Thr Ile Asp Lys Ile Ser Asp Val Ser Thr Ile Val Pro Tyr Ile Gly
1 5 10 15

Pro Ala Leu Asn
20

<210> 13
<211> 25
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 13

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1 5 10 15

Thr Thr Gly Tyr Leu Lys Gly Asn Ser
20 25

<210> 14
<211> 14
<212> PRT

<213> 人工

<220>
<223> 合成构建体

<400> 14

Gly Leu Gln Gly Lys Ile Ala Asp Ala Val Lys Ala Lys Gly
1 5 10

<210> 15
<211> 19
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 15

Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala Met Val Glu
1 5 10 15

Asp Val Asn

<210> 16
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 16

Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg Val Val Ser
1 5 10 15

Asn Ala Asn Lys
20

<210> 17
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 17

Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu
1 5 10 15

[0003]

Met Thr Leu Ala
20

<210> 18
<211> 15
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 18

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp
1 5 10 15

<210> 19
<211> 13
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 19

Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
1 5 10

<210> 20
<211> 16
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 20

Tyr Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val
1 5 10 15

<210> 21
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 21

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr His Val Asp
1 5 10 15

Thr Glu Ser Tyr
20

<210> 22
<211> 15
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 22

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu Gly Val
1 5 10 15

<210> 23
<211> 20
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 23

Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe Asn
1 5 10 15

Val Val Asn Ser
20

<210> 24
<211> 17
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 24

Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu Lys Ile Arg
1 5 10 15

Ile

<210> 25
<211> 13
<212> PRT
<213> 人工

[0004]

<220>
<223> 合成构建体

<220>
<221> X
<222> (1)..(1)
<223> D-丙氨酸

<220>
<221> X
<222> (13)..(13)
<223> D-丙氨酸

<400> 25

Xaa Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Xaa
1 5 10

<210> 26
<211> 13
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<220>
<221> X
<222> (1)..(1)
<223> D-丙氨酸

<220>
<221> X
<222> (3)..(3)
<223> 丙氨酸环己酯

<220>
<221> X
<222> (13)..(13)
<223> D-丙氨酸

<400> 26

Xaa Lys Xaa Val Ala Ala Trp Thr Leu Lys Ala Ala Xaa
1 5 10

<210> 27
<211> 29
<212> PRT
<213> 肺炎链球菌

<400> 27

Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro Ala Pro
1 5 10 15

Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Thr
20 25

<210> 28
<211> 22
<212> PRT
<213> 肺炎链球菌

<400> 28

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
1 5 10 15

Lys Pro Glu Lys Pro Ala
20

<210> 29
<211> 32
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 29

Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro Glu Gln
1 5 10 15

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Lys Pro Glu Lys Pro
20 25 30

<210> 30
<211> 15
<212> PRT
<213> 肺炎链球菌

<400> 30

Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro Ala
1 5 10 15

<210> 31
<211> 14
<212> PRT
<213> 肺炎链球菌

<400> 31

Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Thr
1 5 10

[0005]

<210> 32
<211> 16
<212> PRT
<213> 肺炎链球菌

<400> 32

Gln Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro
1 5 10 15

<210> 33
<211> 16
<212> PRT
<213> 肺炎链球菌

<400> 33

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro
1 5 10 15

<210> 34
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 34

Pro Ala Pro Glu Ala Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro
1 5 10 15

Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys
20 25 30

Pro Glu Lys Thr
35

<210> 35
<211> 25
<212> PRT
<213> 肺炎链球菌

<400> 35

Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala
1 5 10 15

Pro Lys Pro Glu Lys Pro Ala Glu Gln
20 25

<210> 36
<211> 27
<212> PRT
<213> 肺炎链球菌

<400> 36

Pro Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu
1 5 10 15

Lys Pro Ala Glu Gln Pro Lys Pro Glu Lys Thr
20 25

<210> 37
<211> 20
<212> PRT
<213> 肺炎链球菌

<400> 37

Lys Ala Glu Lys Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro
1 5 10 15

Ala Pro Lys Thr
20

<210> 38
<211> 40
<212> PRT
<213> 肺炎链球菌

<400> 38

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Gln Pro
1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
20 25 30

Lys Pro Glu Gln Pro Thr Pro Ala
35 40

<210> 39
<211> 43
<212> PRT
<213> 肺炎链球菌

<400> 39

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro
20 25 30

Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Thr
35 40

<210> 40
<211> 37
[0006] <212> PRT
<213> 肺炎链球菌

<400> 40

Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala
1 5 10 15

Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln Pro Thr
20 25 30

Pro Ala Pro Lys Thr
35

<210> 41
<211> 21
<212> PRT
<213> 肺炎链球菌

<400> 41

Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln Pro Thr
1 5 10 15

Pro Ala Pro Lys Thr
20

<210> 42
<211> 17
<212> PRT
<213> 肺炎链球菌

<400> 42

Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro
1 5 10 15

Gln

<210> 43
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 43

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
1 5 10 15

Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr Gln Pro Glu Lys Pro Ala
20 25 30

Thr Pro Lys Thr
35

<210> 44
<211> 27
<212> PRT
<213> 肺炎链球菌

<400> 44

Pro Lys Pro Glu Gln Pro Ala Lys Pro Glu Lys Pro Ala Glu Glu Pro
1 5 10 15

Thr Gln Pro Glu Lys Pro Ala Thr Pro Lys Thr
20 25

<210> 45
<211> 32
<212> PRT
<213> 肺炎链球菌

<400> 45

Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Ala Pro Ala
1 5 10 15

Pro Gln Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro Ala
20 25 30

<210> 46
<211> 41
<212> PRT
<213> 肺炎链球菌

<400> 46

Pro Glu Thr Pro Ala Pro Ala Pro Lys Pro Glu Thr Pro Ala Pro Ala
1 5 10 15

Pro Glu Ala Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
20 25 30

Pro Ala Pro Lys Pro Glu Lys Ser Ala
35 40

<210> 47
<211> 20
<212> PRT
<213> 肺炎链球菌

<400> 47

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
1 5 10 15

Glu Lys Ser Ala
20

[0007]

<210> 48
<211> 16
<212> PRT
<213> 肺炎链球菌

<400> 48

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Ser Ala
1 5 10 15

<210> 49
<211> 20
<212> PRT
<213> 肺炎链球菌

<400> 49

Lys Ala Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Val Pro
1 5 10 15

Ala Pro Lys Thr
20

<210> 50
<211> 28
<212> PRT
<213> 肺炎链球菌

<400> 50

Pro Ala Pro Ala Pro Lys Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro
1 5 10 15

Ala Pro Ala Pro Ala Pro Lys Pro Glu Lys Ser Ala
20 25

<210> 51
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 51

Pro Ala Pro Glu Gln Pro Thr Glu Pro Thr Gln Pro Glu Lys Pro Ala
1 5 10 15

Glu Glu Thr Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys
20 25 30

Ala Glu Lys Thr
35

<210> 52

<210> 17
<212> PRT
<213> 肺炎链球菌

<400> 52

Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu Lys
1 5 10 15

Thr

<210> 53
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 53

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro
1 5 10 15

Ala Pro Ala Pro Gln Pro Glu Lys Pro Ala Glu Glu Pro Glu Asn Pro
20 25 30

Ala Pro Ala Pro
35

<210> 54
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 54

Ala Pro Ala Pro Lys Pro Glu Thr Pro Ala Pro Ala Pro Glu Ala Pro
1 5 10 15

Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys
20 25 30

Pro Glu Lys Ser
35

<210> 55
<211> 42
<212> PRT
<213> 肺炎链球菌

<400> 55

Ala Pro Ala Pro Glu Thr Pro Ala Pro Glu Ala Pro Ala Glu Gln Pro
1 5 10 15

[0008]

Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys
20 25 30

Pro Ala Glu Gln Pro Lys Pro Glu Lys Thr
35 40

<210> 56
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 56

Pro Ala Pro Glu Gln Pro Thr Glu Pro Thr Gln Pro Glu Lys Pro Ala
1 5 10 15

Glu Glu Thr Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys
20 25 30

Ala Glu Lys Thr
35

<210> 57
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 57

Ala Pro Ala Pro Lys Pro Glu Thr Pro Ala Pro Ala Pro Glu Ala Pro
1 5 10 15

Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys
20 25 30

Pro Glu Lys Ser
35

<210> 58
<211> 42
<212> PRT
<213> 肺炎链球菌

<400> 58

Ala Pro Ala Pro Glu Thr Pro Ala Pro Glu Ala Pro Ala Glu Gln Pro
1 5 10 15

Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys
20 25 30

Pro Ala Glu Gln Pro Lys Pro Glu Lys Thr
35 40

<210> 59
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 59

Ala Pro Ala Pro Lys Pro Glu Thr Pro Ala Pro Ala Pro Glu Ala Pro
1 5 10 15

Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys
20 25 30

Pro Glu Lys Ser
35

<210> 60
<211> 42
<212> PRT
<213> 肺炎链球菌

<400> 60

Ala Pro Ala Pro Glu Thr Pro Ala Pro Glu Ala Pro Ala Glu Gln Pro
1 5 10 15

Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys
20 25 30

Pro Ala Glu Gln Pro Lys Pro Glu Lys Thr
35 40

<210> 61
<211> 43
<212> PRT
<213> 肺炎链球菌

<400> 61

Ala Pro Ala Pro Glu Thr Pro Ala Pro Glu Ala Pro Ala Glu Gln Pro
1 5 10 15

Lys Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys
20 25 30

Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro Ala
35 40

<210> 62
<211> 33
<212> PRT
<213> 肺炎链球菌

[0009] <400> 62

Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
1 5 10 15

Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu His
20 25 30

Pro

<210> 63
<211> 35
<212> PRT
<213> 肺炎链球菌

<400> 63

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Gln Pro
1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
20 25 30

Lys Pro Glu
35

<210> 64
<211> 33
<212> PRT
<213> 肺炎链球菌

<400> 64

Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln
1 5 10 15

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro
20 25 30

Pro

<210> 65
<211> 35
<212> PRT
<213> 肺炎链球菌

<400> 65

Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
1 5 10 15

Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu
20 25 30

Gln Pro Ala
35

<210> 66
<211> 33
<212> PRT
<213> 肺炎链球菌

<400> 66

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Ala Pro
1 5 10 15

Lys Pro Glu Gln Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro
20 25 30

Thr

<210> 67
<211> 35
<212> PRT
<213> 肺炎链球菌

<400> 67

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro
20 25 30

Lys Pro Glu
35

<210> 68
<211> 31
<212> PRT
<213> 肺炎链球菌

<400> 68

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro
1 5 10 15

Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Gln
20 25 30

<210> 69
<211> 35
<212> PRT
<213> 肺炎链球菌

[0010] <400> 69

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Gln Pro
1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
20 25 30

Lys Pro Ala
35

<210> 70
<211> 34
<212> PRT
<213> 肺炎链球菌

<400> 70

Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln Pro Thr
1 5 10 15

Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln
20 25 30

Pro Thr

<210> 71
<211> 21
<212> PRT
<213> 肺炎链球菌

<400> 71

Pro Glu Lys Pro Ala Pro Ala Pro Glu Lys Pro Ala Pro Ala Pro Glu
1 5 10 15

Lys Pro Ala Pro Ala
20

<210> 72
<211> 23
<212> PRT
<213> 肺炎链球菌

<400> 72

Pro Ala Pro Lys Pro Ala Pro Ala Pro Lys Pro Ala Pro Ala Pro Ala
1 5 10 15

Pro Lys Pro Glu Lys Pro Ala
20

<210> 73

<211> 16
<212> PRT
<213> 肺炎链球菌

<400> 73

Pro Ala Pro Ala Pro Thr Pro Glu Ala Pro Ala Pro Ala Pro Lys Pro
1 5 10 15

<210> 74
<211> 23
<212> PRT
<213> 肺炎链球菌

<400> 74

Pro Lys Pro Glu Gln Pro Ala Lys Pro Glu Lys Pro Ala Glu Glu Pro
1 5 10 15

Thr Gln Pro Glu Lys Pro Ala
20

<210> 75
<211> 18
<212> PRT
<213> 肺炎链球菌

<400> 75

Pro Ala Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr Gln Pro Glu Lys
1 5 10 15

Pro Ala

<210> 76
<211> 27
<212> PRT
<213> 肺炎链球菌

<400> 76

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Lys Pro Glu Lys Pro
1 5 10 15

Ala Glu Glu Pro Thr Gln Pro Glu Lys Pro Ala
20 25

<210> 77
<211> 32
<212> PRT
<213> 肺炎链球菌

[0011] <400> 77

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Asn Pro Glu Gln Pro Ala
1 5 10 15

Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr Gln Pro Glu Lys Pro Ala
20 25 30

<210> 78
<211> 29
<212> PRT
<213> 肺炎链球菌

<400> 78

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln
1 5 10 15

Pro Ala Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
20 25

<210> 79
<211> 32
<212> PRT
<213> 肺炎链球菌

<400> 79

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
1 5 10 15

Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr Gln Pro Glu Lys Pro Ala
20 25 30

<210> 80
<211> 32
<212> PRT
<213> 肺炎链球菌

<400> 80

Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala
1 5 10 15

Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr Gln Pro Glu Lys Pro Ala
20 25 30

<210> 81
<211> 38
<212> PRT
<213> 肺炎链球菌

<400> 81

Pro Ala Pro Ala Pro Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro

1 5 10 15

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
20 25 30

Lys Pro Glu Gln Pro Ala
35

<210> 82
<211> 43
<212> PRT
<213> 肺炎链球菌

<400> 82

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro Lys Pro
1 5 10 15

Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
20 25 30

Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
35 40

<210> 83
<211> 43
<212> PRT
<213> 肺炎链球菌

<400> 83

Pro Ala Arg Ala Leu Gln Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
1 5 10 15

Glu Gln Pro Thr Pro Ala Pro Lys Pro Glu Gln Pro Thr Pro Ala Pro
20 25 30

Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
35 40

<210> 84
<211> 23
<212> PRT
<213> 肺炎链球菌

<400> 84

Ser Arg Leu Glu Gln Pro Ser Leu Gln Pro Thr Pro Glu Pro Ser Pro
1 5 10 15

Gly Pro Gln Pro Ala Pro Asn
20

[0012]

<210> 85
<211> 24
<212> PRT
<213> 肺炎链球菌

<400> 85

Arg Pro Glu Glu Pro Ser Pro Gln Pro Thr Pro Glu Pro Ser Pro Ser
1 5 10 15

Pro Gln Pro Ala Pro Ser Asn Pro
20

<210> 86
<211> 36
<212> PRT
<213> 肺炎链球菌

<400> 86

His Trp Val Pro Asp Ser Arg Pro Glu Gln Pro Ser Pro Gln Ser Thr
1 5 10 15

Pro Glu Pro Ser Pro Ser Pro Gln Pro Ala Pro Asn Pro Gln Pro Ala
20 25 30

Pro Ser Asn Pro
35

<210> 87
<211> 32
<212> PRT
<213> 肺炎链球菌

<400> 87

Pro Lys Ser Asn Gln Ile Gly Gln Pro Thr Leu Pro Asn Asn Ser Leu
1 5 10 15

Ala Thr Pro Ser Pro Ser Leu Pro Ile Asn Pro Gly Thr Ser His Glu
20 25 30

<210> 88
<211> 34
<212> PRT
<213> 酿脓链球菌 (Streptococcus pyogenes)

<400> 88

Pro Glu Val Thr Pro Thr Pro Glu Thr Pro Glu Gln Pro Gly Glu Lys
1 5 10 15

Ala Pro Glu Lys Ser Pro Glu Val Thr Pro Thr Pro Glu Thr Pro Glu
20 25 30

Gln Pro

<210> 89
<211> 20
<212> PRT
<213> 酿脓链球菌

<400> 89

Pro Glu Val Thr Pro Thr Pro Glu Thr Pro Glu Gln Pro Gly Glu Lys
1 5 10 15

Ala Pro Glu Lys
20

<210> 90
<211> 17
<212> PRT
<213> 酿脓链球菌

<400> 90

Pro Glu Lys Ser Pro Glu Val Thr Pro Thr Pro Glu Thr Pro Glu Gln
1 5 10 15

Pro

<210> 91
<211> 16
<212> PRT
<213> 酿脓链球菌

<400> 91

Lys Ala Pro Glu Lys Ser Pro Glu Val Thr Pro Thr Pro Glu Met Pro
1 5 10 15

<210> 92
<211> 25
<212> PRT
<213> 酿脓链球菌

<400> 92

Pro Gly Lys Pro Ala Pro Lys Thr Pro Glu Val Pro Gln Lys Pro Asp
1 5 10 15

Thr Ala Pro His Thr Pro Lys Thr Pro
20 25

<210> 93
<211> 18
<212> PRT
<213> 马链球菌(Streptococcus equi)

[0013]

<400> 93

Lys Pro Ser Ala Pro Lys Ala Pro Glu Lys Ala Pro Ala Pro Lys Ala
1 5 10 15

Pro Lys

<210> 94
<211> 24
<212> PRT
<213> 马链球菌

<400> 94

Pro Ala Pro Lys Ala Pro Lys Ala Ser Glu Gln Ser Ser Asn Pro Lys
1 5 10 15

Ala Pro Ala Pro Lys Ser Ala Pro
20

<210> 95
<211> 22
<212> PRT
<213> 酿脓链球菌

<400> 95

Pro Gly Pro Ala Gly Pro Arg Gly Leu Gln Gly Pro Gln Gly Pro Arg
1 5 10 15

Gly Asp Lys Gly Glu Thr
20

<210> 96
<211> 17
<212> PRT
<213> 猪链球菌(Streptococcus suis)

<400> 96

Pro Gln Ala Pro Ser Thr Pro Glu Lys Gln Pro Glu Val Pro Glu Ser
1 5 10 15

Pro

<210> 97
<211> 17
<212> PRT
<213> 猪链球菌

<400> 97

Pro Glu Thr Pro Asp Ala Pro Ser Thr Pro Lys Asp Glu Pro Gln Ala
1 5 10 15

Pro

<210> 98
<211> 28
<212> PRT
<213> 变异链球菌(Streptococcus mutans)

<400> 98

Pro Ala Pro Val Glu Pro Ser Tyr Glu Ala Glu Pro Thr Pro Pro Thr
1 5 10 15

Arg Thr Pro Asp Gln Ala Glu Pro Asn Lys Pro Thr
20 25

<210> 99
<211> 23
<212> PRT
<213> 变异链球菌

<400> 99

Pro Thr Tyr Glu Thr Glu Lys Pro Leu Glu Pro Ala Pro Val Glu Pro
1 5 10 15

Ser Tyr Glu Ala Glu Pro Thr
20

<210> 100
<211> 30
<212> PRT
<213> 变异链球菌

<400> 100

Lys Pro Thr Ala Pro Thr Lys Pro Thr Tyr Glu Thr Glu Lys Pro Leu
1 5 10 15

Lys Pro Ala Pro Val Ala Pro Asn Tyr Glu Lys Glu Pro Thr
20 25 30

<210> 101
<211> 18
<212> PRT
<213> 金黄色葡萄球菌 (Staphylococcus aureus)

<400> 101

Lys Pro Val Val Pro Glu Gln Pro Asp Glu Pro Gly Glu Ile Glu Pro
1 5 10 15

[0014]

Ile Pro

<210> 102
<211> 29
<212> PRT
<213> 金黄色葡萄球菌

<400> 102

Pro Glu Val Pro Ser Glu Pro Glu Thr Pro Thr Pro Pro Thr Pro Glu
1 5 10 15

Val Pro Ala Glu Pro Gly Lys Pro Val Pro Pro Ala Lys
20 25

<210> 103
<211> 28
<212> PRT
<213> 金黄色葡萄球菌

<400> 103

Lys Tyr Thr Pro Lys Lys Pro Asn Lys Pro Ile Tyr Pro Glu Lys Pro
1 5 10 15

Lys Asp Lys Thr Pro Pro Thr Lys Pro Asp His Ser
20 25

<210> 104
<211> 12
<212> PRT
<213> 大消化链球菌(Peptostreptococcus magnus)

<400> 104

Pro Glu Lys Pro Val Glu Pro Ser Glu Pro Ser Thr
1 5 10

<210> 105
<211> 44
<212> PRT
<213> 缺乳链球菌 (Streptococcus dysgalactiae)

<400> 105

Lys Pro Val Glu Pro Ser Glu Pro Ser Thr Pro Asp Val Pro Ser Asn
1 5 10 15

Pro Ser Asn Pro Ser Thr Pro Asp Val Pro Ser Thr Pro Asp Val Pro
20 25 30

Ser Asn Pro Ser Thr Pro Glu Val Pro Ser Asn Pro

35 40

<210> 106
<211> 17
<212> PRT
<213> 缺乳链球菌

<400> 106

Pro Gln Val Glu Pro Asn Val Pro Asp Thr Pro Gln Glu Lys Pro Leu
1 5 10 15

Thr

<210> 107
<211> 31
<212> PRT
<213> 缺乳链球菌

<400> 107

Lys Pro Leu Thr Pro Leu Ala Pro Ser Glu Pro Ser Gln Pro Ser Ile
1 5 10 15

Pro Glu Thr Pro Leu Ile Pro Ser Glu Pro Ser Val Pro Glu Thr
20 25 30

<210> 108
<211> 18
<212> PRT
<213> 无乳链球菌 (Streptococcus agalactiae)

<400> 108

Pro Glu Val Lys Pro Asp Val Lys Pro Glu Ala Lys Pro Glu Ala Lys
1 5 10 15

Pro Ala

<210> 109
<211> 11
<212> PRT
<213> 无乳链球菌

<400> 109

Lys Pro Glu Ala Lys Pro Glu Ala Lys Pro Ala
1 5 10

<210> 110
<211> 20
[0015] <212> PRT
<213> 无乳链球菌

<400> 110

Pro Asp Val Lys Pro Glu Ala Lys Pro Glu Ala Lys Pro Asp Val Lys
1 5 10 15

Pro Glu Ala Lys
20

<210> 111
<211> 14
<212> PRT
<213> 无乳链球菌

<400> 111

Pro Glu Thr Pro Asp Thr Pro Lys Ile Pro Glu Leu Pro Gln
1 5 10

<210> 112
<211> 20
<212> PRT
<213> 无乳链球菌

<400> 112

Pro Asp Thr Pro Gln Ala Pro Asp Thr Pro His Val Pro Glu Ser Pro
1 5 10 15

Lys Thr Pro Glu
20

<210> 113
<211> 29
<212> PRT
<213> 肺炎链球菌

<400> 113

Gln Gln Ala Glu Glu Asp Tyr Ala Arg Arg Ser Glu Glu Glu Tyr Asn
1 5 10 15

Arg Leu Pro Gln Gln Gln Pro Pro Lys Ala Glu Lys Pro
20 25

<210> 114
<211> 31
<212> PRT
<213> 肺炎链球菌

<400> 114

Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr

1 5 10 15

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val
20 25 30

<210> 115
<211> 79
<212> PRT
<213> 肺炎链球菌

<400> 115

Ser Val Ala Met Pro Ser Tyr Ile Gly Ser Ser Leu Glu Phe Thr Lys
1 5 10 15

Asn Asn Leu Ile Gln Ile Val Gly Ile Lys Glu Ala Asn Ile Glu Val
20 25 30

Val Glu Val Thr Thr Ala Pro Ala Gly Ser Ala Glu Gly Met Val Val
35 40 45

Glu Gln Ser Pro Arg Ala Gly Glu Lys Val Asp Leu Asn Lys Thr Arg
50 55 60

Val Lys Ile Ser Ile Tyr Lys Pro Lys Thr Thr Ser Ala Thr Pro
65 70 75

<210> 116
<211> 60
<212> PRT
<213> 肺炎链球菌

<400> 116

Ser Leu Phe Val Glu Ser Ser Val Asp Asp Arg Pro Met Lys Thr Val
1 5 10 15

Ser Gln Asp Thr Asn Ile Pro Ile Tyr Ala Gln Ile Phe Thr Asp Ser
20 25 30

Ile Ala Glu Gln Gly Lys Glu Gly Asp Ser Tyr Tyr Ser Met Met Lys
35 40 45

Tyr Asn Leu Asp Lys Ile Ala Glu Gly Leu Ala Lys
50 55 60

<210> 117
<211> 112
<212> PRT
<213> 肺炎链球菌

<400> 117

[0016]

Asn Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val Ala Gln Tyr
1 5 10 15

Tyr Ile Thr Trp Asn Glu Leu Ser Tyr Asp His Gln Gly Lys Glu Val
20 25 30

Leu Thr Pro Lys Ala Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His
35 40 45

Phe Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn Leu Ser Val
50 55 60

Lys Ile Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Thr Val
65 70 75 80

Tyr Glu Lys Thr Asp Leu Pro Leu Val Arg Lys Arg Thr Ile Ser Ile
85 90 95

Trp Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val Glu Asn Asp
100 105 110

<210> 118
<211> 91
<212> PRT
<213> 肺炎链球菌

<400> 118

Val Glu His Pro Asn Glu Arg Pro His Ser Asp Asn Gly Phe Gly Asn
1 5 10 15

Ala Ser Asp His Val Arg Lys Asn Lys Val Asp Gln Asp Ser Lys Pro
20 25 30

Asp Glu Asp Lys Glu His Asp Glu Val Ser Glu Pro Thr His Pro Glu
35 40 45

Ser Asp Glu Lys Glu Asn His Ala Gly Leu Asn Pro Ser Ala Asp Asn
50 55 60

Leu Tyr Lys Pro Ser Thr Asp Thr Glu Glu Thr Glu Glu Glu Ala Glu
65 70 75 80

Asp Thr Thr Asp Glu Ala Glu Ile Pro Gln Val
85 90

<210> 119
<211> 69
<212> PRT
<213> 肺炎链球菌

<400> 119

Glu Asn Ser Val Ile Asn Ala Lys Ile Ala Asp Ala Glu Ala Leu Leu
1 5 10 15

Glu Lys Val Thr Asp Pro Ser Ile Arg Gln Asn Ala Met Glu Thr Leu
20 25 30

Thr Gly Leu Lys Ser Ser Leu Leu Leu Gly Thr Lys Asp Asn Asn Thr
35 40 45

Ile Ser Ala Glu Val Asp Ser Leu Leu Ala Leu Leu Lys Glu Ser Gln
50 55 60

Pro Ala Pro Ile Gln
65

<210> 120
<211> 28
<212> DNA
<213> 人工

<220>
<223> 合成构建体

<400> 120
gcaagcttat gatatagaaa tttgtaac 28

<210> 121
<211> 27
<212> DNA
<213> 人工

<220>
<223> 合成构建体

<400> 121
ccacataccg ttttcttgtt tccagcc 27

<210> 122
<211> 391
<212> PRT
<213> 肺炎链球菌

<220>
<221> misc_feature
<222> (1)..(2)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (9)..(11)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (14)..(18)
<223> Xaa可以是任何天然存在的氨基酸

[0017]

<220>
<221> misc_feature
<222> (26)..(26)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (32)..(32)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (35)..(35)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (42)..(42)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (51)..(52)
<223> Xaa可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (67)..(67)
<223> Xaa可以是任何天然存在的氨基酸

<400> 122

Xaa Xaa Leu Gly Ala Gly Phe Val Xaa Xaa Xaa Pro Thr Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Glu Ala Pro Val Ala Ser Gln Xaa Lys Ala Glu Lys Asp Xaa
20 25 30

Asp Ala Xaa Lys Arg Asp Ala Glu Asn Xaa Lys Lys Ala Leu Glu Glu
35 40 45

Ala Lys Xaa Xaa Gln Lys Lys Tyr Glu Asp Asp Gln Lys Lys Thr Glu
50 55 60

Glu Lys Xaa Lys Lys Glu Lys Glu Ala Ser Lys Glu Glu Gln Ala Ala
65 70 75 80

Asn Leu Lys Tyr Gln Gln Glu Leu Val Lys Tyr Ala Ser Glu Lys Asp
85 90 95

Ser Val Lys Lys Ala Lys Ile Leu Lys Glu Val Glu Glu Ala Glu Lys
100 105 110

Glu His Lys Lys Lys Arg Ala Glu Phe Glu Lys Val Arg Ser Glu Val
115 120 125

```
Ile Pro Ser Ala Glu Glu Leu Lys Lys Thr Arg Gln Lys Ala Glu Glu
    130                 135                 140

Ala Lys Ala Lys Glu Ala Glu Leu Ile Lys Lys Val Glu Glu Ala Glu
145                 150                 155                 160

Lys Lys Val Thr Glu Ala Lys Gln Lys Leu Asp Ala Glu Arg Ala Lys
                165                 170                 175

Glu Val Ala Leu Gln Ala Lys Ile Ala Glu Leu Glu Asn Glu Val Tyr
            180                 185                 190

Arg Leu Glu Thr Glu Leu Lys Gly Ile Asp Glu Ser Asp Ser Glu Asp
        195                 200                 205

Tyr Val Lys Glu Gly Leu Arg Ala Pro Leu Gln Ser Glu Leu Asp Ala
    210                 215                 220

Lys Arg Thr Lys Leu Ser Thr Leu Glu Glu Leu Ser Asp Lys Ile Asp
225                 230                 235                 240

Glu Leu Asp Ala Glu Ile Ala Lys Leu Glu Lys Asn Val Glu Tyr Phe
                245                 250                 255

Lys Lys Thr Asp Ala Glu Gln Thr Glu Gln Tyr Leu Ala Ala Ala Glu
            260                 265                 270

Lys Asp Leu Ala Asp Lys Lys Ala Glu Leu Glu Lys Thr Glu Ala Asp
        275                 280                 285

Leu Lys Lys Ala Val Asn Glu Pro Glu Lys Pro Ala Glu Glu Thr Pro
    290                 295                 300

Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys Pro Ala
305                 310                 315                 320

Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Thr Asp Asp
                325                 330                 335

Gln Gln Ala Glu Glu Asp Tyr Ala Arg Arg Ser Glu Glu Glu Tyr Asn
            340                 345                 350

Arg Leu Pro Gln Gln Gln Pro Pro Lys Ala Glu Lys Pro Ala Pro Ala
        355                 360                 365

Pro Lys Pro Glu Gln Pro Val Pro Ala Pro Lys Thr Gly Trp Lys Gln
    370                 375                 380

Glu Asn Gly Met Trp Cys Arg
385                 390
```

[0018]

<210> 123
<211> 723
<212> PRT
<213> 肺炎链球菌

<400> 123

```
Met Asn Lys Lys Lys Met Ile Leu Thr Ser Leu Ala Ser Val Ala Ile
1               5                   10                  15

Leu Gly Ala Gly Phe Val Thr Ser Gln Pro Thr Val Val Arg Ala Glu
            20                  25                  30

Glu Ser Pro Val Ala Ser Gln Ser Lys Ala Glu Lys Asp Tyr Asp Ala
        35                  40                  45

Ala Val Lys Lys Ser Glu Ala Ala Lys Lys His Tyr Glu Glu Ala Lys
    50                  55                  60

Lys Lys Ala Glu Asp Ala Gln Lys Lys Tyr Asp Glu Asp Gln Lys Lys
65                  70                  75                  80

Thr Glu Ala Lys Ala Glu Lys Glu Arg Lys Ala Ser Glu Lys Ile Ala
                85                  90                  95

Glu Ala Thr Lys Glu Val Gln Gln Ala Tyr Leu Ala Tyr Leu Gln Ala
            100                 105                 110

Ser Asn Glu Ser Gln Arg Lys Glu Ala Asp Lys Lys Ile Lys Glu Ala
        115                 120                 125

Thr Gln Arg Lys Asp Glu Ala Glu Ala Ala Phe Ala Thr Ile Arg Thr
    130                 135                 140

Thr Ile Val Val Pro Glu Pro Ser Glu Leu Ala Glu Thr Lys Lys Lys
145                 150                 155                 160

Ala Glu Glu Ala Lys Ala Glu Glu Lys Val Ala Lys Arg Lys Tyr Asp
                165                 170                 175

Tyr Ala Thr Leu Lys Leu Ala Leu Ala Lys Lys Glu Val Glu Ala Lys
            180                 185                 190

Glu Leu Glu Ile Glu Lys Leu Gln Tyr Glu Ile Ser Thr Leu Glu Gln
        195                 200                 205

Glu Val Ala Thr Ala Gln His Gln Val Asp Asn Leu Lys Lys Leu Leu
    210                 215                 220

Ala Gly Ala Asp Pro Asp Asp Gly Thr Glu Val Ile Glu Ala Lys Leu
225                 230                 235                 240

Lys Lys Gly Glu Ala Glu Leu Asn Ala Lys Gln Ala Glu Leu Ala Lys
                245                 250                 255

Lys Gln Thr Glu Leu Glu Lys Leu Leu Asp Ser Leu Asp Pro Glu Gly
```

260 265 270

Lys Thr Gln Asp Glu Leu Asp Lys Glu Ala Glu Glu Ala Glu Leu Asp
275 280 285

Lys Lys Ala Asp Glu Leu Gln Asn Lys Val Ala Asp Leu Glu Lys Glu
290 295 300

Ile Ser Asn Leu Glu Ile Leu Leu Gly Gly Ala Asp Pro Glu Asp Asp
305 310 315 320

Thr Ala Ala Leu Gln Asn Lys Leu Ala Ala Lys Lys Ala Glu Leu Ala
325 330 335

Lys Lys Gln Thr Glu Leu Glu Lys Leu Leu Asp Ser Leu Asp Pro Glu
340 345 350

Gly Lys Thr Gln Asp Glu Leu Asp Lys Glu Ala Glu Glu Ala Glu Leu
355 360 365

Asp Lys Lys Ala Asp Glu Leu Gln Asn Lys Val Ala Asp Leu Glu Lys
370 375 380

Glu Ile Ser Asn Leu Glu Ile Leu Leu Gly Gly Ala Asp Ser Glu Asp
385 390 395 400

Asp Thr Ala Ala Leu Gln Asn Lys Leu Ala Thr Lys Lys Ala Glu Leu
405 410 415

Glu Lys Thr Gln Lys Glu Leu Asp Ala Ala Leu Asn Glu Leu Gly Pro
420 425 430

Asp Gly Asp Glu Glu Glu Thr Pro Ala Pro Ala Pro Gln Pro Glu Gln
435 440 445

Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro Lys Pro
450 455 460

Glu Gln Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Pro Ala Pro
465 470 475 480

Lys Pro Glu Gln Pro Ala Lys Pro Glu Lys Pro Ala Glu Glu Pro Thr
485 490 495

Gln Pro Glu Lys Pro Ala Thr Pro Lys Thr Gly Trp Lys Gln Glu Asn
500 505 510

Gly Met Trp Tyr Phe Tyr Asn Thr Asp Gly Ser Met Ala Thr Gly Trp
515 520 525

Leu Gln Asn Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Ser Met
530 535 540

[0019] Ala Thr Gly Trp Val Lys Asp Gly Asp Thr Trp Tyr Tyr Leu Glu Ala
545 550 555 560

Ser Gly Ala Met Lys Ala Ser Gln Trp Phe Lys Val Ser Asp Lys Trp
565 570 575

Tyr Tyr Val Asn Ser Asn Gly Ala Met Ala Thr Gly Trp Leu Gln Tyr
580 585 590

Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Asp Met Ala Thr Gly
595 600 605

Trp Leu Gln Tyr Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Asp
610 615 620

Met Ala Thr Gly Trp Ala Lys Val Asn Gly Ser Trp Tyr Tyr Leu Asn
625 630 635 640

Ala Asn Gly Ala Met Ala Thr Gly Trp Ala Lys Val Asn Gly Ser Trp
645 650 655

Tyr Tyr Leu Asn Ala Asn Gly Ser Met Ala Thr Gly Trp Val Lys Asp
660 665 670

Gly Asp Thr Trp Tyr Tyr Leu Glu Ala Ser Gly Ala Met Lys Ala Ser
675 680 685

Gln Trp Phe Lys Val Ser Asp Lys Trp Tyr Tyr Val Asn Gly Leu Gly
690 695 700

Ala Leu Ala Val Asn Thr Thr Val Asp Gly Tyr Lys Val Asn Ala Asn
705 710 715 720

Gly Glu Trp

<210> 124
<211> 11
<212> PRT
<213> 肺炎链球菌

<400> 124

Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln
1 5 10

<210> 125
<211> 7
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 125

Ile Glu Lys Lys Ile Glu Gly
1 5

<210> 126
<211> 13
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 126

Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Arg Lys
1 5 10

<210> 127
<211> 51
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 127

Gly Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly
1 5 10 15

Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu
20 25 30

Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe Asn Val Val
35 40 45

Asn Ser Lys
50

<210> 128
<211> 67
<212> PRT
<213> 人工

<220>
<223> 合成序列

<400> 128

Gly Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly
1 5 10 15

Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu
20 25 30

[0020] Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe Asn Val Val
35 40 45

Asn Ser Lys Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln
50 55 60

Pro Lys Pro
65

<210> 129
<211> 48
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 129

Gly Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly
1 5 10 15

Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu
20 25 30

Lys Lys Ile Ala Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Arg
35 40 45

<210> 130
<211> 67
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 130

Gly Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly
1 5 10 15

Ile Glu Lys Lys Ile Glu Gly Ile Glu Lys Lys Ile Glu Gly Ile Glu
20 25 30

Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe Asn Val Val
35 40 45

Asn Ser Lys Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln
50 55 60

Pro Lys Pro
65

<210> 131
<211> 79

<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 131

Ser Val Ala Met Pro Ser Tyr Ile Gly Ser Ser Leu Glu Gln Thr Lys
1 5 10 15

Asn Asn Leu Ile Gln Thr Val Gly Ile Lys Glu Ala Asn Ile Glu Val
20 25 30

Val Glu Val Thr Thr Ala Pro Ala Gly Ser Ala Glu Gly Met Val Val
35 40 45

Glu Gln Ser Pro Arg Ala Gly Glu Lys Val Asp Leu Asn Lys Thr Arg
50 55 60

Val Lys Ile Ser Ile Tyr Lys Pro Lys Thr Thr Ser Ala Thr Pro
65 70 75

<210> 132
<211> 31
<212> PRT
<213> 人工

<220>
<223> 合成构建体

<400> 132

Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr
1 5 10 15

Tyr Lys Thr Ala Glu Leu Glu Lys Ala Glu Ser Asp Val Glu Val
20 25 30

<210> 133
<211> 268
<212> PRT
<213> 肺炎链球菌

<400> 133

[0021]

Met Ser Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
1 5 10 15

Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
20 25 30

Cys Gly Pro Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp
35 40 45

Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn
50 55 60

Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu
65 70 75 80

Leu Phe Lys Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser
85 90 95

Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala Gly Ser Gly
100 105 110

Ser Gly His Met His His His His His His Ser Ser Gly Leu Val Pro
115 120 125

Arg Gly Ser Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln
130 135 140

His Met Asp Ser Pro Asp Leu Gly Thr Asp Asp Asp Asp Lys Ala Met
145 150 155 160

Ala Asp Leu Lys Lys Ala Val Asn Glu Pro Glu Lys Pro Ala Glu Glu
165 170 175

Thr Pro Ala Pro Ala Pro Lys Pro Glu Gln Pro Ala Glu Gln Pro Lys
180 185 190

Pro Ala Pro Ala Pro Gln Pro Ala Pro Ala Pro Lys Pro Glu Lys Thr
195 200 205

Asp Asp Gln Gln Ala Glu Glu Asp Tyr Ala Arg Arg Ser Glu Glu Glu
210 215 220

Tyr Asn Arg Leu Pro Gln Gln Gln Pro Pro Lys Ala Glu Lys Pro Ala
225 230 235 240

Pro Ala Pro Lys Pro Glu Gln Pro Val Pro Ala Pro Lys Pro Glu Gln
245 250 255

Pro Val Pro Ala Pro Lys Thr Gly Trp Lys Gln Glu
260 265

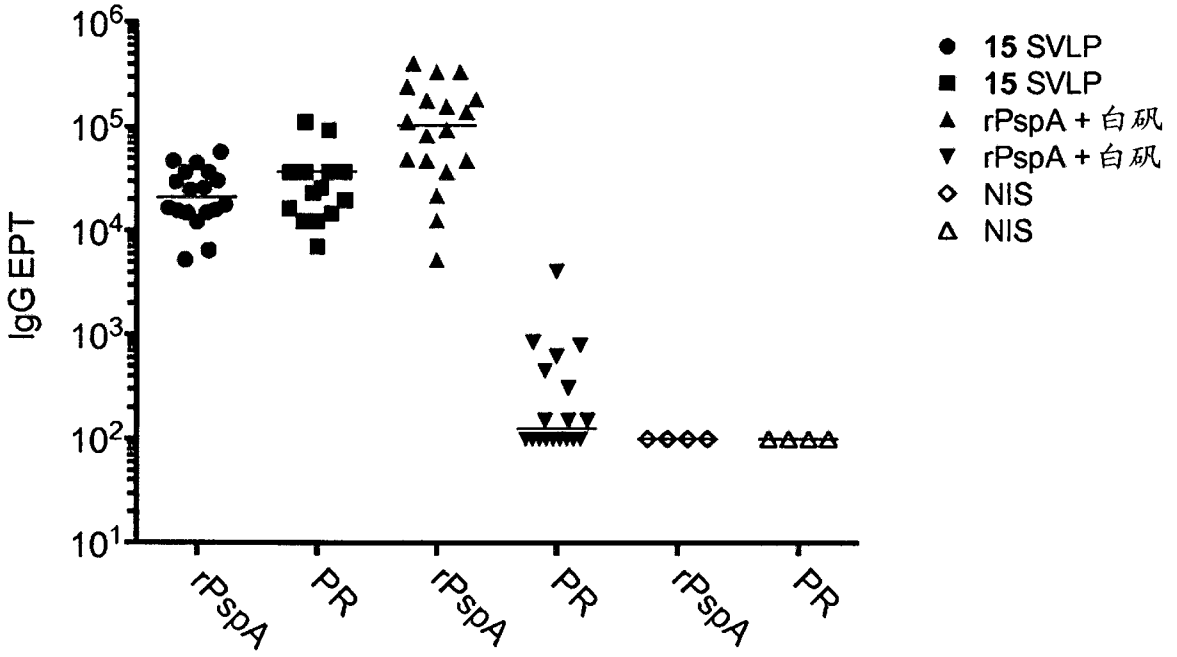

IgG EPT
10^6
10^5
10^4
10^3
10^2
10^1
rPspA
PR
rPspA
PR
rPspA
PR
15 SVLP
15 SVLP
rPspA + 白矾
rPspA + 白矾
NIS
NIS

图1

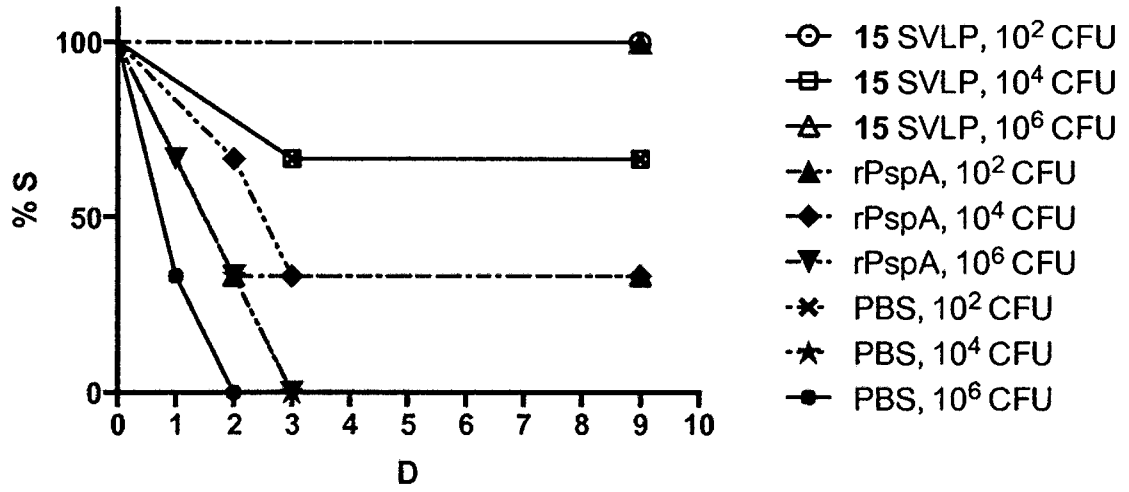

% S
100
50
0
0 1 2 3 4 5 6 7 8 9 10
D
15 SVLP, 10^2 CFU
15 SVLP, 10^4 CFU
15 SVLP, 10^6 CFU
rPspA, 10^2 CFU
rPspA, 10^4 CFU
rPspA, 10^6 CFU
PBS, 10^2 CFU
PBS, 10^4 CFU
PBS, 10^6 CFU

图2

Abstract

The invention relates to lipopeptides consisting of a peptide chain comprising a parallel coiled-coil domain, a proline-rich peptide antigen, and a lipid moiety, all covalently linked, which aggregate to synthetic virus-like particles. Proline-rich peptide antigens considered contain negatively and positively charged amino acid, and at least 15% of the amino acids are proline. Such synthetic virus-like particles carrying proline-rich antigens derived from pneumococcal proteins are useful as vaccines against infectious diseases caused by Gram-positive bacteria such as *Streptococcus pneumoniae*.