COMPOSITIONS OF PNEUMOCOCCAL ANTIGENS

The present invention relates to compositions and pharmaceutical compositions comprising a first, a second and a third pneumococcal antigen or the respective nucleic acids encoding said pneumococcal antigens, wherein said pneumococcal antigens are derived from the non-proline block (NPB) and proline-rich region (PR) of the Pneumococcal surface protein A (PspA) and from the surface-exposed pneumococcal protein PhtE, as well as uses of said compositions and pharmaceutical compositions, in particular for the prevention or the treatment of *Streptococcus pneumoniae* infections and diseases.

RELATED ART

Streptococcus pneumoniae, also known as pneumococcus, is the leading cause of bacterial pneumonia worldwide and is a leading cause of meningitis and bacterial blood infection (sepsis), conditions that are classified as invasive pneumococcal disease (IPD). The affected populations are primarily young children, elderly adults and immunocompromised individuals.

Currently approved vaccines target pneumococcal surface polysaccharides, also known as capsular antigens, and comprise either unconjugated pneumococcal surface polysaccharides, then referred to as pneumococcal polysaccharide vaccines (PPSV), or pneumococcal surface polysaccharides conjugated to a protein carrier, then referred to as pneumococcal conjugate vaccines (PCV). There are close to one hundred pneumococcal serotypes yet identified, each characterized by capsular antigen. Two types of pneumococcal vaccines both targeting capsular polysaccharides are currently licensed: (a) the 23-valent pneumococcal polysaccharide-based vaccine (PPSV23) and (b) the 7-, 10-, 13-, 15- and 20-valent pneumococcal conjugate vaccines (PCV7, PCV10, PCV13, PCV15, PCV20). PPSV23 is poorly immunogenic in children under two years of age and do not generate an immune memory. Conjugation of a pneumococcal polysaccharide to a carrier protein turned polysaccharide-based vaccines from T-cell independent to T-cell dependent antigens, enhancing their immunogenicity and evoking an efficient immune memory. However, all currently available pneumococcal vaccines still have limitations. Both PPSVs and PCVs are serotype-based vaccines and therefore they elicit only serotype-specific immunity. In addition, emergence of replacement serotypes has repeatedly occurred after their introduction, even after the use of expanded valency PCVs, not to mention the vaccine-failures reported following the immunization with PCVs. In addition, PCVs are

very complicated and expensive to manufacture (T. Lagousi et al., Vaccines 2019, 7, 9; and references cited therein).

A promising alternative to capsular polysaccharide-based vaccines is the use of pneumococcal proteins (PnPs) for the construction of novel vaccines. Pneumococcus has several proteins that are well-conserved among different serotypes, most of which are surface-displayed and thus antibody accessible. Pneumococcal proteins (PnPs) may induce broader immune responses, stimulating humoral and cellular immunity, while being easier to manufacture and less expensive. However, such approach raised issues mainly associated with sequence/level of expression variability, chemical instability, as well as possible undesirable reactogenicity and autoimmune properties. To overcome these shortcomings, research has been directed towards the identification of highly-conserved antigenic regions within PnPs that could retain the benefits of protein antigens (T. Lagousi et al., Vaccines 2019, 7, 9; and references cited therein such as C. Giefing et al., J. Exp. Med. (2008) 205:117–131; E. Beghetto et al., FEMS Microbiol. Lett. (2006) 262;14–21). However, the total length of these antigenic fragments (thousands or even up to then thousands of amino acids) hampers the construction of synthetic analogues that could be used as stable vaccine components.

Therefore, recent developments focused on the identification of peptides within the PnPs comprising antigenic or immunodominant regions or epitopes for further evaluation such as within the histidine triad (Pht) protein family including the four highly conserved surface-exposed proteins (PhtA, PhtB, PhtD, and PhtE), the Choline-binding protein D (CbpD) or the zinc metalloproteinase B (ZmpB) (T. Lagousi, et al., J. Biol. Chem. (2015) 290:27500–27510; T. Papastamatiou et al., Infection and Immunity (2018) 86(3):e00673-17). Peptide-based vaccines do not only offer beneficial chemical stability and easier production processes as compared to protein-based vaccines, but peptide-based vaccines are considered safer than protein-based vaccines as they allow the exclusion of antigen fragments associated with autoimmune responses or toxicity.

Pneumococcal surface protein A (PspA) is a widely studied pneumococcal protein which is expressed on the surface of all isolated strains. PspA contains a variable α-helical N-terminal domain, an antigenically conserved proline-rich region (PRR), which is often interrupted by a non-proline block (NPB), and a choline-binding domain that anchors the protein to the bacterial outer cell wall (C.C. Daniels, et al., Infect. Immun. (2010) 78:2163–2172). Due to the high degree of amino-acid sequence variability among different pneumococcal strains, several PspA fragments mainly containing the N-terminal region, have been studied as vaccine candidates so far (D.E. Briles et al., J. Infect. Dis. (2000) 182:1694–1701; D.E. Briles et al., Infect. Immun.

(2000) 68:796–800; Nabors et al., Vaccine (2000) 18 :1743–1754; WO2018/102774). Immunization of mice with a distinct 100-amino-acid fragment located at the more conserved N-terminal region of PspA induced protection in mice against pneumococcal lethal challenge (C.F.M. Vadesilho et al. Clin. Vaccine Immunol. (2014) 21:940–948). Except from the N-terminal domains, immunization with recombinant proline-rich (rPR) molecules and passive immunization with monoclonal antibodies reactive with either NPB or PR epitopes, alone or in combination with N-terminal fragments, were shown to be protective against IPD in mice (S.K. Hollingshead et al., Infect. Immun. (2000) 68:5889–5900; C.C. Daniels, et al., Infect. Immun. (2010) 78:2163–2172 ; A.T. Moreno et al., Clin. Vaccine Immunol. (2010) 17:439–446; Q. O. King et al., J. Infect. Dis. (2009) 200:537–545). In a more recent study, synthetic virus-like particles (SVLPs) carrying distinct B-cell epitope mimetics derived from the PR region of PspA were found to be immunogenic in mice without additional adjuvants and elicited protective antibodies (M. Tamborrini et al., Vaccines (2015), 3, 850–874; WO2015/082501).

However, despite the promising developments achieved so far on the identification of antigenic regions, peptides, and/or B-cell epitopes within pneumococcal proteins and their evaluation as pneumococcal vaccine candidates, there is still a great and still increasing need for vaccines that allow efficient and broad coverage as pneumococcal vaccine candidates and against pneumococcal diseases.

SUMMARY OF THE INVENTION

It has surprisingly found that the inventive specific combination of the three pneumococcal antigens comprising epitopes derived from surface-exposed virulence proteins of *Streptococcus pneumoniae* provides 100% protection in a lethal mouse pneumonia model upon serotype 3 intranasal challenge. Moreover, besides the conferred 100% protection from lethal sepsis, the inventive specific combination of the three pneumococcal antigens further significantly inhibits bacterial organ colonization and bacteria presence in the blood. Notably, when each of the single pneumococcal antigens are applied, only partial protection and solely moderate inhibition of bacterial organ colonization is observed confirming the synergistic effect of the inventive specific combination of the three pneumococcal antigens. Beyond serotype 3, the inventive specific combination significantly protects against lethal sepsis upon intranasal infection with the highly virulent serotype 8. Furthermore, the inventive composition comprising said three pneumococcal antigens represents potent immunogens for inducing antibodies specific for these pneumococcal antigens and epitopes, and are, in addition, able to

recognize *in vitro* the surface of a broad set of serotypes, suggesting its ability to provide cross-protection after infection with several serotypes. Thus, the present invention, provides compositions able to provide protection against *Streptococcus pneumoniae*, and thus for prophylactic and therapeutic treatment of *Streptococcus pneumoniae* infections and disease.

Thus, in one aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope or a nucleic acid encoding said first pneumococcal antigen, preferably said first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX$_{19}$QQQ (SEQ ID NO:1),

wherein X$_{19}$ represents an amino acid selected from T and P; or

a variant of said amino acid sequence, wherein up to 2 amino acids in other than in position denoted with X in SEQ ID NO:1 are replaced by any amino acid; and

(b) a second pneumococcal antigen comprising, preferably consisting of, a second pneumococcal peptide epitope or a nucleic acid encoding said second pneumococcal antigen, preferably said second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

X$_1$X$_2$PX$_4$PEX$_7$X$_8$AX$_{10}$X$_{11}$PX$_{13}$PX$_{15}$X$_{16}$APX$_{19}$X$_{20}$APAPX$_{25}$PEX$_{28}$PA

(SEQ ID NO:2),

wherein X$_1$ represents an amino acid selected from E, P or A;

wherein X$_2$ represents an amino acid selected from T, A or P;

wherein X$_4$ represents an amino acid selected from A, T or Q;

wherein X$_7$ represents an amino acid selected from A, P or Q;

wherein X$_8$ represents an amino acid selected from P, Q or E;

wherein X$_{10}$ represents an amino acid selected from E or P;

wherein X$_{11}$ represents an amino acid selected from Q or A;

wherein X$_{13}$ represents an amino acid selected from K or A;

wherein X$_{15}$ represents an amino acid selected from A or E;

wherein X$_{16}$ represents an amino acid selected from P or Q;

wherein X$_{19}$ represents an amino acid selected from Q, K or E;

wherein X$_{20}$ represents an amino acid selected from P or Q;

wherein X$_{25}$ represents an amino acid selected from K, A or Q;

wherein X$_{28}$ represents an amino acid selected from K or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:2 are replaced by any amino acid, and

(c) a third pneumococcal antigen comprising, preferably consisting of, a third pneumococcal peptide epitope or a nucleic acid encoding said third pneumococcal antigen, preferably said third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;

wherein X_4 represents an amino acid selected from S or P;

wherein X_7 represents an amino acid selected from D or N;

wherein X_{12} represents an amino acid selected from Y, I or F;

wherein X_{13} represents an amino acid selected from N, P or F;

wherein X_{15} represents an amino acid selected from K, S or D;

wherein X_{17} represents an amino acid selected from P, L, H or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in position denoted with X in SEQ ID NO:3 are replaced by any amino acid; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids.

In another aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$QQAEEDYARRSEEEYNRLX_{19}QQQ$ (SEQ ID NO:1),

wherein X_{19} represents an amino acid selected from T and P;

(b) a second pneumococcal antigen comprising, preferably consisting of, a second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$

(SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;
wherein X_8 represents an amino acid selected from P, Q or E;
wherein X_{10} represents an amino acid selected from E or P;
wherein X_{11} represents an amino acid selected from Q or A;
wherein X_{13} represents an amino acid selected from K or A;
wherein X_{15} represents an amino acid selected from A or E;
wherein X_{16} represents an amino acid selected from P or Q;
wherein X_{19} represents an amino acid selected from Q, K or E;
wherein X_{20} represents an amino acid selected from P or Q;
wherein X_{25} represents an amino acid selected from K, A or Q;
wherein X_{28} represents an amino acid selected from K or Q; and

(c) a third pneumococcal antigen comprising, preferably consisting of, a third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;
wherein X_4 represents an amino acid selected from S or P;
wherein X_7 represents an amino acid selected from D or N;
wherein X_{12} represents an amino acid selected from Y, I or F;
wherein X_{13} represents an amino acid selected from N, P or F;
wherein X_{15} represents an amino acid selected from K, S or D;
wherein X_{17} represents an amino acid selected from P, L, H or Q; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids.

In another aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first nucleic acid encoding a first pneumococcal antigen, preferably a first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX$_{19}$QQQ (SEQ ID NO:1),

wherein X_{19} represents an amino acid selected from T and P; and

(b) a second nucleic acid encoding a second pneumococcal antigen, preferably a second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$

(SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;

wherein X_8 represents an amino acid selected from P, Q or E;

wherein X_{10} represents an amino acid selected from E or P;

wherein X_{11} represents an amino acid selected from Q or A;

wherein X_{13} represents an amino acid selected from K or A;

wherein X_{15} represents an amino acid selected from A or E;

wherein X_{16} represents an amino acid selected from P or Q;

wherein X_{19} represents an amino acid selected from Q, K or E;

wherein X_{20} represents an amino acid selected from P or Q;

wherein X_{25} represents an amino acid selected from K, A or Q;

wherein X_{28} represents an amino acid selected from K or Q; and

(c) a third nucleic acid encoding a third pneumococcal antigen, preferably a third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;

wherein X_4 represents an amino acid selected from S or P;

wherein X_7 represents an amino acid selected from D or N;

wherein X_{12} represents an amino acid selected from Y, I or F;

wherein X_{13} represents an amino acid selected from N, P or F;

wherein X_{15} represents an amino acid selected from K, S or D;

wherein X_{17} represents an amino acid selected from P, L, H or Q; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids;

and wherein preferably at least one of, preferably all of, said first nucleic acid, said second nucleic acid and said third nucleic acid is a mRNA.

Further aspects and embodiments of the present invention will be become apparent as this description continues.

DESCRIPTION OF FIGURES

FIG. 1A: Kaplan-Meier survival curves after challenge with serotype 3 in animals immunized with single pneumococcal peptide epitopes or inventive pneumococcal peptide epitope combinations on SVLPs.

FIG. 1B: Kaplan-Meier survival curves after challenge with serotype 8 in animals immunized with inventive pneumococcal peptide epitope combinations on SVLPs.

FIG. 2A and FIG. 2B: Vaccine efficacy against bacteremia and organs colonization. Epitope combination significantly reduces *S. pneumoniae* (Fig. 2A) blood CFUs 3- and 6-days post-infection (two-way ANOVA; $p<0,001$; $p\leq0,0042$) and (Fig. 2B) lung (one-way ANOVA; $p\leq0,0024$; $p\leq0,0322$), liver ($p<0,0001$; $p\leq0,0023$) and spleen ($p<0,0001$) CFUs at the end of the study (day 10).

FIG. 3: Kaplan-Meier survival curves after challenge with serotype 3 in animals immunized with various inventive pneumococcal peptide epitope combinations on SVLPs.

FIG. 4A and FIG. 4B: Whole cell ELISA assay to assess IgG antibody binding onto the surface of various bacterial serotypes. IgG antibodies from mice immunized three times with empty SVLP as a control, the inventive combination of NPB-PR1 + PhtE on SVLPs, or with PCV13 were tested for their ability to bind serotypes included in the PCV13 vaccine (Fig. 4A) as well as non-PCV13 vaccine serotypes (Fig. 4B).

FIG. 5A, FIG. 5B and FIG. 5C: IgG antibody levels against the three peptide epitopes NPB (FIG. 5A), PR1 (FIG. 5B) and PhtE (FIG. 5C) received upon immunization of mice with inventive compositions comprising the differently branched pneumococcal antigens **BPA-2** and **BPA-4**. Mice were immunized three times (days 0, 14 and 28) with SVLP based inventive compositions containing the three pneumococcal peptide epitopes. Within these figures, BPA-2 refers to the SVLP based inventive compositions of the three pneumococcal peptide epitopes comprising the branched pneumococcal antigen **BPA-2**, while BPA-4 in these figures refers to the SVLP based inventive compositions of the three pneumococcal peptide epitopes comprising the branched pneumococcal antigen **BPA-4**. Individual animal sera isolated on day 42 were tested at a 1:100 serum dilution in an ELISA assay. Statistical analyses were performed using

2-way ANOVA tests. No statistical differences were observed for any of the epitopes, when the same dose of the two differently branched pneumococcal antigens **BPA-2** and **BPA-4** was tested.

FIG. 6: IgG antibody levels against various *S. pneumoniae* capsular polysaccharides (CPS) in sera isolated from mice 42 days after immunizing three times concurrently with the SVLP based composition and vaccine comprising the three pneumococcal peptide epitopes and in particular Conjugate **22** and Conjugate **3** and PCV13, as determined with an ELISA assay. The x-axis depicts the serotypes from which the tested polysaccharides are derived.

FIG. 7: Mice were immunized once with the preferred inventive SVLP based composition comprising Conjugate **22** and Conjugate **3** (referred to as NPB/PR1-SVLP + PhtE-SVLP), or the SVLP vehicle control (referred to as Empty SVLP). Cellular responses following said single immunization were measured. Hereto, splenocytes were isolated on day 16 and restimulated with the agents shown. IFN-γ secretion was determined using an ELISPOT assay. Naïve mice were untreated control mice. ST3 pneumococcus refers to serotype 3 *streptococcus pneumoniae*.

FIG. 8A, FIG. 8B and FIG. 8C: IgG antibody levels against the respective peptide epitopes included in the mRNA vaccine candidate: NPB (FIG. 8A), PR (FIG. 8B), PhtE (FIG. 8C). Mice were immunized twice on days 0 and 21 with three different doses (10µg, 2µg, 0.5µg) of LNP particles containing individual mRNA transcripts for the respective NPB, PR and PhtE peptide epitopes. Individual animal sera isolated on day 35 were tested at a 1:20 serum dilution in an ELISA assay. The x-axis depicts the animal ID used in this study.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. The herein described and disclosed embodiments, preferred embodiments and very preferred embodiments should apply to all aspects and other embodiments, preferred embodiments and very preferred embodiments irrespective of whether is specifically again referred to. Thus, all embodiments and preferred and very preferred embodiments of the pneumococcal antigens, pneumococcal peptide epitopes, lipid building blocks, and the conjugates and all of its components including peptide moieties, lipid moieties and linkers described herein are applicable to all aspects and other embodiments of the present invention, even though not all embodiments and preferred and very preferred embodiments are not

necessarily again repeated and reiterated.

Throughout this specification and the claims, which follow, unless the context requires otherwise, the term “comprise” and its variations such as “comprises” and “comprising” etc., are to be understood as a non-exhaustive wording and imply the inclusion of a stated feature or element but not the exclusion of any other feature or element. The term “comprise” and its variations cover the term “consisting of”. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents, unless the content clearly dictates otherwise. The terms “reduce”, “inhibit” or “decrease”, as used herein, include a just detectable reduction but also a reduction down to zero (reduction by 100%).

The term "antigen", as used herein, refers typically to a substance which may be recognized by the immune system, preferably by the adaptive immune system, and is capable of triggering an antigen-specific immune response, e.g. by inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T-lymphocytes as part of an adaptive immune response. Typically, an antigen may be or may comprise a peptide, protein, epitope or epitope mimetic that may encompass one or more B- or T-cell epitopes, which may be presented by the MHC to T-cells. Antigens, as used herein, are typically capable of being bound by an antibody, B- or T-cell receptor if presented by MHC molecules. In the sense of the present invention an antigen may be the product of translation of a provided nucleic acid molecule, preferably an mRNA.

The term “peptide”, as used herein, is any peptide-bond-linked chain of amino acids, regardless of length, secondary and tertiary structure, number of subunits or post-translational and artificial modification. Thus, the term “peptide” is to be understood as covering the terms “polypeptide”, “protein”, “amino acid chain” and “polypeptide chain”. By convention and as used and effected herein, peptide sequences are written from N-terminus to C-terminus, left to right. Peptides can be an open linear peptide chain or cyclic peptides, and may include at least one chemical modification, such as lipidation, glycosylation and phosphorylation. Peptides can be produced by chemical synthesis, RNA translation and/or recombinant processes.

The term “amino acid”, as used herein, refers to organic compounds containing the functional groups amine (-NH2) and carboxylic acid (-COOH) and its zwitterions, typically and preferably, along with a side chain specific to each amino acid. The term “amino acid” typically and preferably includes amino acids that occur naturally, such as proteinogenic amino acids (produced by RNA-translation), non-proteinogenic amino acids (produced by other metabolic mechanisms, e.g. posttranslational modification), standard or canonical amino acids (that are directly encoded by the codons of the genetic code) and non-standard or non-canonical amino

acids (not directly encoded by the genetic code). Naturally occurring amino acids include non-eukaryotic and eukaryotic amino acids. The term "amino acid", as used herein, also includes unnatural amino acids that are chemically synthesized; alpha- (α-), beta- (β-), gamma- (γ-) and delta- (δ-) etc. amino acids as well as mixtures thereof in any ratio; and, if applicable, any isomeric form of an amino acid, i.e. its D-stereoisomers (labelled with a lower-case initial letter) and L-stereoisomers (labelled with a capital initial letter) (alternatively addressed by the (R) and (S) nomenclature) as well as mixtures thereof in any ratio, preferably in a racemic ratio of 1:1. Amino acids in this invention are preferably in L-configuration, unless mentioned specifically as D-configuration. The term "D-stereoisomer", "L-stereoisomer", "D-amino acid" or "L-amino acid" refers to the chiral alpha carbon of the amino acids. Amino acid can include one or more modifications and/or attached groups, for example protecting groups used for peptide synthesis, such as Boc, Fmoc or both. The term "deletion" refers herein to a position in an amino acid sequence that is not occupied by an amino acid. In a preferred embodiment of the present invention, an amino acid is a naturally occurring amino acid, wherein said naturally occurring amino acid is in its L-configuration, in its D-configuration, or in a mixture of any ratio of said L-configuration and D-configuration. In a further preferred embodiment of the present invention, an amino acid is a naturally occurring amino acid, wherein said naturally occurring amino acid is in its L-configuration.

The term "epitope", (also referred to as "antigenic determinant") as used herein, is the portion of an antigen that can be recognized by or interacts with the immune system, and specifically which can be recognized by antibodies, B-cells, or T-cells. For example, the epitope is the specific portion of the antigen to which an antibody, a B-cell or a T-cell binds. B-lymphocytes responding to antigenic determinants produce antibodies, whereas T-lymphocytes respond to antigenic determinants by proliferation and establishment of effector functions critical for the mediation of cellular and/or humoral immunity. The term "epitope" as used herein comprises conformational epitopes and linear epitopes, based on their structure and interaction with the paratope, i.e. the part of an antibody that binds to the epitope.

The term "pneumococcal antigen", as used herein, refers to an antigen that elicits an antigen specific immune response against any organism of the pneumococcal genus, such as a *Streptococcus pneumoniae* organism. In some embodiments, a pneumococcal antigen elicits an antigen specific immune response against *S. pneumoniae* organisms of multiple species. Pneumococcal antigens comprise pneumococcal peptide epitopes as defined herein. Said pneumococcal peptide epitopes are typically and preferably epitopes derived from peptides within the pneumococcal proteins (PnPs) such as within the histidine triad (Pht) protein family

including the highly conserved surface-exposed proteins (PhtD and PhtE), or are derived specifically from the Pneumococcal surface protein A (PspA) and hereby from the conserved proline-rich region (PRR) or the non-proline block (NPB).

In a preferred embodiment, said pneumococcal antigen comprises, preferably, consists of pneumococcal peptide epitopes as defined herein, and said pneumococcal antigen has a length of at most 50 amino acids, preferably of at most 45 amino acids, further preferably of at most 40, 39, 38, 37, 36, 35, 34, 33, 32, 31 or 30 amino acids. In another preferred embodiment, said pneumococcal antigen comprises, preferably, consists of pneumococcal peptide epitopes as defined herein, and said pneumococcal antigen has a length of at most 35 amino acids, preferably of at most 32 amino acids, further preferably of at most 30. In another preferred embodiment, said pneumococcal antigen consists of said pneumococcal peptide epitope.

In a preferred embodiment, said first pneumococcal antigen comprises, preferably, consists of, said first pneumococcal peptide epitope, and said first pneumococcal antigen has a length of at most 50 amino acids, preferably of at most 45 amino acids, further preferably of at most 40, 39, 38, 37, 36, 35, 34, 33, 32, 31 or 30 amino acids. In another preferred embodiment, said first pneumococcal antigen comprises, preferably, consists of said first pneumococcal peptide epitope, and said first pneumococcal antigen has a length of at most 35 amino acids, preferably of at most 32 amino acids, further preferably of at most 30. In a preferred embodiment, said second pneumococcal antigen comprises, preferably, consists of, said second pneumococcal peptide epitope, and said second pneumococcal antigen has a length of at most 50 amino acids, preferably of at most 45 amino acids, further preferably of at most 40, 39, 38, 37, 36, 35, 34, 33, 32, 31 or 30 amino acids. In another preferred embodiment, said second pneumococcal antigen comprises, preferably, consists of said second pneumococcal peptide epitope, and said second pneumococcal antigen has a length of at most 35 amino acids, preferably of at most 32 amino acids, further preferably of at most 30. In a preferred embodiment, said third pneumococcal antigen comprises, preferably, consists of, said third pneumococcal peptide epitope, and said third pneumococcal antigen has a length of at most 50 amino acids, preferably of at most 45 amino acids, further preferably of at most 40, 39, 38, 37, 36, 35, 34, 33, 32, 31 or 30 amino acids. In another preferred embodiment, said third pneumococcal antigen comprises, preferably, consists of said third pneumococcal peptide epitope, and said third pneumococcal antigen has a length of at most 35 amino acids, preferably of at most 32 amino acids, further preferably of at most 30. In a preferred embodiment, said first pneumococcal antigen consists of said first pneumococcal peptide epitope. In another preferred embodiment, said second pneumococcal antigen consists of said second

pneumococcal peptide epitope. In another preferred embodiment, said third pneumococcal antigen consists of said third pneumococcal peptide epitope.

The term "variant of SEQ ID NO: ..." or "variant of sequence ..." as used herein, refers to an amino acid sequence in which preferably a defined number of amino acids are replaced or deleted as compared to the referenced and non-variant parent sequence. In case of replacement, the defined number of amino acids to be replaced corresponds to said defined number of amino acids exchanging the replaced. Thus, in case three amino acids are replaced of a certain amino acid sequence, then three amino acids are exchanging the replaced three amino acids. The term "variant of SEQ ID NO: ..." or "variant of sequence ..." as used herein, and when referring to a variant of a pneumococcal peptide epitope of a specific sequence, refers typically and preferably to an amino acid sequence that maintains the ability to elicit an antigen-specific response to the pneumococcal peptide epitope of said specific sequence and confers the desired biological activity.

The term "N-terminus", as used herein, refers to an end of a peptide having a free (-NH_2) or modified amino or amine group. Preferred N-terminal modifications are those that protect the N-terminus from proteolytic degradation. N-terminal modifications in accordance with the invention include but are not limited to acetylation, attachment of at least one polymer, preferably polyethylene glycol (PEGylation) or poly(lactic acid), or attachment of at least one amino acid, preferably of at least one D-amino acid, or attachment of at least one compound, such as a cell penetrating peptide, nucleic acid, a carbamate, such as fluorenylmethoxycarbamate or benzyloxy carbamate, aldehyde, hydrazinonicotinic acid, 4-formyl benzamide, methyl, myristoyl, prenyl group, palmitoyl, ubiquitin, 7-methoxycoumarin acetic acid (Mca), dansyl, formyl, 4-diniphenyl, pyroglutamyl, urea, carbamate, sulphonamide, alkylamine, fatty acids, such as palmitic acids, radioligand, quencher, fluorescein or another dye or label such as biotin.

The term "C-terminus", as used herein, refers to an end of a peptide having a free (-COOH) or modified carboxyl group. Preferred C-terminal modifications are those that protect the C-terminus from proteolytic degradation. C-terminal modifications in accordance with the invention include but are not limited to amidation or attachment of at least one amino acid, preferably of at least one D-amino acid, or attachment of at least one compound, such as a cell penetrating peptide, nucleic acid, polyethylene glycol (PEGylation), thiol, ester, aldehyde, sulphonamide, pNA (para-nitroanilide), Amc (7-amino-4-methylcoumarinyl), hydrazide, hydroxamic acid, chloromethyl ketone, biotin, radioligand, quencher, Abz or other dyes and

labels. Herein and by general convention, peptide sequences are written from N-terminal on the left to C-terminal on the right (according to the direction of translation).

The term “coiled coil peptide chain segment”, as used herein, refers to a sequence of a peptide chain capable of forming a coiled coil with at least one other coiled coil peptide chain segment. A coiled coil is a peptide structure in which at least two coiled coil peptide chain segments, each having preferably an alpha helical secondary structure, are associated into a bundle. Coiled coil peptide chain segments of the invention contain multiple repeat units, typically and preferably consecutively linked to each other. The repeat units of the coiled coil peptide chain segment may be identical or may be different, e.g. may contain at least one discontinuity, such as an insertion, deletion or exchange of at least one, preferably exactly 1, 2, 3 or 4 amino acids within the repeat unit.

The term “alkyl” refers to a straight or branched chain of saturated hydrocarbons. Thus, -C_1-C_6 alkyl groups contain 1 to 6 carbon atoms. Examples of a -C_1-C_6 alkyl group include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, isopropyl, isobutyl, sec-butyl and tert-butyl, isopentyl and neopentyl.

The term “alkenyl” means an aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched. Thus, a -C_2-C_6 alkenyl group is an alkenyl group containing between 2 and 6 carbon atoms. Some alkenyl groups have 2 to about 4 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl, or propyl are attached to a linear alkenyl chain. As used herein, the term “alkenyl” includes groups that contain a carbon-carbon double bond anywhere within the carbon chain.

The term "hydrocarbyl" as used herein means a straight or branched, preferably straight, alkyl or alkenyl group, of typically and preferably at least 7 carbon atoms and at most 50 carbon atoms. In a preferred embodiment, said hydrocarbyl refers to straight alkyl or alkenyl consisting of between 8 and 50 carbon atoms, preferably between 8 and 25 carbon 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. Branched alkyl or alkenyl are preferably 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.

The term "adjuvant", as used herein in the broadest sense, is typically a pharmacological and/or immunological agent that may modify, e.g. enhance, the effect of other agents, such as a drug or vaccine, and thus, in a broad sense refers to a broad spectrum of substances. Typically, these substances are able to increase the immunogenicity of antigens. For example, adjuvants may be recognized by the innate immune systems and, e.g., may elicit an innate immune

response. Adjuvants, as used herein, typically do not elicit an adaptive immune response; insofar, adjuvants, as used herein do not qualify as antigens in accordance with the present invention. Their mode of action is distinct from the effects triggered by antigens resulting in an adaptive immune response. Thus adjuvants, as used herein, refers to non-specific stimulators of the immune response and/or substances that may allow generation of a depot in the host which when combined with the compositions, vaccines or pharmaceutical compositions of the present invention may provide for a more enhanced immune response. Adjuvants are known in the art and include, by way of example, complete and incomplete Freund's adjuvant, aluminum containing adjuvants such as aluminum hydroxide and other aluminum salts, modified muramyl dipeptide mineral gels such as aluminum hydroxide, surface active substances such as lyso lecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and human adjuvants such as BCG (Bacille Calmette Guerin) and Corynebacterium parvum, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, MF-59, OM-174, OM-197, OM-294 as well as immunostimulatory substances, preferably selected from TLR ligands such as poly(I:C) or a CpG-DNA, or substances such as lipopolysaccharides. The adjuvants may also comprise mixtures of these substances.

The term “immune response”, as used herein, may typically be a specific reaction of the adaptive immune system to a particular antigen (so called specific or adaptive immune response) or an unspecific reaction of the innate immune system (so called unspecific or innate immune response), or a combination thereof.

The term “adaptive immune response”, as used herein typically refers to be an antigen-specific response of the immune system. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells.

The term “innate immune system”, as used herein, is also known as non-specific (or unspecific) immune system, typically comprises the cells and mechanisms that defend the host from infection by other organisms in a nonspecific manner. This means that the cells of the innate system may recognize and respond to pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host.

The term “carrier”, as used herein and in the context of the invention, is an entity or a substance, typically and preferably a particle, that (i) may provide a foundation for attachment, by covalent or non-covalent binding, preferably by covalent binding, of the pneumococcal antigens, preferably the pneumococcal peptide epitopes, of the present invention, typically and preferably for presenting said antigens and/or epitopes as displayed on its surface to the immune

system, (ii) may facilitate transport and/or complexation of the pneumococcal antigens, preferably the pneumococcal peptide epitopes, and/or preferably the nucleic acids, preferably mRNAs, encoding for the pneumococcal antigens, preferably the pneumococcal peptide epitopes, typically and preferably as cargo; a carrier may be associated to its cargo by covalent or non-covalent interaction. With respect to "foundation for attachment", said carrier may comprise individual components such as the coat proteins of a virus or a virus-like particle or the lipid building blocks (LBB's) of the preferred SVLPs as described herein, which individual components are responsible for the formation of the final carrier and carrier particle, and to which said pneumococcal antigens, preferably the pneumococcal peptide epitopes, are linked for its purpose such as, for example, presenting the pneumococcal antigens on the surface of the carriers. Depending on the mode of linking of said pneumococcal antigens to said components of said carriers or the actual way of formation of the carrier and carrier particle, the eventual formation and generation of the carriers may occur prior to said linking of said pneumococcal antigens to said individual components or thereafter. For example, when said pneumococcal antigens, preferably said pneumococcal peptide epitopes, are fused with or within coat proteins of a virus or a virus-like particle, such linking occur prior to the formation of the eventual (modified) virus or virus-like particle. The same is true for the described lipid building blocks (LBB's) to which said pneumococcal antigens, preferably the pneumococcal peptide epitopes, are linked to form, first, conjugates, which then assemble to the SVLPs. With respect to the term "complexation" and in a preferred embodiment, at least one, typically and preferably all of the nucleic acids, preferably mRNA, encoding for the pneumococcal antigen, preferably the pneumococcal peptide epitopes of the invention may be complexed with lipids to form one or more liposomes, lipoplexes, or lipid nanoparticles, wherein preferably said complexation is by way of non-covalent interaction and binding, and wherein further said complexation is by way of encapsulation. Therefore, in one embodiment, the inventive composition comprises liposomes, lipoplexes, and/or lipid nanoparticles comprising the at least one nucleic acids, preferably mRNAs, further preferably wherein the inventive composition comprises liposomes, lipoplexes, and/or lipid nanoparticles comprising all of said nucleic acids, preferably mRNAs, encoding for said first, second, and third pneumococcal antigens, preferably encoding for said first, second, and third pneumococcal peptide epitopes. The "bi-epitope carrier", as used herein and in the context of the invention, shall refer to a carrier as defined and described herein, to which two antigens, typically and preferably two of the inventive pneumococcal antigens, preferably of the pneumococcal peptide epitopes, are linked, while the term "multi-epitope carrier", as used herein and in the context of the invention, shall

refer to a carrier as defined and described herein, to which more than two antigens, typically and preferably, more than two antigens of the inventive pneumococcal antigens, preferably of the pneumococcal peptide epitopes, are linked. The term "single epitope carrier", as used herein and in the context of the invention, shall refer to a carrier as defined and described herein, to which one antigen, typically and preferably one of the inventive pneumococcal antigens, preferably of the pneumococcal peptide epitopes, is linked. The same terminology is used when referring to the conjugates which form said SVLPs, namely to a single epitope conjugate, to a bi-epitope epitope conjugate , or to a multi-epitope conjugate, as well as when referring to the single, bi-epitope and multi-epitope LBBs are referred to which single, bi-epitope and multi-epitope pneumococcal antigens, preferably pneumococcal peptide epitopes, are linked.

The term "attached" or "attachment", as used herein, refers to covalent binding as well as non-covalent binding, wherein covalent binding is preferred.

The term "immunogenic composition" as used herein refers to a composition that is capable of eliciting an immune response, such as an antibody or innate immune response such as cellular immune response, when administered to a subject. The immunogenic compositions of the present invention may or may not be immuno-protective or therapeutic. When the immunogenic compositions of the present invention prevent, ameliorate, palliate or eliminate disease from the subject, then the immunogenic composition may optionally be referred to as a vaccine. As used herein, however, the term immunogenic composition is not intended to be limited to vaccines. In some embodiments, an immunogenic composition includes a polypeptide or peptide antigen such as the pneumococcal antigens the pneumococcal peptide epitopes in accordance with the present invention, or in other embodiments, an immunogenic composition includes a nucleic acid encoding a polypeptide or peptide antigen such as pneumococcal antigens the pneumococcal peptide epitopes in accordance with the present invention. An immunogenic composition can include molecules that induce an immune response against multiple antigens.

Thus, in one aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope or a nucleic acid encoding said first pneumococcal antigen, preferably said first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX$_{19}$QQQ (SEQ ID NO:1),

wherein X$_{19}$ represents an amino acid selected from T and P; or

a variant of said amino acid sequence, wherein up to 2 amino acids in other than in position denoted with X in SEQ ID NO:1 are replaced by any amino acid; and

(b) a second pneumococcal antigen comprising, preferably consisting of, a second pneumococcal peptide epitope or a nucleic acid encoding said second pneumococcal antigen, preferably said second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$

(SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;

wherein X_8 represents an amino acid selected from P, Q or E;

wherein X_{10} represents an amino acid selected from E or P;

wherein X_{11} represents an amino acid selected from Q or A;

wherein X_{13} represents an amino acid selected from K or A;

wherein X_{15} represents an amino acid selected from A or E;

wherein X_{16} represents an amino acid selected from P or Q;

wherein X_{19} represents an amino acid selected from Q, K or E;

wherein X_{20} represents an amino acid selected from P or Q;

wherein X_{25} represents an amino acid selected from K, A or Q;

wherein X_{28} represents an amino acid selected from K or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:2 are replaced by any amino acid, and

(c) a third pneumococcal antigen comprising, preferably consisting of, a third pneumococcal peptide epitope or a nucleic acid encoding said third pneumococcal antigen, preferably said third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;

wherein X_4 represents an amino acid selected from S or P;

wherein X_7 represents an amino acid selected from D or N;

wherein X_{12} represents an amino acid selected from Y, I or F;

wherein X_{13} represents an amino acid selected from N, P or F;

wherein X_{15} represents an amino acid selected from K, S or D;

wherein X_{17} represents an amino acid selected from P, L, H or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:3 are replaced by any amino acid, and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids.

In a further aspect, the present invention provides a composition comprising a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope, a second pneumococcal antigen comprising, preferably consisting of, a second pneumococcal peptide epitope, and a third pneumococcal antigen comprising, preferably consisting of, a third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope is derived from the non-proline block (NPB) of the Pneumococcal surface protein A (PspA), said second pneumococcal peptide epitope is derived from the proline-rich region (PR) of the Pneumococcal surface protein A (PspA), and said third pneumococcal peptide epitope is derived from the surface-exposed pneumococcal protein PhtE of the histidine triad (Pht) protein family.

In another aspect, the present invention provides a composition comprising

(a) a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX$_{19}$QQQ (SEQ ID NO:1),

wherein X_{19} represents an amino acid selected from T and P; or

a variant of said amino acid sequence, wherein up to 2 amino acids in other than in position denoted with X in SEQ ID NO:1 are replaced by any amino acid,

(b) a second pneumococcal antigen comprising a second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$

(SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;

wherein X_8 represents an amino acid selected from P, Q or E;
wherein X_{10} represents an amino acid selected from E or P;
wherein X_{11} represents an amino acid selected from Q or A;
wherein X_{13} represents an amino acid selected from K or A;
wherein X_{15} represents an amino acid selected from A or E;
wherein X_{16} represents an amino acid selected from P or Q;
wherein X_{19} represents an amino acid selected from Q, K or E;
wherein X_{20} represents an amino acid selected from P or Q;
wherein X_{25} represents an amino acid selected from K, A or Q;
wherein X_{28} represents an amino acid selected from K or Q; or
a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:2 are replaced by any amino acid, and

(c) a third pneumococcal antigen comprising a third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;
wherein X_4 represents an amino acid selected from S or P;
wherein X_7 represents an amino acid selected from D or N;
wherein X_{12} represents an amino acid selected from Y, I or F;
wherein X_{13} represents an amino acid selected from N, P or F;
wherein X_{15} represents an amino acid selected from K, S or D;
wherein X_{17} represents an amino acid selected from P, L, H or Q; or
a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:3 are replaced by any amino acid, and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, each independently consists of a length of at most 50 amino acids, further preferably of at most 45 amino acids.

In another aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first pneumococcal antigen comprising, preferably consisting of, a first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$QQAEEDYARRSEEEYNRLX_{19}QQQ$ (SEQ ID NO:1),

wherein X_{19} represents an amino acid selected from T and P;

(b) a second pneumococcal antigen comprising, preferably consisting of, a second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$ (SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;

wherein X_8 represents an amino acid selected from P, Q or E;

wherein X_{10} represents an amino acid selected from E or P;

wherein X_{11} represents an amino acid selected from Q or A;

wherein X_{13} represents an amino acid selected from K or A;

wherein X_{15} represents an amino acid selected from A or E;

wherein X_{16} represents an amino acid selected from P or Q;

wherein X_{19} represents an amino acid selected from Q, K or E;

wherein X_{20} represents an amino acid selected from P or Q;

wherein X_{25} represents an amino acid selected from K, A or Q;

wherein X_{28} represents an amino acid selected from K or Q; and

(c) a third pneumococcal antigen comprising, preferably consisting of, a third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;

wherein X_4 represents an amino acid selected from S or P;

wherein X_7 represents an amino acid selected from D or N;

wherein X_{12} represents an amino acid selected from Y, I or F;

wherein X_{13} represents an amino acid selected from N, P or F;

wherein X_{15} represents an amino acid selected from K, S or D;

wherein X_{17} represents an amino acid selected from P, L, H or Q; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids.

In another aspect, the present invention provides a composition comprising, preferably consisting of,

(a) a first nucleic acid encoding a first pneumococcal antigen, preferably a first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX$_{19}$QQQ (SEQ ID NO:1),

wherein X$_{19}$ represents an amino acid selected from T and P; and

(b) a second nucleic acid encoding a second pneumococcal antigen, preferably a second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

X$_1$X$_2$PX$_4$PEX$_7$X$_8$AX$_{10}$X$_{11}$PX$_{13}$PX$_{15}$X$_{16}$APX$_{19}$X$_{20}$APAPX$_{25}$PEX$_{28}$PA

(SEQ ID NO:2),

wherein X$_1$ represents an amino acid selected from E, P or A;

wherein X$_2$ represents an amino acid selected from T, A or P;

wherein X$_4$ represents an amino acid selected from A, T or Q;

wherein X$_7$ represents an amino acid selected from A, P or Q;

wherein X$_8$ represents an amino acid selected from P, Q or E;

wherein X$_{10}$ represents an amino acid selected from E or P;

wherein X$_{11}$ represents an amino acid selected from Q or A;

wherein X$_{13}$ represents an amino acid selected from K or A;

wherein X$_{15}$ represents an amino acid selected from A or E;

wherein X$_{16}$ represents an amino acid selected from P or Q;

wherein X$_{19}$ represents an amino acid selected from Q, K or E;

wherein X$_{20}$ represents an amino acid selected from P or Q;

wherein X$_{25}$ represents an amino acid selected from K, A or Q;

wherein X$_{28}$ represents an amino acid selected from K or Q; and

(c) a third nucleic acid encoding a third pneumococcal antigen, preferably a third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

YX$_2$TX$_4$HGX$_7$HYHYX$_{12}$X$_{13}$GX$_{15}$VX$_{17}$YDA (SEQ ID NO:3),

wherein X$_2$ represents an amino acid selected from V, A or I;

wherein X$_4$ represents an amino acid selected from S or P;

wherein X$_7$ represents an amino acid selected from D or N;

wherein X$_{12}$ represents an amino acid selected from Y, I or F;

wherein X_{13} represents an amino acid selected from N, P or F;

wherein X_{15} represents an amino acid selected from K, S or D;

wherein X_{17} represents an amino acid selected from P, L, H or Q; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids;

and wherein preferably at least one of, preferably all of, said first nucleic acid, said second nucleic acid and said third nucleic acid is a mRNA.

The full-length PspA protein sequence of several *S. pneumoniae* strains such as ST2, ST3, ST4, ST11A, ST14, ST15A, ST19F, ST22F, ST24F and ST33F was retrieved from the NCBI protein database and an alignment was performed using the Clustal Omega tool. The alignment was focused on the PR and NPB domains of the proteins to identify, specifically for the PR domains, a) conserved motifs, b) the repetition of conserved short signatures, and c) conserved or systematically appearing amino acid alternations within a certain amino acid stretch leading to SEQ ID NO:2 and its preferred specific sequences thereof, leading to the generation of amino acid sequences SEQ ID NO:1 and SEQ ID NO:2, in particular of the generation of amino acid sequence SEQ ID NO:2.

The full-length PhtE protein sequence of several *S. pneumoniae* strains such as ST2, ST3, ST4, ST15B, ST19A and ST24A was retrieved from the NCBI protein database and an alignment was performed using the Clustal Omega tool. The Zn^{2+}-binding histidine triad (HT) motif of all proteins – being repeated five times within one protein sequence – was then isolated and a consensus as well as SEQ ID NO:3 and its preferred specific sequences thereof were derived based on conserved or systematically appearing amino acid alternations.

In a preferred embodiment, at least one, preferably all of said amino acid replacements leading to said variants are conservative amino acid substitutions.

In a preferred embodiment, said amino acid replacements leading to said variants are conservative, wherein said conservative amino acid replacements and substitutions, respectively, refer to an exchange of amino acids within the same functional group, i.e. the functional group of aliphatic amino acids, polar amino acids with uncharged side chains, aromatic amino acids, basic amino acids, acidic amino acids and their amides. Thus, in a preferred embodiment, at least one, preferably all of said replacements for generating said variants are conservative amino acid substitutions by one amino acid or several amino acids of the same functional group. More preferably, said functional groups with interchangeable amino acids are as follows:

a. Glycine, Alanine, Valine, Leucine, Isoleucine
b. Serine, Cysteine, Threonine, Asparagine, Glutamine
c. Phenylalanine, Tyrosine, Tryptophan
d. Histidine, Lysine, Arginine
e. Aspartate, Glutamate, Asparagine, Glutamine

In a preferred embodiment, at least one of said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprise a C-terminal D-Alanine, preferably all of said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprise a C-terminal D-Alanine. In a preferred embodiment, said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprise a C-terminal D-Alanine. In a preferred embodiment, said first pneumococcal peptide epitope comprises a C-terminal D-Alanine. In a preferred embodiment, said second pneumococcal peptide epitope comprises a C-terminal D-Alanine. In a preferred embodiment, said third pneumococcal peptide epitope comprises a C-terminal D-Alanine.

In a preferred embodiment, at least one of said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprises an amidated C-terminal D-Alanine, preferably all of said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprise an amidated C-terminal D-Alanine.

In a further preferred embodiment, said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope comprise an amidated C-terminal D-Alanine.

Said modifications are believed to increase the stability and protect the C-termini of said pneumococcal peptide epitopes (A. Ghasparian, *et al.*, ChemBioChem 2011, 12, 100-109).

In a preferred embodiment, said first pneumococcal peptide epitope comprises an amidated C-terminal D-Alanine. In a preferred embodiment, said second pneumococcal peptide epitope comprises an amidated C-terminal D-Alanine. In a preferred embodiment, said third pneumococcal peptide epitope comprises an amidated C-terminal D-Alanine.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID

NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19, wherein said first pneumococcal peptide epitope further comprises a C-terminal D-stereoisomeric alanine (D-Alanine; D-Ala).

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19, wherein said first pneumococcal peptide epitope further comprises a C-terminal D-stereoisomeric alanine (D-Alanine; D-Ala) and a C-terminal amidation, thus a C-terminal amidated D-Alanine.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21:

QQAEEDYARRSEEEYNRLTQQQ	(SEQ ID NO:4)
QQAEEDYARRSEEEYNRLTQQQa	(SEQ ID NO:5)
QQAEEDYARRSEEEYNRLTQQQa-NH_2	(SEQ ID NO:6)
QQAEEDYARRSEEEYNRLPQQQ	(SEQ ID NO:7)
QQAEEDYARRSEEEYNRLPQQQa	(SEQ ID NO:8)
QQAEEDYARRSEEEYNRLPQQQa-NH_2	(SEQ ID NO:9)
EKTDDQQAEEDYARRSEEEYNRLTQQQ	(SEQ ID NO:10)
EKTDDQQAEEDYARRSEEEYNRLTQQQa	(SEQ ID NO:11)
EKTDDQQAEEDYARRSEEEYNRLTQQQa-NH_2	(SEQ ID NO:12)
EKTDDQQAEEDYARRSEEEYNRLPQQQ	(SEQ ID NO:13)
EKTDDQQAEEDYARRSEEEYNRLPQQQa	(SEQ ID NO:14)
EKTDDQQAEEDYARRSEEEYNRLPQQQa-NH_2	(SEQ ID NO:15)
EKSADQQAEEDYARRSEEEYNRLTQQQ	(SEQ ID NO:16)
EKSADQQAEEDYARRSEEEYNRLTQQQa	(SEQ ID NO:17)
EKSADQQAEEDYARRSEEEYNRLTQQQa-NH_2	(SEQ ID NO:18)
EKSADQQAEEDYARRSEEEYNRLPQQQ	(SEQ ID NO:19)
EKSADQQAEEDYARRSEEEYNRLPQQQa	(SEQ ID NO:20)
EKSADQQAEEDYARRSEEEYNRLPQQQa-NH_2	(SEQ ID NO:21)

wherein a represents D-Ala and wherein a-NH_2 represents amidated D-Ala, i.e. D-alanine with amidation of the C-terminus of the D-Alanine.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID

NO:4 to SEQ ID NO:6.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:5.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:6.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:7.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:8.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:9.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:10.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:11.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:12.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:13.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:14.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:15.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:16.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:17.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:18.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:19.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises,

preferably consists of, the amino sequence of SEQ ID NO:20.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:21.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEAX_8AX_{10}X_{11}PKPX_{15}X_{16}APX_{19}PAPAPX_{25}PEX_{28}PA$ (SEQ ID NO:22),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_8 represents an amino acid selected from P, Q or E;

wherein X_{10} represents an amino acid selected from E or P;

wherein X_{11} represents an amino acid selected from Q or A;

wherein X_{15} represents an amino acid selected from A or E;

wherein X_{16} represents an amino acid selected from P or Q;

wherein X_{19} represents an amino acid selected from Q or K;

wherein X_{25} represents an amino acid selected from K, A or Q; and

wherein X_{28} represents an amino acid selected from K or Q.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID NO:110.

In a further preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID NO:110, wherein said second pneumococcal peptide epitope comprises a C-terminal D-Alanine.

In a further preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID NO:110, wherein said second pneumococcal peptide epitope comprises a C-terminal amidated D-Ala.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100:

ETPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:23)
ETPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:24)
ETPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:25)
PTPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:26)
PTPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:27)
PTPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:28)
ATPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:29)
ATPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:30)
ATPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:31)
EAPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:32)
EAPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:33)
EAPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:34)
EPPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:35)
EPPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:36)
EPPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:37)
PAPAPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:38)
PAPAPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:39)
PAPAPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:40)
ETPTPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:41)
ETPTPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:42)
ETPTPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:43)
ETPQPEAPAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:44)

ETPQPEAPAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:45)
ETPQPEAPAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:46)
PAPTPEAPAPAPKPAPAPKPAPAPAPEKPA	(SEQ ID NO:47)
PAPTPEAPAPAPKPAPAPKPAPAPAPEKPAa	(SEQ ID NO:48)
PAPTPEAPAPAPKPAPAPKPAPAPAPEKPAa-NH_2	(SEQ ID NO:49)
PAPAPEAPAPAPKPEQAPQPAPAPKPEKPA	(SEQ ID NO:50)
PAPAPEAPAPAPKPEQAPQPAPAPKPEKPAa	(SEQ ID NO:51)
PAPAPEAPAPAPKPEQAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:52)
ETPAPEAQAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:53)
ETPAPEAQAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:54)
ETPAPEAQAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:55)
ETPAPEAEAEQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:56)
ETPAPEAEAEQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:57)
ETPAPEAEAEQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:58)
ETPAPEAQAEQPKPAPAPQPAPAPKPEQPA	(SEQ ID NO:59)
ETPAPEAQAEQPKPAPAPQPAPAPKPEQPAa	(SEQ ID NO:60)
ETPAPEAQAEQPKPAPAPQPAPAPKPEQPAa-NH_2	(SEQ ID NO:61)
EAPAPEAEAPAPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:62)
EAPAPEAEAPAPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:63)
EAPAPEAEAPAPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:64)
ETPAPEAPAPQPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:65)
ETPAPEAPAPQPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:66)
ETPAPEAPAPQPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:67)
ETPAPEAPAEAPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:68)
ETPAPEAPAEAPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:69)
ETPAPEAPAEAPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:70)
ETPAPEAPAPAPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:71)
ETPAPEAPAPAPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:72)
ETPAPEAPAPAPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:73)
PAPAPEAPAPAPKPAPAPQPAPAPKPEKPA	(SEQ ID NO:74)
PAPAPEAPAPAPKPAPAPQPAPAPKPEKPAa	(SEQ ID NO:75)
PAPAPEAPAPAPKPAPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:76)
ETPAPEAPAEQPKPEPAPQPAPAPKPEKPA	(SEQ ID NO:77)
ETPAPEAPAEQPKPEPAPQPAPAPKPEKPAa	(SEQ ID NO:78)

ETPAPEAPAEQPKPEPAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:79)
ETPAPEAPAEQPKPAQAPQPAPAPKPEKPA	(SEQ ID NO:80)
ETPAPEAPAEQPKPAQAPQPAPAPKPEKPAa	(SEQ ID NO:81)
ETPAPEAPAEQPKPAQAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:82)
ETPAPEAPAEQPKPEQAPQPAPAPKPEKPA	(SEQ ID NO:83)
ETPAPEAPAEQPKPEQAPQPAPAPKPEKPAa	(SEQ ID NO:84)
ETPAPEAPAEQPKPEQAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:85)
PAPAPEAPAEQPKPEQAPQPAPAPKPEKPA	(SEQ ID NO:86)
PAPAPEAPAEQPKPEQAPQPAPAPKPEKPAa	(SEQ ID NO:87)
PAPAPEAPAEQPKPEQAPQPAPAPKPEKPAa-NH_2	(SEQ ID NO:88)
ETPAPEAPAEQPKPAPAPKPAPAPKPEKPA	(SEQ ID NO:89)
ETPAPEAPAEQPKPAPAPKPAPAPKPEKPAa	(SEQ ID NO:90)
ETPAPEAPAEQPKPAPAPKPAPAPKPEKPAa-NH_2	(SEQ ID NO:91)
PAPAPEAPAEQPKPAPAPKPAPAPKPEKPA	(SEQ ID NO:92)
PAPAPEAPAEQPKPAPAPKPAPAPKPEKPAa	(SEQ ID NO:93)
PAPAPEAPAEQPKPAPAPKPAPAPKPEKPAa-NH_2	(SEQ ID NO:94)
ETPAPEAPAEQPKPAPAPQPAPAPAPEKPA	(SEQ ID NO:95)
ETPAPEAPAEQPKPAPAPQPAPAPAPEKPAa	(SEQ ID NO:96)
ETPAPEAPAEQPKPAPAPQPAPAPAPEKPAa-NH_2	(SEQ ID NO:97)
ETPAPEAPAEQPKPAPAPQPAPAPQPEKPA	(SEQ ID NO:98)
ETPAPEAPAEQPKPAPAPQPAPAPQPEKPAa	(SEQ ID NO:99)
ETPAPEAPAEQPKPAPAPQPAPAPQPEKPAa-NH_2	(SEQ ID NO:100)
ETPAPEAPAEQPKPAPAPKPAPAPQPEKPA	(SEQ ID NO:101)
ETPAPEAPAEQPKPAPAPKPAPAPQPEKPAa	(SEQ ID NO:102)
ETPAPEAPAEQPKPAPAPKPAPAPQPEKPAa-NH_2	(SEQ ID NO:103)
PAPAPEAPAPAPKPAPAPKPAPAPAPEKPA	(SEQ ID NO:104)
PAPAPEAPAPAPKPAPAPKPAPAPAPEKPAa	(SEQ ID NO:105)
PAPAPEAPAPAPKPAPAPKPAPAPAPEKPAa-NH_2	(SEQ ID NO:106)
ETPAPEAPAEQPKPAPAPQPAPAPKPEQPA	(SEQ ID NO:107)
ETPAPEAPAEQPKPAPAPQPAPAPKPEQPAa	(SEQ ID NO:108)
ETPAPEAPAEQPKPAPAPQPAPAPKPEQPAa-NH_2	(SEQ ID NO:109)
PAPAPEAPAPAPKPEQAPQPAPAPKPEQPA	(SEQ ID NO:110)
PAPAPEAPAPAPKPEQAPQPAPAPKPEQPAa	(SEQ ID NO:111)
PAPAPEAPAPAPKPEQAPQPAPAPKPEQPAa-NH_2	(SEQ ID NO:112)

wherein a represents D-Alanine and wherein a-NH_2 represents amidated D-Alanine, i.e. D-alanine with amidation of the C-terminus of the D-Alanine.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23-25, 38-40, 47-52, 59-64, 71-76, 83-88, 92-94, 101-106 and 110-112.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:24.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:25.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:26.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:27.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:28.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:29.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:30.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:31.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:32.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:33.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:34.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:35.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:36.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:37.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:38.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:39.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:40.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:41.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:42.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:43.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:44.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:45.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:46.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:47.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:48.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:49.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:50.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:51.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:52.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:53.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:54.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:55.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:56.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:57.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:58.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:59.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:60.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:61.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:62.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:63.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:64.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:65.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:66.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:67.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:68.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:69.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:70.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:71.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:72.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:73.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:74.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:75.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:76.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:77.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:78.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:79.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:80.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:81.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:82.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:83.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:84.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:85.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:86.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:87.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:88.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:89.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:90.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:91.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:92.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:93.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:94.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:95.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:96.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:97.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:98.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:99.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:100.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:101.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:102.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:103.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:104.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:105.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:106.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:107.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:108.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:109.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:110.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:111.

In a preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:112.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO: 113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170, wherein said third pneumococcal peptide epitope comprises a C-terminal D-Alanine.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO: 113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:125,

SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170, wherein said third pneumococcal peptide epitope comprises a C-terminal amidated D-Ala.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172:

YVTSHGDHYHYYNGKVPYDA	(SEQ ID NO:113)
YVTSHGDHYHYYNGKVPYDAa	(SEQ ID NO:114)
YVTSHGDHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:115)
YITSHGDHYHYYNGKVPYDA	(SEQ ID NO:116)
YITSHGDHYHYYNGKVPYDAa	(SEQ ID NO:117)
YITSHGDHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:118)
YVTPHGDHYHYYNGKVPYDA	(SEQ ID NO:119)
YVTPHGDHYHYYNGKVPYDAa	(SEQ ID NO:120)
YVTPHGDHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:121)
YVTSHGNHYHYYNGKVPYDA	(SEQ ID NO:122)
YVTSHGNHYHYYNGKVPYDAa	(SEQ ID NO:123)
YVTSHGNHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:124)
YVTSHGDHYHYINGKVPYDA	(SEQ ID NO:125)
YVTSHGDHYHYINGKVPYDAa	(SEQ ID NO:126)
YVTSHGDHYHYINGKVPYDAa-NH_2	(SEQ ID NO:127)
YVTSHGDHYHYYPGKVPYDA	(SEQ ID NO:128)
YVTSHGDHYHYYPGKVPYDAa	(SEQ ID NO:129)
YVTSHGDHYHYYPGKVPYDAa-NH_2	(SEQ ID NO:130)
YVTSHGDHYHYYNGSVPYDA	(SEQ ID NO:131)
YVTSHGDHYHYYNGSVPYDAa	(SEQ ID NO:132)
YVTSHGDHYHYYNGSVPYDAa-NH_2	(SEQ ID NO:133)
YVTSHGDHYHYYNGKVLYDA	(SEQ ID NO:134)
YVTSHGDHYHYYNGKVLYDAa	(SEQ ID NO:135)
YVTSHGDHYHYYNGKVLYDAa-NH_2	(SEQ ID NO:136)
YITPHGDHYHYYNGKVPYDA	(SEQ ID NO:137)
YITPHGDHYHYYNGKVPYDAa	(SEQ ID NO:138)
YITPHGDHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:139)

YITPHGDHYHYYPGKVPYDA	(SEQ ID NO:140)
YITPHGDHYHYYPGKVPYDAa	(SEQ ID NO:141)
YITPHGDHYHYYPGKVPYDAa-NH_2	(SEQ ID NO:142)
YVTSHGDHYHYIPGKVPYDA	(SEQ ID NO:143)
YVTSHGDHYHYIPGKVPYDAa	(SEQ ID NO:144)
YVTSHGDHYHYIPGKVPYDAa-NH_2	(SEQ ID NO:145)
YVTSHGDHYHYIPGSVPYDA	(SEQ ID NO:146)
YVTSHGDHYHYIPGSVPYDAa	(SEQ ID NO:147)
YVTSHGDHYHYIPGSVPYDAa-NH_2	(SEQ ID NO:148)
YITSHGDHYHYIPGSVPYDA	(SEQ ID NO:149)
YITSHGDHYHYIPGSVPYDAa	(SEQ ID NO:150)
YITSHGDHYHYIPGSVPYDAa-NH_2	(SEQ ID NO:151)
YVTSHGDHYHYIPGKVLYDA	(SEQ ID NO:152)
YVTSHGDHYHYIPGKVLYDAa	(SEQ ID NO:153)
YVTSHGDHYHYIPGKVLYDAa-NH_2	(SEQ ID NO:154)
YVTPHGDHYHYIPGKVPYDA	(SEQ ID NO:155)
YVTPHGDHYHYIPGKVPYDAa	(SEQ ID NO:156)
YVTPHGDHYHYIPGKVPYDAa-NH_2	(SEQ ID NO:157)
YVTPHGDHYHYIPGSVLYDA	(SEQ ID NO:158)
YVTPHGDHYHYIPGSVLYDAa	(SEQ ID NO:159)
YVTPHGDHYHYIPGSVLYDAa-NH_2	(SEQ ID NO:160)
YITPHGDHYHYYNGKVPYDA	(SEQ ID NO:161)
YITPHGDHYHYYNGKVPYDAa	(SEQ ID NO:162)
YITPHGDHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:163)
YITPHGDHYHYYPGKVPYDA	(SEQ ID NO:164)
YITPHGDHYHYYPGKVPYDAa	(SEQ ID NO:165)
YITPHGDHYHYYPGKVPYDAa-NH_2	(SEQ ID NO:166)
YITSHGNHYHYYNGKVPYDA	(SEQ ID NO:167)
YITSHGNHYHYYNGKVPYDAa	(SEQ ID NO:168)
YITSHGNHYHYYNGKVPYDAa-NH_2	(SEQ ID NO:169)
YITSHGNHYHYYNGKVLYDA	(SEQ ID NO:170)
YITSHGNHYHYYNGKVLYDAa	(SEQ ID NO:171)
YITSHGNHYHYYNGKVLYDAa-NH_2	(SEQ ID NO:172)

wherein a represents D-Alanine and wherein a-NH_2 represents amidated D-Alanine, i.e. D-

alanine with amidation of the C-terminus of the D-Alanine.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:114.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:115.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:116.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:117.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:118.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:119.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:120.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:121.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:122.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:123.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:124.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:125.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:126.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:127.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:128.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:129.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:130.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:131.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:132.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:133.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:134.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:135.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:136.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:137.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:138.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:139.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:140.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:141.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:142.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:143.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:144.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:145.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:146.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:147.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:148.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:149.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:150.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:151.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:152.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:153.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:154.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:155.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:156.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:157.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:158.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:159.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:160.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:161.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:162.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:163.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:164.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:165.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:166.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:167.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:168.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:169.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:170.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:171.

In a preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:172.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19.

In a further preferred embodiment, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110.

In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises,

preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170, and wherein preferably said first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal D-Alanine, or preferably wherein first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal amidated D-Alanine.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170, and wherein said first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal D-Alanine, and wherein preferably first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal amidated D-Alanine.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170, and wherein said first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal D-Alanine, or preferably wherein first, said second, or said third pneumococcal peptide epitope comprises a C-terminal amidated D-

Alanine.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170, and wherein first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal amidated D-Alanine.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4, 7, 10, 13, 16, and 19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107 and 110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, 116, 119, 122, 125, 128, 131, 134, 137, 140, 143, 146, 149, 152, 155, 158, 161, 164, 167 and 170, and wherein said first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal D-Alanine, or preferably wherein first, said second, and/or said third pneumococcal peptide epitope comprises a C-terminal amidated D-Alanine.

In a preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID NO:110; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113, SEQ ID NO:116, SEQ

ID NO:119, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19, wherein said first pneumococcal peptide epitope further comprises a C-terminal D-Alanine; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID NO:110, wherein said second pneumococcal peptide epitope comprises a C-terminal D-Ala; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO: 113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170, wherein said third pneumococcal peptide epitope comprises a C-terminal D-Ala.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19, wherein said first pneumococcal peptide epitope further comprises a C-terminal amidated D-Alanine; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:98, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, and SEQ ID

NO:110, wherein said second pneumococcal peptide epitope comprises a C-terminal amidated D-Ala; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO: 113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:134, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:149, SEQ ID NO:152, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167 and SEQ ID NO:170, wherein said third pneumococcal peptide epitope comprises a C-terminal amidated D-Ala.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4 to 21; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23 to 112; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to 172.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4 to 6; said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23 to 25; and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to 115.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:115.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4; said second pneumococcal peptide

epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:25; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:115.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:25; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:6; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:6; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:25; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:6; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:115.

In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:6; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NOs:25; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:115.

In a preferred embodiment, said composition comprises said first pneumococcal antigen, preferably said first pneumococcal peptide epitope, said second pneumococcal antigen, preferably said second pneumococcal peptide epitope, and said third pneumococcal antigen, preferably said third pneumococcal peptide epitope in an equimolar ratio. Thus, in a preferred embodiment, said first pneumococcal antigen, preferably said first pneumococcal peptide epitope, said second pneumococcal antigen, preferably said second pneumococcal peptide

epitope, and said third pneumococcal antigen, preferably said third pneumococcal peptide epitope is comprised in the inventive composition at equal concentrations. In a preferred embodiment, said composition comprises said first pneumococcal peptide epitope, said second pneumococcal peptide epitope, and said third pneumococcal peptide epitope in an equimolar ratio. Thus, in a preferred embodiment, said first pneumococcal peptide epitope, said second pneumococcal peptide epitope, and said third pneumococcal peptide epitope is comprised in the inventive composition at equal concentrations.

In a further preferred embodiment, at least two, preferably exactly two, of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of at least one, preferably exactly one, connecting moiety forming a multi-epitope pneumococcal antigen, wherein preferably said connecting moiety is a branching moiety forming a multi-epitope pneumococcal antigen, preferably forming a multi-epitope branched pneumococcal antigen.

The terms “connecting moiety” and “branching moiety”, as used herein, refers to a chemical moiety able to connect said first, said second and/or said third pneumococcal antigens typically and preferably by way of covalently linkages either in linear or in a branched manner. The linking of said first, said second and/or said third pneumococcal antigens in a branched manner shall typically and preferably refer to the linking of said pneumococcal antigens via a side chain functionality of an amino acid residue comprised in one of said pneumococcal antigens.

In a further preferred embodiment, at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of at least one connecting moiety, preferably by way of at least one branching moiety forming a multi-epitope pneumococcal antigen, preferably forming a multi-epitope branched pneumococcal antigen.

In a further preferred embodiment, at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of exactly one connecting moiety, preferably by way of exactly one branching moiety, forming a multi-epitope pneumococcal antigen, preferably forming a multi-epitope branched pneumococcal antigen.

In a further preferred embodiment, at least two, preferably exactly two, of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of at least one, preferably exactly one, connecting moiety, forming a multi-epitope pneumococcal antigen.

In a further preferred embodiment, at least two, preferably exactly two, of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of at least one, preferably exactly one, branching moiety forming a multi-epitope pneumococcal antigen, preferably a multi-epitope branched pneumococcal antigen, wherein preferably said branching moiety comprises a lysine or a cysteine residue, preferably a lysine residue.

In a further preferred embodiment, two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope pneumococcal antigen, preferably a branched bi-epitope pneumococcal antigen, wherein preferably said branching moiety comprises a lysine or a cysteine residue, preferably a lysine residue.

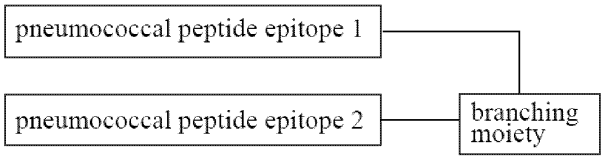
In a further preferred embodiment, said branching moiety comprises, preferably consists of, a lysine or a cysteine residue. In a further preferred embodiment, said branching moiety comprises preferably consists of, a lysine residue. In a further preferred embodiment, said branching moiety comprises, preferably consists of, a cysteine residue.

In a further preferred embodiment, said branching moiety consists of a lysine or a cysteine residue. In a further preferred embodiment, said branching moiety consists of a lysine residue. In a further preferred embodiment, said branching moiety consists of a cysteine residue.

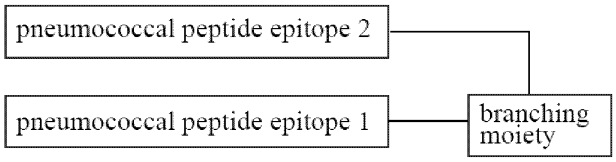
In a further preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope.

In a further preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue.

The structure of a branched pneumococcal antigen comprising a first and a second pneumococcal peptide epitope is schematically illustrated below, wherein said branched pneumococcal antigen comprises a branching moiety allowing to covalently link two or more, preferably the represented two pneumococcal peptide epitopes leading to a bi-epitope branched pneumococcal antigen.



It is of note that the present invention also provides bi-epitope branched pneumococcal antigens having different including the opposite branching with respect to the main peptide chain as illustrated below.



In a preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, and wherein said branching moiety comprises, preferably consists of, an amino acid residue, wherein one of said first and said second pneumococcal antigen is linked to the N-terminus of said amino acid residue and the other of said first and said second pneumococcal antigen is linked to the side chain of said amino acid residue.

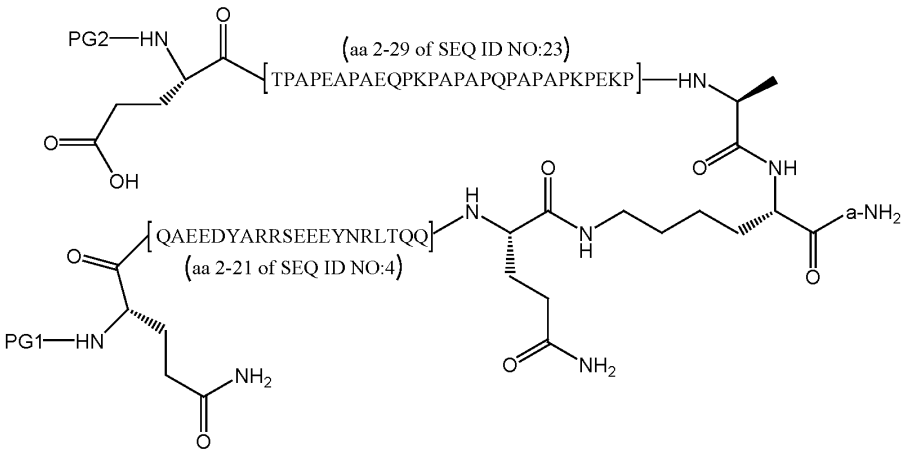
In a further preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said second pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said second pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue.

In a further preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said second pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said second

pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21 and wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100.

In a further preferred embodiment, said first pneumococcal antigen and said second pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said second pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said second pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4, and wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23.

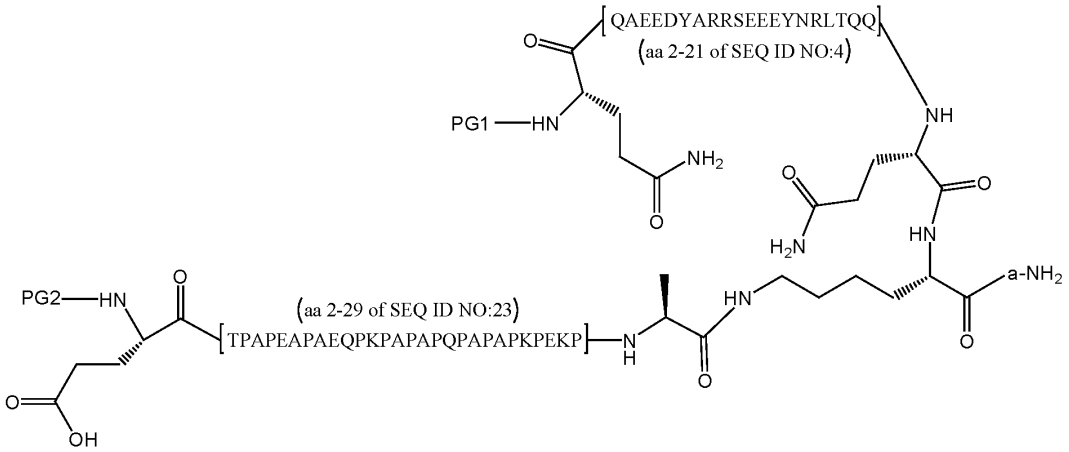
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-1***, wherein PG1 and PG2 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-1*

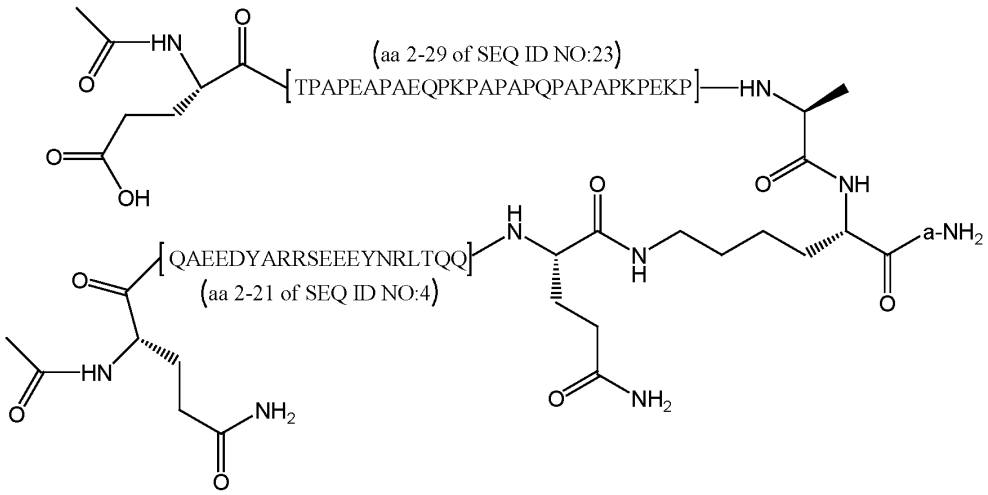
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-3***, wherein PG1 and PG2 represents independently an amino protecting group, and

wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



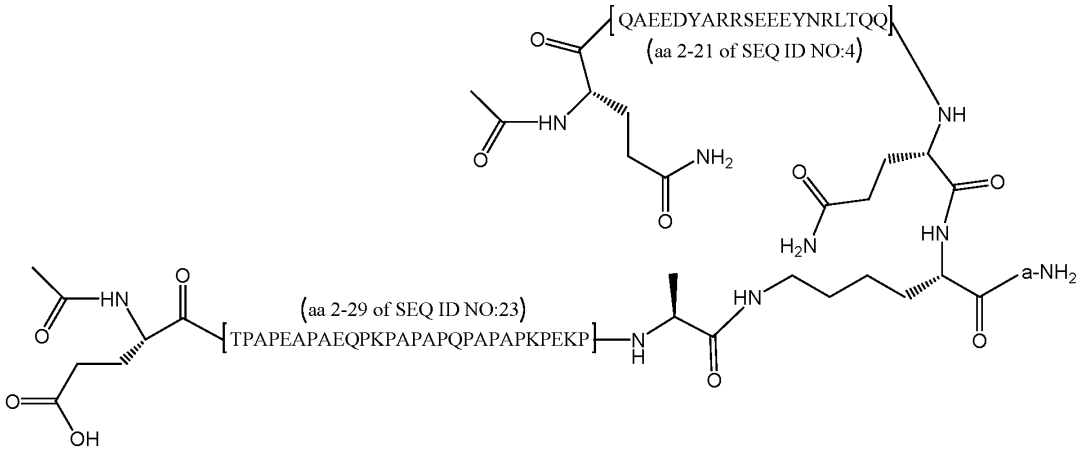
BPA-3*

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-1**, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



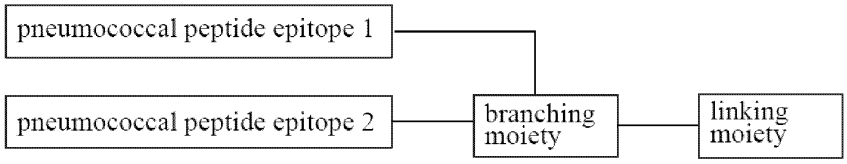
BPA-1

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-3**, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.

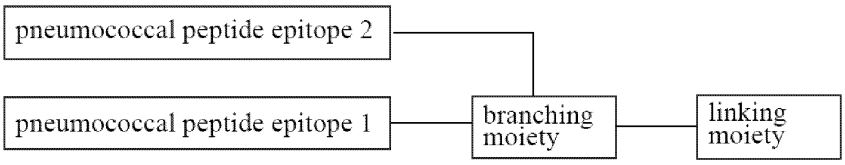


BPA-3

If said branched pneumococcal antigen, preferably said branched bi-epitope pneumococcal antigen, is to be further connected to a carrier such as, preferably, to a SVLP, and hereby to a LBB of a SVLP, said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, typically and preferably further comprises a linking moiety allowing to covalently link said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, to said carrier such as, preferably, to said SVLP, and hereby to a LBB of a SVLP as illustrated below.



It is of note that the present invention also provides branched pneumococcal antigens, preferably bi-epitope branched pneumococcal antigens, having the opposite branching with respect to the main chain as depicted below.



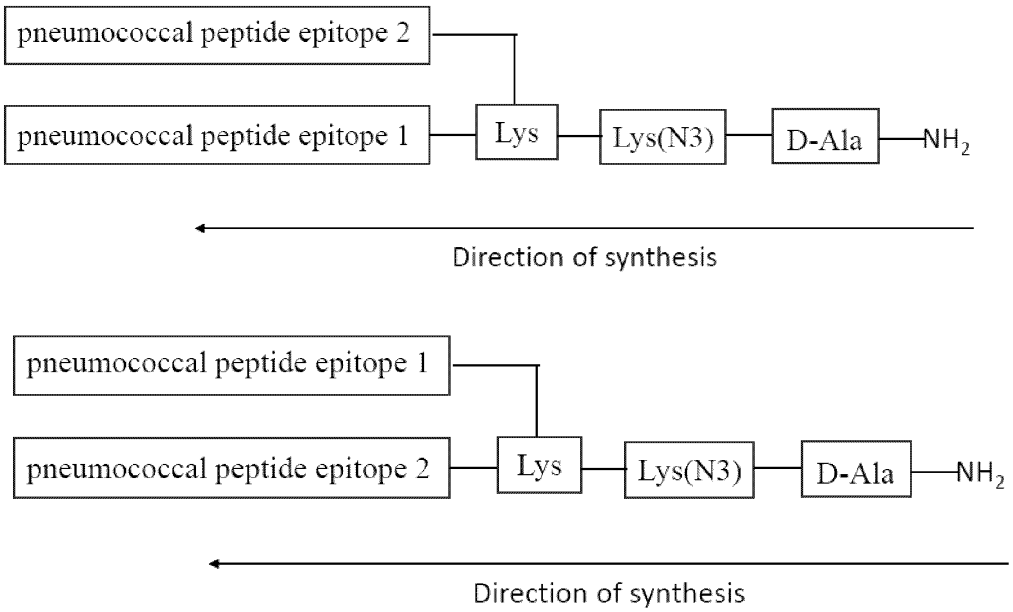
The syntheses of said branched bi-epitope pneumococcal antigens are described herein by way of the syntheses of the preferred bi-epitope branched pneumococcal antigens **BPA-2** and **BPA-4**, both comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the second pneumococcal peptide epitope of SEQ ID NO:23 but having the opposite branching with respect to the main chain. Said preferred bi-epitope branched pneumococcal antigens

BPA-2 and **BPA-4** comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the second pneumococcal peptide epitope of SEQ ID NO:23 further comprises a lysine residue as branching moiety and an additional lysine residue as linking moiety which linking moiety is modified to contain a terminal azide group allowing for linking to a carrier, preferably, as further described herein, to a SVLP, and hereby to a LBB of a SVLP.

In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group. In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group able to link to the linker comprising an alkyne group typically and preferably as described herein in a click-chem reaction. Preferred linking moieties are linked to the connecting moiety, preferably to said branching moiety, by way of classical peptide synthesis. Thus, preferred linking moieties comprising an azide group able to link to the linker comprising an alkyne group in accordance with the present invention are derived from N-alpha-(9-Fluorenylmethyloxycarbonyl)-epsilon-azido-L-norleucine (CAS 159610-89-6), (S)-6-azido-2-(Fmoc-amino)hexanoic acid, N-alpha-(9-Fluorenylmethyloxycarbonyl)-3-azido-L-alanine, N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-homoalanine, N-alpha-(((9H-fluoren-9-yl)-methoxy)carbonyl)-N-gamma-(2-azidoethyl)-L-asparagine, (2S,3S)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2S,3R)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (2R,3S)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (S)-3-(9-Fluorenylmethyloxy-carbonyl)amino-4-azido-butanoic acid, N-alpha-(9-Fluorenylmethyl-oxycarbonyl)-delta-azido-L-ornithine, trans-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, cis-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, and/or N-alpha-(9-Fluorenylmethyloxy-carbonyl)-O-(2-azidoethyl)-L-tyrosine.

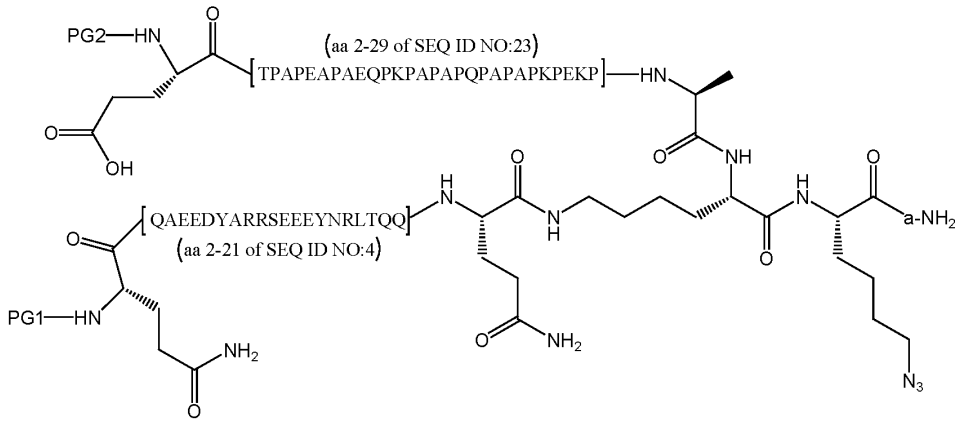
The preferred branched bi-epitope pneumococcal antigens **BPA-2** and **BPA-4** further comprise N-terminal amine protecting groups such as acetyl groups as well as an amidated C-terminal D-alanine residue which is believed to increase the stability and protect the C-terminus of said branched bi-epitope pneumococcal antigen.

The schematic structures of preferred branched bi-epitope pneumococcal antigens is shown below.



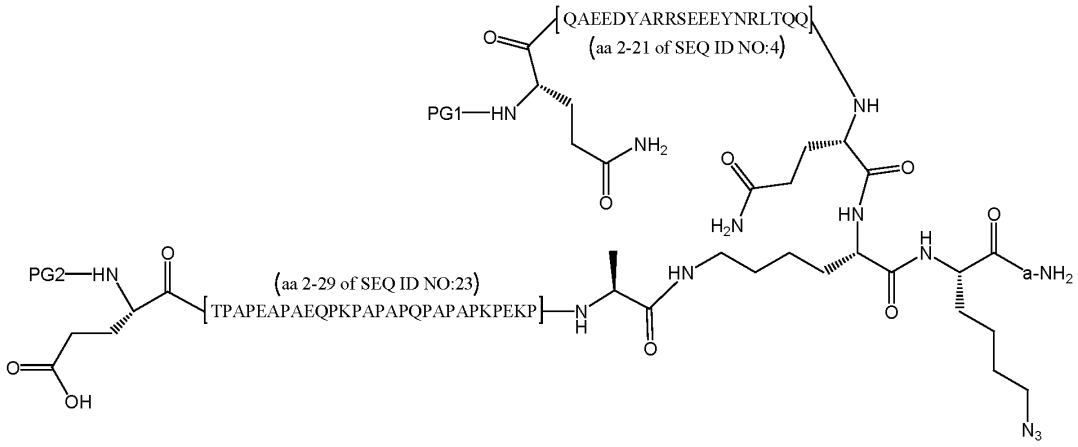
It is of note and as shown in the illustrations, said synthesis is, preferably, effected starting from the C-terminus, and the synthesis of pneumococcal peptide epitope 1 or pneumococcal peptide epitope 2 will be completed before the synthesis of the pneumococcal peptide epitope 2 or pneumococcal peptide epitope 1 is initiated. Hereto, the lysine residue representing the branching moiety is, preferably, protected with a protecting group that can be released by, by way of example and preferably, 1% TFA after synthesis of the pneumococcal peptide epitope 1 or pneumococcal peptide epitope 2.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-2***, wherein PG1 and PG2 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-2*

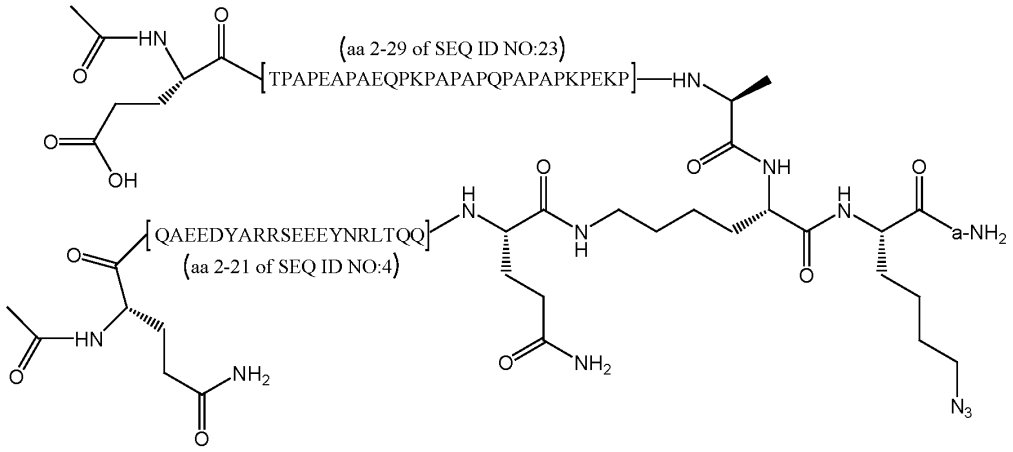
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-4***, wherein PG1 and PG2 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap "a".



BPA-4*

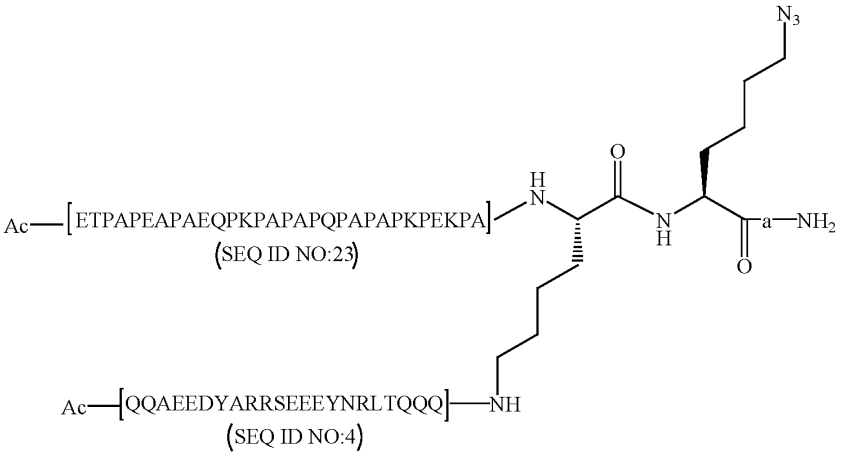
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-2** or **BPA-4**.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-2.** The chemical structure of said preferred branched bi-epitope pneumococcal antigen **BPA-2** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, while D-Ala is represented as a small cap "a".



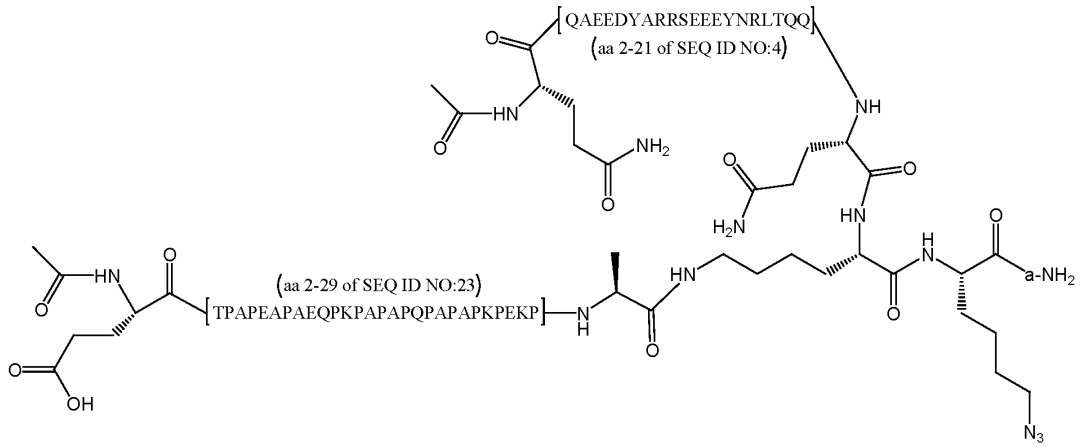
BPA-2

The more simplified representation of **BPA-2** is as follows:



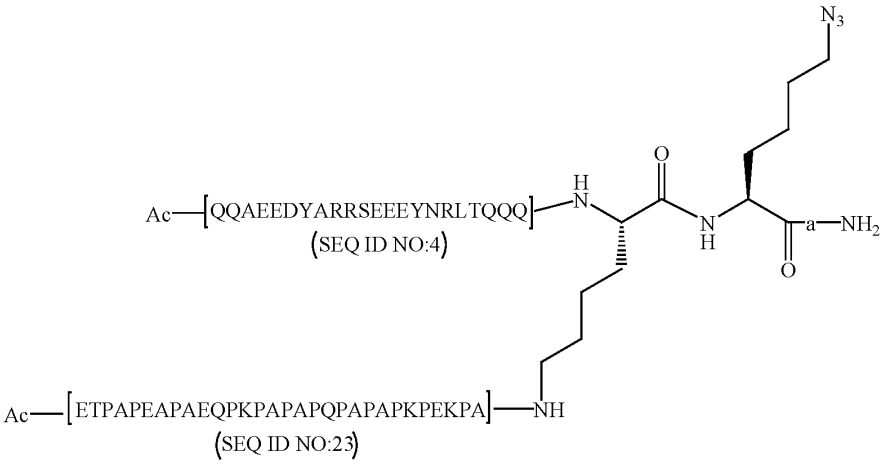
wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-4.** The chemical structure of said preferred branched bi-epitope pneumococcal antigen **BPA-4** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, while D-Ala is represented as a small cap "a".



BPA-4

The more simplified representation of **BPA-4** is as follows:

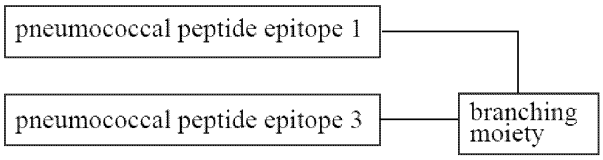


wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23.

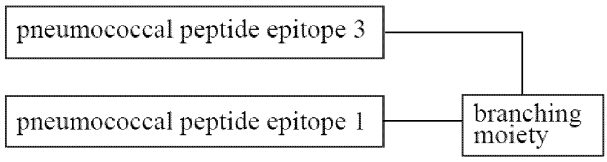
It is of note that the present invention also allows branched pneumococcal antigens, preferably branched bi-epitope pneumococcal antigens, having the opposite branching with respect to the main chain as referred above.

In a further preferred embodiment, said first pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, comprising said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue.

The structure of a branched pneumococcal antigen comprising a first and a third pneumococcal peptide epitope is schematically illustrated below, wherein said branched pneumococcal antigen comprises a branching moiety allowing to covalently link two or more, preferably the represented two pneumococcal peptide epitopes leading to a bi-epitope branched pneumococcal antigen.



It is of note that the present invention also provides bi-epitope branched pneumococcal antigens having different including the opposite branching with respect to the main peptide chain as illustrated below.



In a preferred embodiment, said first pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, and wherein said branching moiety comprises, preferably consists of, an amino acid residue, wherein one of said first and said third pneumococcal antigen is linked to the N-terminus of said amino acid residue and the other of said first and said third pneumococcal antigen is linked to the side chain of said amino acid residue.

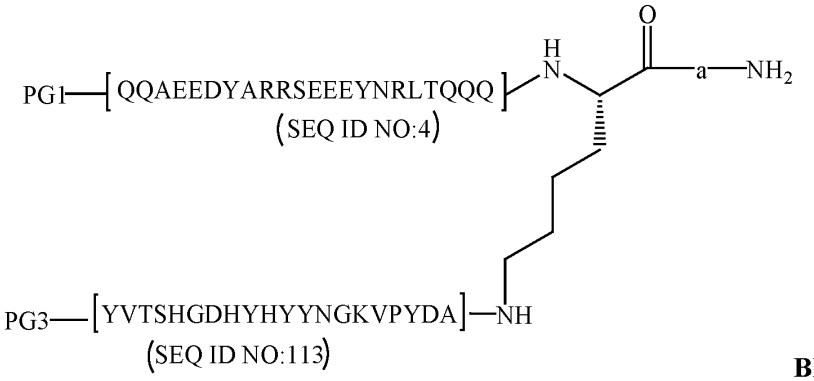
In a further preferred embodiment, said first pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue.

In a further preferred embodiment, said first pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172.

In a further preferred embodiment, said first pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said first pneumococcal peptide epitope and said

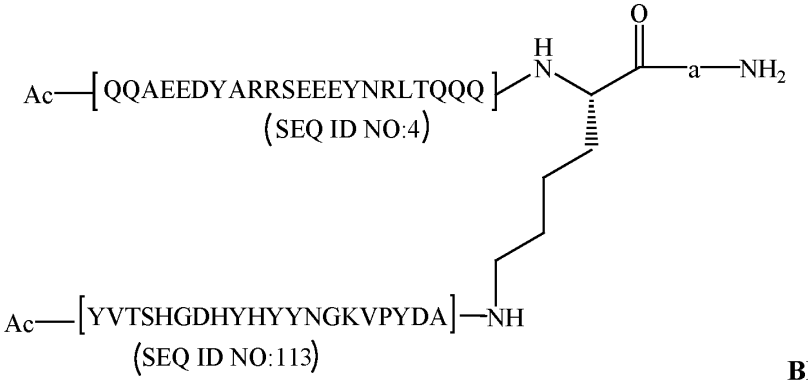
third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said first and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said first and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4, and wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-5***, wherein PG1 and PG3 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-5*

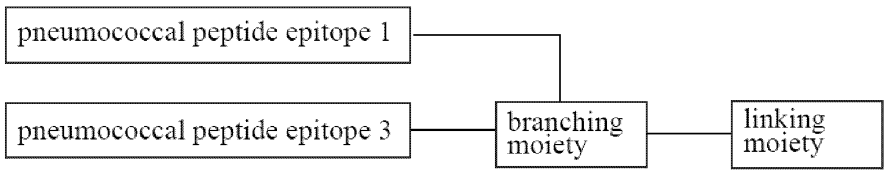
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-5**, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



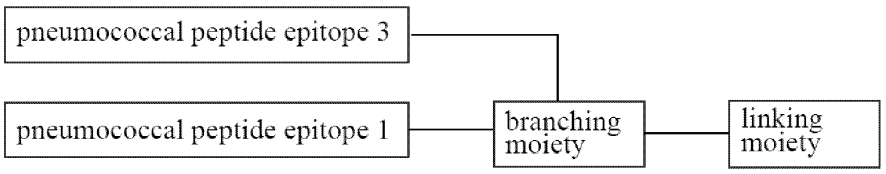
BPA-5,

wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:113.

If said branched pneumococcal antigen, preferably said branched bi-epitope pneumococcal antigen, is to be further connected to a carrier such as, preferably, to a SVLP, and hereby to a LBB of a SVLP, said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, typically and preferably further comprises a linking moiety allowing to covalently link said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, to said carrier such as, preferably, to said SVLP, and hereby to a LBB of a SVLP as illustrated below.



It is of note that the present invention also provides branched pneumococcal antigens, preferably bi-epitope branched pneumococcal antigens, having the opposite branching with respect to the main chain as depicted below.



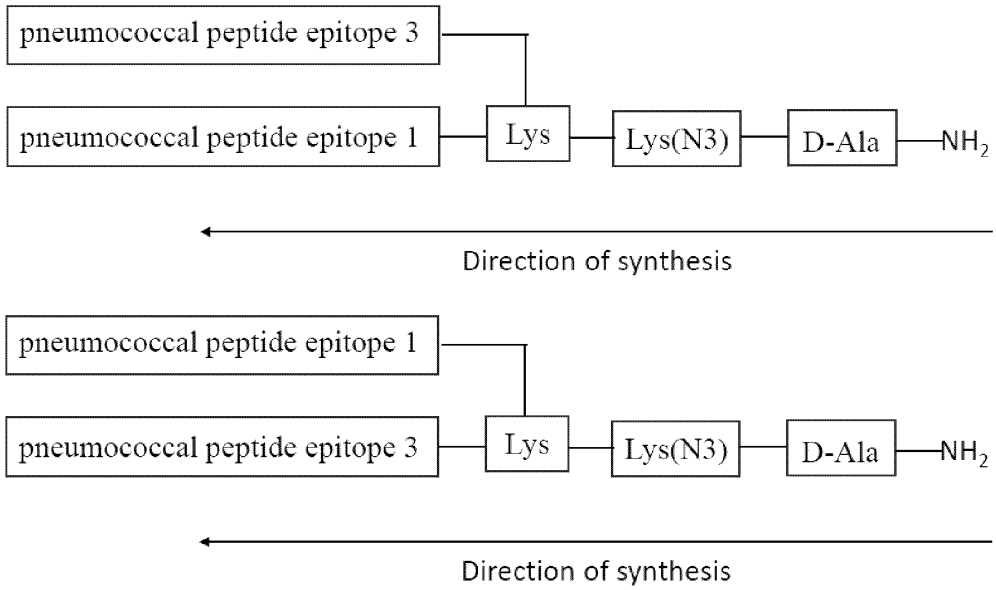
The syntheses of said branched bi-epitope pneumococcal antigens are described herein by way of the syntheses of the preferred bi-epitope branched pneumococcal antigens **BPA-5** comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the third pneumococcal peptide epitope of SEQ ID NO:113. Said preferred bi-epitope branched pneumococcal antigen **BPA-5** comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the third pneumococcal peptide epitope of SEQ ID NO:113 further comprises a lysine residue as branching moiety and an additional lysine residue as linking moiety which linking moiety is modified to contain a terminal azide group allowing for linking to a carrier, preferably, as further described herein, to a SVLP, and hereby to a LBB of a SVLP.

In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group. In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group able to link to the linker comprising an alkyne group typically and preferably as described herein in a click-chem reaction. Preferred linking moieties are linked to the connecting moiety, preferably to said branching moiety, by way of classical peptide synthesis. Thus, preferred linking moieties comprising an azide group able to link to

the linker comprising an alkyne group in accordance with the present invention are derived from N-alpha-(9-Fluorenylmethyloxycarbonyl)-epsilon-azido-L-norleucine (CAS 159610-89-6), (S)-6-azido-2-(Fmoc-amino)hexanoic acid, N-alpha-(9-Fluorenylmethyloxycarbonyl)-3-azido-L-alanine, N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-homoalanine, N-alpha-(((9H-fluoren-9-yl)-methoxy)carbonyl)-N-gamma-(2-azidoethyl)-L-asparagine, (2S,3S)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2S,3R)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (2R,3S)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (S)-3-(9-Fluorenylmethyloxy-carbonyl)amino-4-azido-butanoic acid, N-alpha-(9-Fluorenylmethyl-oxycarbonyl)-delta-azido-L-ornithine, trans-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, cis-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, and/or N-alpha-(9-Fluorenylmethyloxy-carbonyl)-O-(2-azidoethyl)-L-tyrosine.

The preferred branched bi-epitope pneumococcal antigen **BPA-5** further comprise N-terminal amine protecting groups such as acetyl groups as well as an amidated C-terminal D-alanine residue which is believed to increase the stability and protect the C-terminus of said branched bi-epitope pneumococcal antigen.

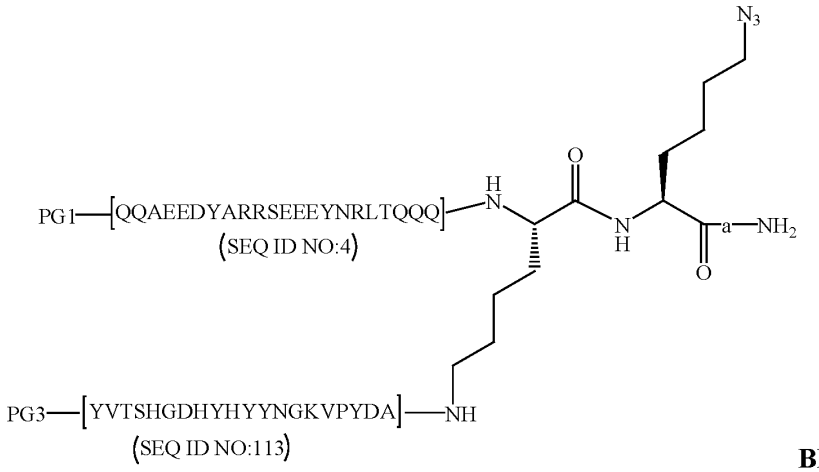
The schematic structures of preferred branched bi-epitope pneumococcal antigens is shown below.



It is of note and as shown in the illustrations, said synthesis is, preferably, effected starting from the C-terminus, and the synthesis of pneumococcal peptide epitope 1 or pneumococcal

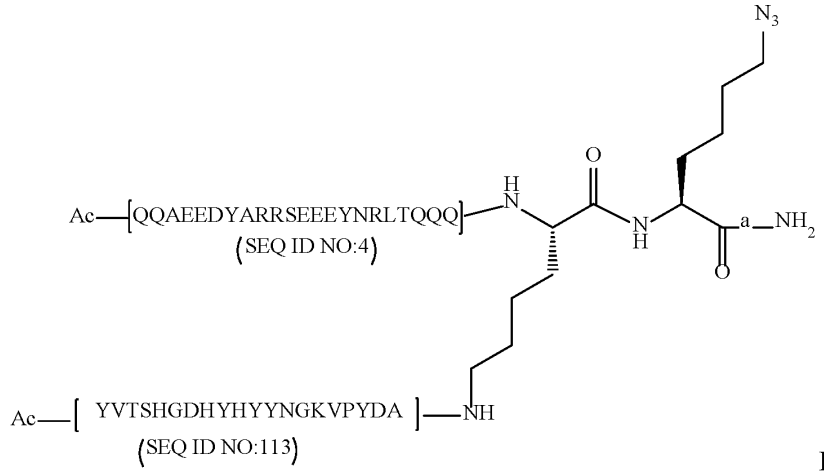
peptide epitope 3 will be completed before the synthesis of the pneumococcal peptide epitope 3 or pneumococcal peptide epitope 1 is initiated. Hereto, the lysine residue representing the branching moiety is, preferably, protected with a protecting group that can be released by, by way of example and preferably, 1% TFA after synthesis of the pneumococcal peptide epitope 1 or pneumococcal peptide epitope 3.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-6***, wherein PG1 and PG3 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-6*

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-6**. The chemical structure of said preferred branched bi-epitope pneumococcal antigen **BPA-6** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, while D-Ala is represented as a small cap “a”.



BPA-6,

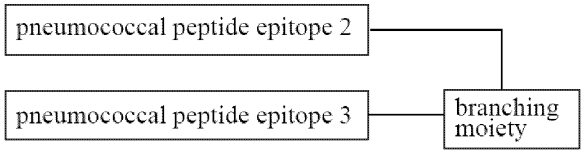
wherein Ac represents acetyl residues as amino protecting group protecting the N-

terminus of the SEQ ID NO:4 and SEQ ID NO:113.

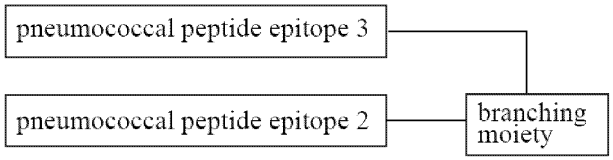
It is of note that the present invention also allows branched pneumococcal antigens, preferably branched bi-epitope pneumococcal antigens, having the opposite branching with respect to the main chain as referred above.

In a further preferred embodiment, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, comprising said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue.

The structure of a branched pneumococcal antigen comprising a second and a third pneumococcal peptide epitope is schematically illustrated below, wherein said branched pneumococcal antigen comprises a branching moiety allowing to covalently link two or more, preferably the represented two pneumococcal peptide epitopes leading to a bi-epitope branched pneumococcal antigen.



It is of note that the present invention also provides bi-epitope branched pneumococcal antigens having different including the opposite branching with respect to the main peptide chain as illustrated below.



In a preferred embodiment, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, and wherein said branching moiety comprises, preferably consists of, an amino acid residue, wherein one of said second and said third pneumococcal antigen is linked to the N-terminus of said amino acid residue and the other of said second and said third pneumococcal antigen is linked to the side chain of said amino acid residue.

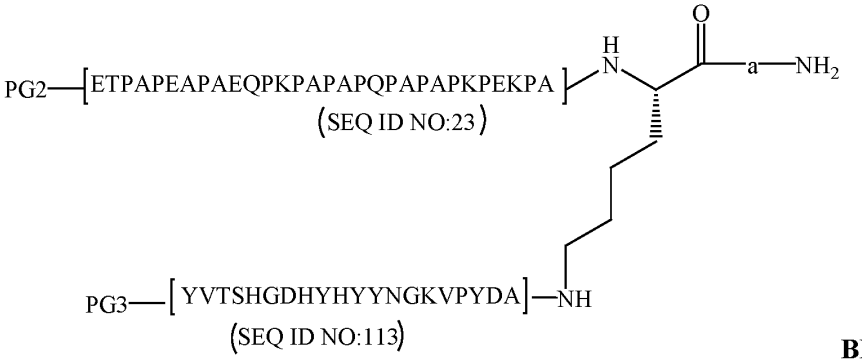
In a further preferred embodiment, said second pneumococcal antigen and said third

pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said second and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said second and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue.

In a further preferred embodiment, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said second and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said second and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172.

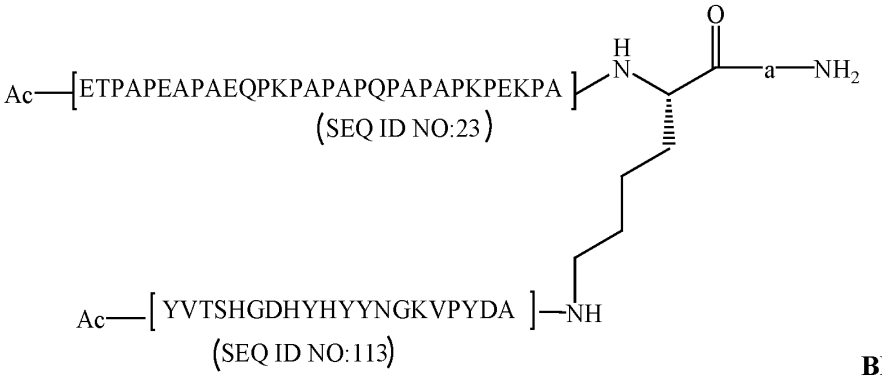
In a further preferred embodiment, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked by way of a branching moiety forming a bi-epitope branched pneumococcal antigen comprising said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said branching moiety comprises, preferably consists of, a lysine residue, wherein one of said second and said third pneumococcal antigen is linked to the N-terminus of said lysine residue and the other of said second and said third pneumococcal antigen is linked to the side chain of said lysine residue via the amine functionality of said side chain of said lysine residue, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23, and wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-7***, wherein PG2 and PG3 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-7*

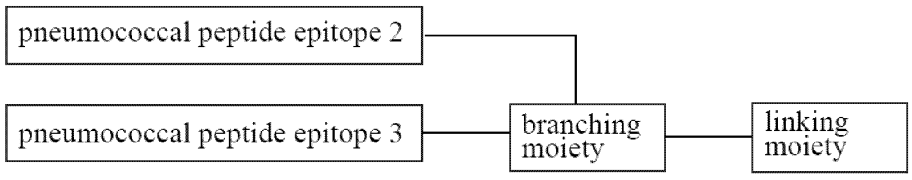
In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-7**, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap "a".



BPA-7,

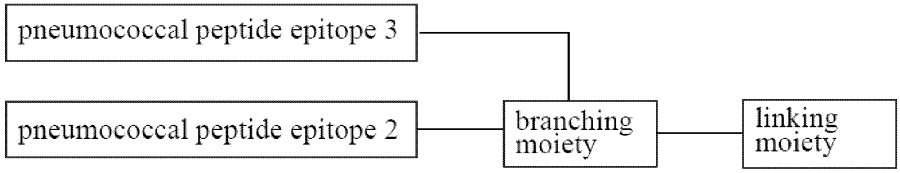
wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:23 and SEQ ID NO:113.

If said branched pneumococcal antigen, preferably said branched bi-epitope pneumococcal antigen, is to be further connected to a carrier such as, preferably, to a SVLP, and hereby to a LBB of a SVLP, said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, typically and preferably further comprises a linking moiety allowing to covalently link said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, to said carrier such as, preferably, to said SVLP, and hereby to a LBB of a SVLP as illustrated below.



It is of note that the present invention also provides branched pneumococcal antigens,

preferably bi-epitope branched pneumococcal antigens, having the opposite branching with respect to the main chain as depicted below.



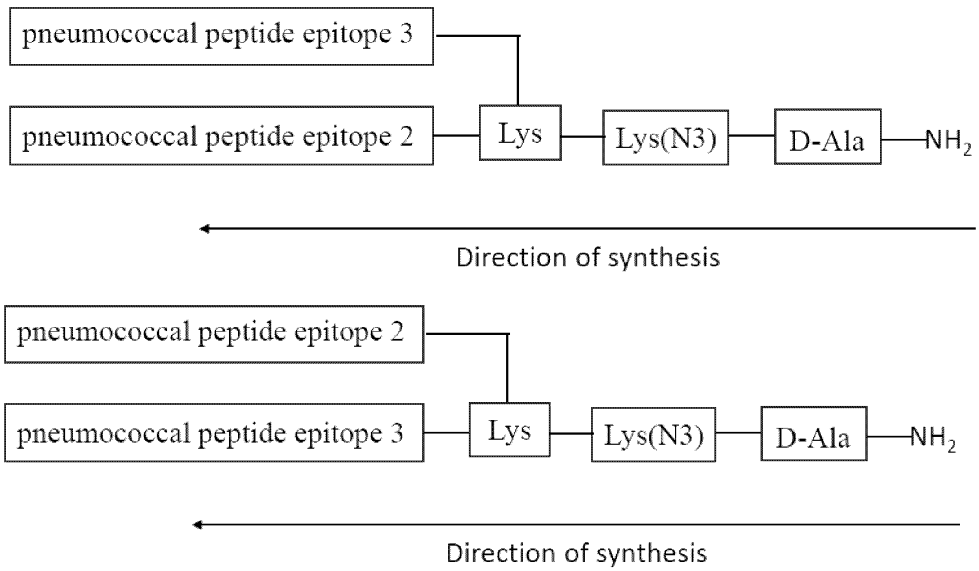
The syntheses of said branched bi-epitope pneumococcal antigens are described herein by way of the syntheses of the preferred bi-epitope branched pneumococcal antigens **BPA-7** comprising the second pneumococcal peptide epitope of SEQ ID NO:23 and the third pneumococcal peptide epitope of SEQ ID NO:113. Said preferred bi-epitope branched pneumococcal antigen **BPA-7** comprising the second pneumococcal peptide epitope of SEQ ID NO:23 and the third pneumococcal peptide epitope of SEQ ID NO:113 further comprises a lysine residue as branching moiety and an additional lysine residue as linking moiety which linking moiety is modified to contain a terminal azide group allowing for linking to a carrier, preferably, as further described herein, to a SVLP, and hereby to a LBB of a SVLP.

In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group. In a preferred embodiment, said linking moiety comprises an azide group, preferably a terminal azide group able to link to the linker comprising an alkyne group typically and preferably as described herein in a click-chem reaction. Preferred linking moieties are linked to the connecting moiety, preferably to said branching moiety, by way of classical peptide synthesis. Thus, preferred linking moieties comprising an azide group able to link to the linker comprising an alkyne group in accordance with the present invention are derived from N-alpha-(9-Fluorenylmethyloxycarbonyl)-epsilon-azido-L-norleucine (CAS 159610-89-6), (S)-6-azido-2-(Fmoc-amino)hexanoic acid, N-alpha-(9-Fluorenylmethyloxycarbonyl)-3-azido-L-alanine, N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-homoalanine, N-alpha-(((9H-fluoren-9-yl)-methoxy)carbonyl)-N-gamma-(2-azidoethyl)-L-asparagine, (2S,3S)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2S,3R)-2-(9-Fluorenylmethyl-oxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (2R,3S)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azido-butanoic acid, (2R,3R)-2-(9-Fluorenylmethyloxycarbonyl)amino-3-azidobutanoic acid, (S)-3-(9-Fluorenylmethyloxy-carbonyl)amino-4-azido-butanoic acid, N-alpha-(9-Fluorenylmethyl-oxycarbonyl)-delta-azido-L-ornithine, trans-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, cis-N-alpha-(9-Fluorenylmethyloxycarbonyl)-4-azido-L-proline, and/or N-

alpha-(9-Fluorenylmethyloxy-carbonyl)-O-(2-azidoethyl)-L-tyrosine.

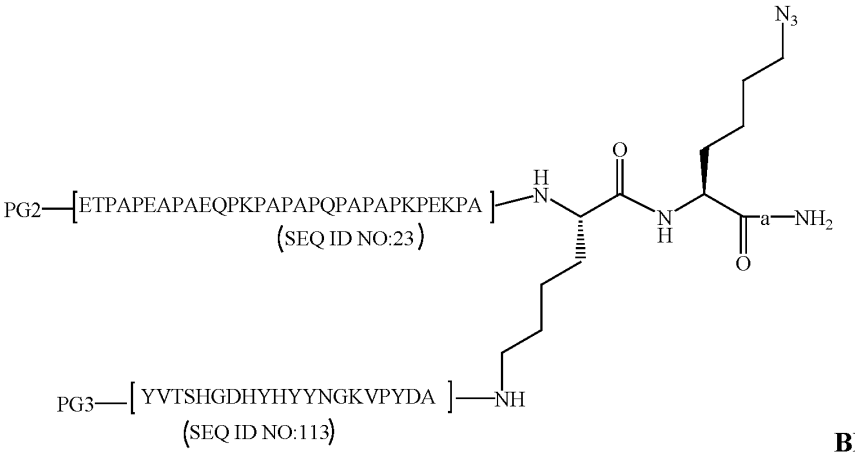
The preferred branched bi-epitope pneumococcal antigen **BPA-7** further comprise N-terminal amine protecting groups such as acetyl groups as well as an amidated C-terminal D-alanine residue which is believed to increase the stability and protect the C-terminus of said branched bi-epitope pneumococcal antigen.

The schematic structures of preferred branched bi-epitope pneumococcal antigens is shown below.



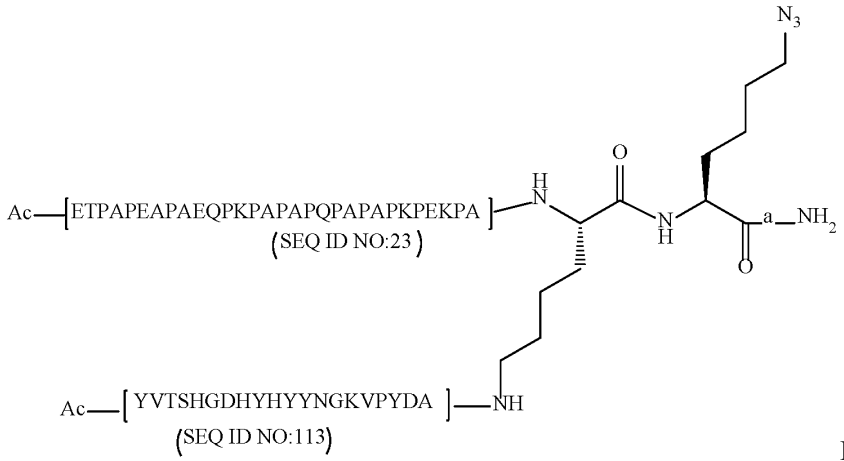
It is of note and as shown in the illustrations, said synthesis is, preferably, effected starting from the C-terminus, and the synthesis of pneumococcal peptide epitope 2 or pneumococcal peptide epitope 3 will be completed before the synthesis of the pneumococcal peptide epitope 3 or pneumococcal peptide epitope 2 is initiated. Hereto, the lysine residue representing the branching moiety is, preferably, protected with a protecting group that can be released by, by way of example and preferably, 1% TFA after synthesis of the pneumococcal peptide epitope 2 or pneumococcal peptide epitope 3.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-8***, wherein PG2 and PG3 represents independently an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap “a”.



BPA-8*

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is **BPA-8**. The chemical structure of said preferred branched bi-epitope pneumococcal antigen **BPA-8** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, while D-Ala is represented as a small cap "a".



BPA-8,

wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:113.

It is of note that the present invention also allows branched pneumococcal antigens, preferably branched bi-epitope pneumococcal antigens, having the opposite branching with respect to the main chain as referred above.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap “a”, and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113.

Carriers

In a preferred embodiment, said composition further comprises at least one carrier.

The at least one carrier as referred herein can be one or more carriers in accordance with the present invention and said one or more carriers can be same or can be different. The herein described embodiments for said one or more carriers are typically applicable to any of said one or more carriers, except where referred to and explicitly described differently.

In a preferred embodiment, said composition further comprises at least one carrier, wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, or at least one of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to or is complexed with said at least one carrier.

In a preferred embodiment, said carrier is a particle able to provide a foundation for attachment, by covalent or non-covalent binding, preferably by covalent binding, of the pneumococcal antigens, preferably the pneumococcal peptide epitopes in accordance with the present invention, typically and preferably on its surface for presenting said antigens and/or epitopes to the immune system.

Thus, in a preferred embodiment, said composition further comprises at least one carrier, wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to said at least one carrier.

In a preferred embodiment, said composition further comprises at least one carrier, wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to said at least one carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least one carrier, wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is covalently linked to said at least one carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least one carrier, wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is non-covalently linked to said at least one carrier.

In a preferred embodiment, said composition further comprises at least one carrier, wherein said first, said second and said third pneumococcal antigen are linked to said at least one carrier, either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said first and said second carrier.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said first carrier and wherein the other of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said second carrier.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said first and said second carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked, either directly or by way of a linker, to said first carrier and one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked, either directly or by way of a linker, to said second carrier.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked to said first and said second carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked, either directly or by way of a linker, to said first carrier and one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are covalently linked, either directly or by way of a linker, to said second carrier.

In a preferred embodiment, said composition further comprises at least a first and a second carrier, wherein at least two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are non-covalently linked to said first and said second carrier.

In a preferred embodiment, said first carrier and said second carrier are the same. In a preferred embodiment, said first carrier and said second carrier are identical. In another preferred embodiment, said first carrier and said second carrier are different.

In a preferred embodiment, said composition further comprises at least a first carrier, a second carrier, and a third carrier, wherein one each of said first pneumococcal antigen, said

second pneumococcal antigen and said third pneumococcal antigen is linked to said first carrier, and said second carrier and said third carrier.

In a preferred embodiment, said composition further comprises at least a first carrier, a second carrier, and a third carrier, wherein one each of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to said first carrier, and said second carrier and said third carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least a first carrier, a second carrier, and a third carrier, wherein one each of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is covalently linked to said first carrier, said second carrier and said third carrier, independently either directly or by way of a linker.

In a preferred embodiment, said composition further comprises at least a first carrier, a second carrier, and a third carrier, wherein one each of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is non-covalently linked to said first carrier, and said second carrier and said third carrier.

In a preferred embodiment, said first carrier, said second carrier and said third carrier are the same. In a preferred embodiment, said first carrier, said second carrier and said third carrier are identical. In another preferred embodiment, said first carrier, said second carrier and said third carrier are different.

In a preferred embodiment, said carrier is a particle able to transport and/or allow complexation of the pneumococcal antigens, preferably the pneumococcal peptide epitopes, and/or, in particular and preferably the nucleic acids, preferably mRNAs, encoding for the pneumococcal antigens, preferably the pneumococcal peptide epitopes. Thus, said nucleic acids, preferably mRNAs, encoding for the pneumococcal antigens, preferably the pneumococcal peptide epitopes, represent the cargo of said carrier, wherein the carrier may be associated to its cargo by covalent or non-covalent interaction.

Thus, in a preferred embodiment, said composition further comprises at least one carrier, wherein at least one, preferably all, of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is complexed with said at least one carrier.

In a preferred embodiment, said composition further comprises one single carrier, wherein all of said nucleic acids encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are complexed with said one single carrier, and wherein preferably all of said nucleic acids encoding said first pneumococcal

antigen, said second pneumococcal antigen and said third pneumococcal antigen are encapsulated in said one single carrier.

In a preferred embodiment, each of said at least one carrier is the same. In a preferred embodiment, each of said at least one carrier is identical. In another preferred embodiment, each of said at least one carrier is different.

In a further preferred embodiment, said carrier suitable for use in the present invention is a virus, a viral capsid particle, a bacteriophage, a virus-like particle (VLP) such as a virus-like particle of an RNA-bacteriophage, a synthetic virus-like particle (SVLP), a bacterial pilus or a structure formed from bacterial pilin, a bacterial flagellum, a lipid, synthetic or a natural polymer, a lipid nanoparticle (LNP) such as solid lipid nanoparticle (SLN) or a lipid-based nanoparticle such as a liposome or a lipid nano emulsion (LNE). Such carriers and particles are known to the skilled person in the art and have been described (KT Gause et al, ACS Nano. 2017, 11(1):54-68; M Bröker et al, Vaccine 2017, 35:3286–3294 and references cited therein). In a further preferred embodiment, said carrier is a synthetic virus-like particle (SVLP). In a further preferred embodiment, said carrier is a lipid nanoparticle (LNP).

In a preferred embodiment, said carrier has an inherent repetitive structure capable of presenting the antigens of the present invention in a repetitive manner, such a virus, a viral capsid particle, a bacteriophage, a virus-like particle (VLP) such as a virus-like particle of an RNA-bacteriophage or a synthetic virus-like particle (SVLP). Any virus known in the art having a repetitive coat protein structure may be selected as a carrier for the present invention; examples of suitable viruses include sindbis and other alphaviruses, rhabdoviruses (e.g. vesicular stomatitis virus), picornaviruses, togaviruses, orthomyxoviruses, polyomaviruses (e.g., polyomavirus BK, polyomavirus JC, avian polyomavirus BFDV), parvoviruses, rotaviruses, Norwalk virus, foot and mouth disease virus, a retrovirus, Hepatitis B virus, Tobacco mosaic virus, Flock House Virus, and human Papillomavirus, and RNA-bacteriophages.

In another preferred embodiment, said at least one carrier, preferably said one single carrier, is a liposome, wherein at least one, preferably all, of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is complexed with, preferably encapsulated in said liposome.

In another preferred embodiment, said at least one carrier, preferably said one single carrier, is a lipid nanoparticle (LNP), wherein at least one, preferably all, of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is complexed with, preferably encapsulated in said lipid nanoparticle

(LNP).In another preferred embodiment, said at least one carrier, preferably said one single carrier, is a lipid nanoparticle (LNP), wherein at least one, preferably all, of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is complexed with, preferably encapsulated in said lipid nanoparticle (LNP), wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:4; said second pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:23; and said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence of SEQ ID NO:113.

Synthetic virus-like particles (SVLPs)

In a preferred embodiment, said at least one carrier is a synthetic virus-like particle (SVLP). In a preferred embodiment, all of said at least one carrier is a synthetic virus-like particle (SVLP). In a preferred embodiment, each of said SVLP is the same. In a preferred embodiment, each of said SVLP is identical. In another preferred embodiment, each of said SVLP is different.

Synthetic virus-like particles (SVLPs), in particular, SVLP-based vaccine candidates using synthetic lipopeptide building blocks to which antigens are conjugated and which typically spontaneously self-assemble into highly immunogenic nanoparticles are known by the skilled person (A. Ghasparian, *et al.*, ChemBioChem 2011, 12, 100-109; T. Riedel, *et al.*, ChemBioChem 2011, 12, 2829; R. Sharma, *et al.*, PLoS One 2012, 7, e43248; WO2008/068017; WO2015/082501; WO2020/127728). Said nanoparticles and SVLPs are typically and preferably, have a size of 10-40 nm, typically and preferably as measured by DLS and as described herein, and comprise preferably about 50-90 or 60-90 copies of said conjugates. Since said antigens are displayed across the outer surface of such nanoparticles and SVLPs, strong immune responses against the antigens, in particular, B cell responses are induced. Importantly, SVLPs contain no genetic information and cannot replicate in cells. Typically, said lipopeptide building blocks comprise coiled-coil peptide sequences as well as T-helper cell epitopes.

Thus, and as used herein, a “synthetic virus-like-particle (SVLP)” typically and preferably refers to an assembly of components, typically arranged in a repetitive fashion, which bear multiple copies of an antigen such as the pneumococcal antigens, pneumococcal peptide epitopes in accordance with the present invention for display and, typically and preferably, delivery to immunocompetent cells.

In a further preferred embodiment, said composition further comprises at least one carrier,

wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said at least one carrier, either directly or by way of a linker, wherein said at least one carrier is a synthetic virus-like particle (SVLP).

In a further preferred embodiment, at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to said at least one carrier independently either directly or by way of a linker, wherein preferably said at least one carrier is a synthetic virus-like particle (SVLP).

In a further preferred embodiment, at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is covalently linked to said at least one carrier independently either directly or by way of a linker, wherein preferably said at least one carrier is a synthetic virus-like particle (SVLP).

In a preferred embodiment, said carrier is a synthetic virus-like particle (SVLP), wherein said synthetic virus-like particle (SVLP) comprises at least one conjugate, wherein said conjugate comprises a lipopeptide building block (LBB) and at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

In a further preferred embodiment, said composition further comprises two carriers, wherein two of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to one of said carriers, and wherein the other of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to the other carrier, wherein preferably said two carriers are synthetic virus-like particles (SVLP).

In a further preferred embodiment, said composition further comprises three carriers, wherein each of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to one of said three carriers, wherein preferably said three carriers are synthetic virus-like particles (SVLP).

In a further preferred embodiment, said composition further comprises one carrier, wherein said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said one carrier, wherein preferably said one carrier is a synthetic virus-like particle (SVLP).

In a preferred embodiment, said composition further comprises a first carrier, a second carrier, and a third carrier. In a preferred embodiment, said first carrier, said second carrier, and said third carrier is a synthetic virus-like particle (SVLP). In a preferred embodiment, said composition further comprises a bi-epitope carrier, and a single third carrier. In a preferred embodiment, said bi-epitope carrier and a single third carrier is a synthetic virus-like particle (SVLP).

In a preferred embodiment, said synthetic virus-like particle (SVLP) comprises at least one conjugate, and wherein said conjugate comprises a lipopeptide building block (LBB) and at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked, either directly or via a linker, to said lipopeptide building block.

In a preferred embodiment, said synthetic virus-like particle (SVLP) comprises at least one conjugate, and wherein said conjugate comprises a lipopeptide building block (LBB) and at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked, either directly or via a linker, to said peptide moiety.

In a preferred embodiment, said synthetic virus-like particle (SVLP) comprises at least one conjugate, and wherein said conjugate comprises a lipopeptide building block (LBB) and at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked at one end to said lipid moiety, either directly or via a coupling moiety, and at the other end to said first pneumococcal antigen, said second pneumococcal antigen and/or said third pneumococcal antigen, either directly or via a linker.

In a preferred embodiment, said composition comprises

(a) a first carrier, wherein said first pneumococcal antigen are linked to said first carrier, either directly or via a linker;

(b) a second carrier, wherein said second pneumococcal antigen are linked to said second carrier, either directly or via a linker; and

(c) a third carrier, wherein said third pneumococcal antigen are linked to said third carrier, either directly or via a linker.

In a further preferred embodiment, said composition comprises

(a) a first synthetic virus-like particle (SVLP) as said first carrier, wherein said first SVLP comprises at least one first conjugate, wherein said first conjugate comprises a first lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said first lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker;

(b) a second synthetic virus-like particle (SVLP) as said second carrier, wherein said second SVLP comprises at least one second conjugate, wherein said second conjugate comprises a second lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said second lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said second pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker; and

(c) a third synthetic virus-like particle (SVLP) as said third carrier, wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

In a further preferred embodiment, said composition comprises

(a) a first synthetic virus-like particle (SVLP), wherein said first SVLP comprises at least one first conjugate, wherein said first conjugate comprises a first lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said first lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker;

(b) a second synthetic virus-like particle (SVLP), wherein said second SVLP comprises at least one second conjugate, wherein said second conjugate comprises a second lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said second lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said second pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker; and

(c) a third synthetic virus-like particle (SVLP), wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

In a preferred embodiment, said first LBB, said second LBB and said third LBB are the same. In a preferred embodiment, said first LBB, said second LBB and said third LBB are identical. In another preferred embodiment, said first LBB, said second LBB and said third LBB are different. In another preferred embodiment, at least one of said first LBB, said LBB and said third LBB is different than the other. In a further preferred embodiment, said first lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said first lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said first lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said second lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said second lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said second lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5.** In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ

ID NO:172. In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

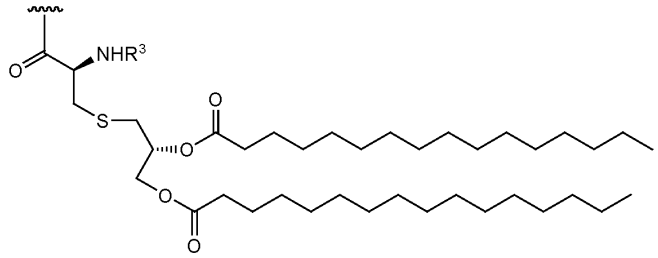
(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), preferably said third SVLP, wherein said single epitope SVLP carrier, preferably said third SVLP, comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first

pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap "a", and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.In a further preferred embodiment, said composition comprises

(a) a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) a single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), preferably said third synthetic virus-like particle (SVLP), wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third

lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

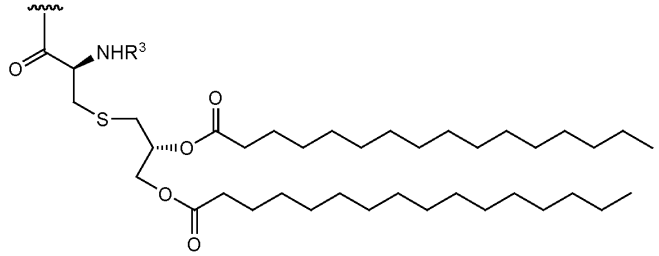
(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

In a preferred embodiment, said bi-epitope LBB and said single epitope LBB are the same. In a preferred embodiment, said bi-epitope LBB and said single epitope LBB are identical. In another preferred embodiment, said bi-epitope LBB and said single epitope LBB are different. In a preferred embodiment, said bi-epitope LBB and said third LBB are the same. In a preferred embodiment, said bi-epitope LBB and said third LBB are identical. In another preferred embodiment, said bi-epitope LBB and said third LBB are different. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably H or $-C(O)C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide

epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap “a”, and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists

of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.The preferred synthetic virus-like particles (SVLPs) are typically produced by a self-assembly process, e.g. in aqueous solution. This method may involve dissolving the lipopeptide building block 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. SVLPs are, inter alia, disclosed in WO 2008/068017 and Ghasparian A et al., ChemBioChem 2011, 12, 100-109, the disclosure of which is incorporated by reference herein. The conjugates as herein described will typically and preferably self-assemble in aqueous solutions to helical lipopeptide bundles (HLB) and further to synthetic virus-like particles (SVLP). The self-assembly process includes (1) the oligomerization of the coiled-coil domains of peptide moiety of the lipopeptide building block, to form a bundle, preferably a helical lipopeptide bundle (HLB), more preferably a parallel helical lipopeptide bundle of a defined oligomerization state, preferably a trimeric bundle; and (2) the oligomerization of the bundles to an SVLP. As a result of step (1), the lipid moieties attached to the peptide chains within each HLB also self-assemble at one end of the bundle, and multiple copies of the pneumococcal antigens of the invention are presented on the surface of the HLB or SVLP, i.e. the other, non-lipophilic end of the bundle or SVLP. The HLB can self-assemble, resulting in the formation of SVLP. The process is driven by the self-association of the lipid tails attached to each building block, which then occupy the central lipid core of the SVLP. In this way, the peptide chains in each helical bundle are oriented outwards, towards the bulk solvent. The size and composition of the SVLP thus determines the final size and shape of the SVLPs, the diameters of which are typically in the nanometer range (10-40 nm).

In some embodiments, the SVLP have a diameter of less than 100 nm, preferably of less than 50 nm. In another more preferred embodiment, said SVLP have a diameter between 10 nm and 40 nm, e.g. between about 15 nm and about 40 nm. Preferably, the diameter is measured via Dynamic Light Scattering (DLS) and transmission electron microscopy (as described in WO 2008/068017, Ghasparian A et al., 2011), preferably by DLS and typically and preferably as described herein. Preferably, said SVLP of the invention are monodisperse particles. Preferably, said SVLP shows a polydispersity index of between 0.05 and 0.30. Typically and preferably, the SVLP of the invention comprises about 30-150, preferably 60-90, copies of the SVLP conjugate.

Typically and preferably, the SVLPs in accordance with the present invention are composed of protein and lipid components, as found in real viruses, and have physical

dimensions resembling those of viruses, but are completely of synthetic origin, i.e. are produced by chemical synthesis, and contain no genetic information and cannot replicate in cells. It is believed that the presentation of multiple copies of the pneumococcal antigens on the surface of the SVLP enhances B-cell receptor affinity to the pneumococcal antigens through an avidity effect and facilitates uptake and presentation of the particle pneumococcal antigens by immunocompetent cells. Thus, the SVLPs are, therefore, be viewed as carriers and/or delivery vehicles for the pneumococcal antigens, in particular for the purpose of raising efficient immune responses against said pneumococcal antigens in an animal. Typically, the lipid chains of the SVLP conjugates are buried in the core of the SVLP, while the pneumococcal antigens of the invention are exposed on the SVLP surface.

The conjugates forming the SVLPs are designed in such a way that the coiled-coil domain in the lipopeptide will assemble to a defined helical bundle (e.g. dimeric, trimeric, tetrameric, pentameric, hexameric or heptameric bundle of helices, preferably a trimeric bundle of helices). This association leads to the formation of the helical lipopeptide bundles, which further, typically, self-assemble into a synthetic virus-like particle (SVLP) with dimensions as described. Preferably, said coiled coil peptide chain segments of said peptide moieties of said conjugate form in said bundle a left-handed alpha-helical coiled coil, wherein the coiled coil peptide chain segments have a parallel orientation in said coiled coil. In a preferred embodiment, said SVLP is formed by self-assembly of trimeric bundles of SVLP conjugates, i.e. each bundle consists of three conjugates. Preferably, said SVLP has a diameter of 20-40, preferably, of 25-35 nm, typically and preferably as determined by DLS and as described herein. In a preferred embodiment, said SVLP comprises about 60-90 copies of the SVLP conjugate. Preferably, said SVLP may shows a polydispersity index of about 0.05-0.3.

In a further aspect, the present invention provides for a method for producing an SVLP of the invention comprising the steps of:

(a) providing a lipid building block with at least one coupling moiety;

(b) providing at least one pneumococcal antigen of the invention comprising a pneumococcal peptide epitope;

(c) forming a conjugate by covalently binding said lipid building block to said pneumococcal antigen, either directly or via a linker,

d) forming a SVLP by self-assembling of said conjugates;

wherein preferably said pneumococcal antigens are displayed in an ordered and repetitive way on the surface of the SVLP.

Preferably, for obtaining a mixture of different single epitope SVLPs, equal amounts

from each of the desired different single epitope SVLPs are combined leading to an inventive composition. In a further preferred embodiment, and for obtaining an inventive composition comprising a mixture of SVLPs comprising said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen, conjugates comprising said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are mixed, typically and preferably, in an equimolar ratio with respect to said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen.

Peptide moiety

The lipopeptide building blocks (LBBs) as referred herein comprises a peptide moiety. (which is herein also referred to and abbreviated as **PM**). Said peptide moieties can be same or can be different in the LBBs referred herein, be it for said first LBB, said second LBB, said third LBB, or said multi-epitope lipopeptide building block (LBB) and/or for any other LBB. The below described embodiments are applicable for any LBB as used in the present invention, except where referred to and explicitly described differently.

In one embodiment, said peptide moiety comprises more than one coiled coil peptide chain segment. Coiled coil peptide chain segments contain multiple repeat units, preferably consecutively linked to each other. The repeat units of the coiled coil peptide chain segment may be identical or may be different, e.g. may contain at least one discontinuity, such as an insertion, deletion or exchange of at least one, preferably exactly 1, 2, 3 or 4 amino acids within the repeat unit. In a preferred embodiment, said coiled coil peptide chain segment of said peptide moiety consists of 2 to 10 repeat units including 2, 3, 4, 5, 6, 7, 8, 9 and 10 repeat units, preferably 3 to 8 repeat units including 3, 4, 5, 6, 7, 8 repeat units, more preferably four repeat units.

In a preferred embodiment, said coiled coil peptide chain segment of said peptide moiety consists of 3 to 8 repeat units including 3, 4, 5, 6, 7, 8 repeat units. In a preferred embodiment, said coiled coil peptide chain segment of said peptide moiety comprises or preferably consists of 4 repeat units. In a most preferred embodiment, said coiled coil peptide chain segment of said peptide moiety consists of 4 repeat units. In a preferred embodiment, said 4 repeat units are consecutively linked to each other. Coiled coil peptide chain segments of the invention are based on canonical repeat units, typically and preferably canonical tandem heptad repeats that form right-handed amphipathic alpha-helices, which then assemble to form helical bundles with left-handed coiled coils. Design rules are discussed in more detail, for example, in Woolfson,

D.N., Adv. Prot. Chem. 2005, 70, 79-112.

In a preferred embodiment, said repeat unit of the coiled coil peptide chain segments consists of seven amino acids (a, b, c, d, e, f and g). Preferably, in the coiled-coil peptide chain segment, 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; wherein none, one or two of all the a and d positions comprise an amino acid with a polar non-charged residue; and wherein none or one of all the a and d positions comprise an amino acid with a polar cationic residue or an acylated derivative thereof, or with a polar anionic residue, or glycine. Preferably, 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.

In a preferred embodiment, said heptad motif consist of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid. In a preferred embodiment, said repeat unit consists of the sequence IEKKIE-X0, wherein X0 represents an amino acid provided that said X0 is not proline. In another preferred embodiment, said repeat unit consists of the sequence IEKKIE-X0, wherein X0 represents an amino acid, wherein said amino acid is a naturally occurring amino acid, wherein said naturally occurring amino acid is in its L-configuration, in its D-configuration, or in a mixture of any ratio thereof, provided that said amino acid is not proline. In another preferred embodiment, said repeat unit consists of the sequence IEKKIE-X0, wherein X0 represents an amino acid, wherein said amino acid is a naturally occurring amino acid in its L-configuration.

In a preferred embodiment, said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176). In a preferred embodiment, said repeat unit consists of the sequence selected from IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176). In a preferred embodiment, said repeat unit consists of the sequence IEKKIEA (SEQ ID NO:175). In a very preferred embodiment, said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176).

In a preferred embodiment, said coiled coil peptide chain segment comprises, or preferably consists of, the sequence selected from $(IEKKIEG)_4$ (SEQ ID NO:177), $(IEKKIEA)_4$ (SEQ ID NO:178) or $(IEKKIES)_4$ (SEQ ID NO:179). In a preferred embodiment, said coiled coil peptide chain segment consists of the sequence selected from $(IEKKIEG)_4$ (SEQ ID

NO:177), $(IEKKIEA)_4$ (SEQ ID NO:178) or $(IEKKIES)_4$ (SEQ ID NO:179). In a very preferred embodiment, said coiled coil peptide chain segment comprises, or preferably consists of, the sequence SEQ ID NO:178 or SEQ ID NO:179. In a very preferred embodiment, said coiled coil peptide chain segment of the peptide moiety consists of the sequence SEQ ID NO:178 or SEQ ID NO:179. In a very preferred embodiment, said coiled coil peptide chain segment comprises, or preferably consists of, the sequence $(IEKKIES)_4$ (SEQ ID NO:179). In a very preferred embodiment, said coiled coil peptide chain segment of the peptide moiety consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179).

In a preferred embodiment, said peptide moiety comprises a coiled coil peptide chain segment and further a T-helper cell epitope. In a preferred embodiment, said peptide moiety further comprises a T-helper cell epitope, wherein said T-helper cell epitope comprises or preferably consists of a sequence selected from the group consisting of (i) SEQ ID NO:180 to SEQ ID NO:216 and (ii) SEQ ID NO:180 to SEQ ID NO:216, wherein one, two, or three amino acids are replaced by other amino acids or are deleted. In a preferred embodiment, said peptide moiety further comprises a T-helper cell epitope, wherein said T-helper cell epitope consists of a sequence selected from the group consisting of (i) SEQ ID NO:180 to SEQ ID NO:216 and (ii) SEQ ID NO:180 to SEQ ID NO:216, wherein one, two, or three amino acids are replaced by other amino acids or are deleted. In a preferred embodiment, said peptide moiety further comprises a T-helper cell epitope, wherein said T-helper cell epitope comprises a sequence selected from the group consisting of SEQ ID NO:180 to SEQ ID NO:216. In a preferred embodiment, said T-helper cell epitope consists of a sequence selected from the group consisting of SEQ ID NO:180 to SEQ ID NO:216.

In one embodiment, said peptide moiety further comprises 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. Suitable T-helper cell epitopes are known to the skilled person in the art and are described, e.g., in Weber *et al.,* Advanced Drug Delivery Reviews, 2009, 61:11, 965-976; Caro-Aguilar e*t al.*, Infect. Immun., 2002, 70:7, 3479-3492; Mishra *et al.,* Immunology, 1993, 79:3, 362-367; Kobayashi *et al.,* Cancer Research, 2000, 60:18, 5228-523; Fraser *et al.,* Vaccine, 2014, 32:24, 2896-2903; Grabowska *et al.,* Int. J. Cancer, 2015, 136:1, 212–224 and WO1998/023635A1. More preferred T-helper cell epitopes included in the peptide moiety are those listed in WO 2015/082501 such TT830-843, TT1064-1079, TT1084-1099, TT947-968, TT1174-1189, DTD271-290, DTD321-340, DTD331-350, DTD351-370, DTD411-430, DTD431-450, TT632-651, CTMOMP36-60, TraT1, TraT2, TraT3, HbcAg50-69, HbSAg19-33, HA307-319, MA17-31, MVF258-277, MVF288-302,

CS.T3, SM Th, PADRE1 and PADRE2 as well as variants thereof in which one, two, or three amino acids are inserted, replaced by other amino acids or deleted.

Preferred T-helper epitopes that can be incorporated into said peptide moiety are any one selected from the group 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 cell epitopes

T-helper cell epitope	SEQ ID NO:	Sequence [a)]
CS.T3	180	IEKKIAKMEKASSVFNVVNS
	181	EKKIAKMEKASSVFNV
	182	EKKIAKMEKASSVFNVV
	183	EKKIAKMEKASSVFNVVN
	184	EKKIAKMEKASSVFNVVNS
	185	DIEKKIAKMEKASSVFNVVN
	186	DIEKKIAKMEKASSVFNV
	187	DIEKKIAKMEKASSVFNVV
	188	DIEKKIAKMEKASSVFNVVN
	189	IEKKIAKMEKASSVFNV
	190	IEKKIAKMEKASSVFNVV
	191	IEKKIAKMEKASSVFNVVN
TT830-843	192	QYIKANSKFIGITE
TT1064-1079	193	IREDNNITLKLDRCNN
TT1084-1099	194	VSIDKFRIFCKANPK
TT947-968	195	FNNFTVSFWLRVPKVSASHLET
TT1174-1189	196	LKFIIKRYTPNNEIDS
DTD271–290	197	PVFAGANYAAWAVNVAQVID
DTD321–340	198	VHHNTEEIVAQSIALSSLMV
DTD331–350	199	QSIALSSLMVAQAIPLVGEL
DTD351–370	200	VDIGFAAYNFVESIINLFQV
DTD411–430	201	QGESGHDIKITAENTPLPIA
DTD431–450	202	GVLLPTIPGKLDVNKSKTHI
TT632-651	203	TIDKISDVSTIVPYIGPALN
CTMOMP36-60	204	ALNIWDRFDVFCTLGATTGYLKGNS
TraT1	205	GLQGKIADAVKAKG
TraT2	206	GLAAGLVGMAADAMVEDVN
TraT3	207	STETGNQHHYQTRVVSNANK
HbcAg50-69	208	PHHTALRQAILCWGELMTLA

HbSAg19-33	209	FFLLTRILTIPQSLD
HA307-319	210	PKYVKQNTLKLAT
MA17-31	211	YSGPLKAEIAQRLEDV
MVF258-277	212	GILESRGIKARITHVDTESY
MVF288-302	213	LSEIKGVIVHRLEGV
SM Th	214	KWFKTNAPNGVDEKIRI
PADRE1 [b)]	215	aKFVAAWTLKAAa
PADRE2 [b)]	216	aK-Chx-VAAWTLKAAa
[a)] References: *Eur. J. Immunol.* **2001,** *31,* 3816–3824; *JID* **2000**, *181,* 1001-1009; US 5,759,551; *Nature* **1988**, *336*, 778-780; *Immunity* **1994**, *1*, 751–761. [b)] „a“ denotes D-Ala and „Chx“ denotes cyclohexylalanine.		

In a very preferred embodiment, said peptide moiety further comprises a T-helper cell epitope, wherein said T-helper cell epitope comprises or preferably consists of the amino acid sequence of SEQ ID NO:180. In a preferred embodiment, said peptide moiety further comprises a T-helper cell epitope, wherein said T-helper cell epitope consists of the amino acid sequence of SEQ ID NO:180.

In a preferred embodiment, said peptide moiety has a length of 12 to 200 amino acids. In a preferred embodiment, said peptide moiety has a length of 21 to 120 amino acids. In a preferred embodiment, said peptide moiety has a length of 21 to 80 amino acids. In a preferred embodiment, said peptide moiety has a length of 21 to 70 amino acids. In a preferred embodiment, said peptide moiety has a length of 21 to 60 amino acids. In a preferred embodiment, said peptide moiety has a length of 21 to 50 amino acids. In a preferred embodiment, said peptide moiety has a length of 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 49 or 50 amino acids. In a preferred embodiment, said peptide moiety has a length of 28 to 48 amino acids. In a preferred embodiment, said peptide moiety is a non-human sequence, typically and preferably to avoid the risk of autoimmune disorders when applied in the vaccination of humans.

In a further preferred embodiment, the last C terminal amino acid of said peptide moiety is a D-amino acid, preferably D-Ala. Preferably, the C-terminus is either an amide ($CONH_2$) or a free carboxyl terminus, more preferably $CONH_2$.

In a further preferred embodiment, said peptide moiety comprises (i) an N-terminal amino acid sequence, wherein said N-terminal amino acid sequence comprises or preferably consists of fibroblast-stimulating lipopeptide FSL-1 (S-(2,3-bispalmitoyloxypropyl)- or PAM2-CGDPKHPKSF; SEQ ID NO:217), FSL-2 (S-(2,3-bispalmitoyloxypropyl)- or PAM2-

CGDPKHPKSR, SEQ ID NO:218) or FSL-3 (S-(2,3-bisstearyloxypropyl)-CGDPKHPKSF; SEQ ID NO:217), Mycoplasma fermentans-derived peptide MALP-2 (S-(2,3-bispalmitoyloxypropyl)- or PAM2-CGNNDESNISFKEK; SEQ ID NO:219), GG, and/or GX, wherein X is Asx or Ser and/or (ii) a C-terminal amino acid sequence, wherein said C-terminal amino acid sequence comprises or preferably consists of a sequence recognized by an enzyme as cleavage site. Preferably said C-terminal amino acid sequence is an amino acid sequence of 5 consecutive amino acids. More preferably, said C-terminal amino acid sequence comprises or preferably consists of sequence KKKCa (SEQ ID NO:220).

In a very preferred embodiment, said peptide moiety comprises (i) an N-terminal amino acid sequence, wherein said N-terminal amino acid sequence comprises or preferably consists of GG and/or GX where X is Asx or Ser and/or (ii) a C-terminal amino acid sequence, wherein said C-terminal amino acid sequence comprises or preferably consists of sequence KKKCa (SEQ ID NO:220). In a more preferred embodiment, said peptide moiety comprises (i) an N-terminal amino acid sequence, wherein said N-terminal amino acid sequence comprises or preferably consists of GG and (ii) a C-terminal amino acid sequence, wherein said C-terminal amino acid sequence comprises or preferably consists of sequence KKKCa (SEQ ID NO:220). In an even more preferred embodiment, said peptide moiety comprises (i) an N-terminal amino acid sequence, wherein said N-terminal amino acid sequence consists of GG and (ii) a C-terminal amino acid sequence, wherein said C-terminal amino acid sequence consists of sequence KKKCa (SEQ ID NO:220).

In a very preferred embodiment, said peptide moiety comprises or preferably consists of a sequence selected from the group consisting of

SEQ ID NO:221: $GG(IEKKIEA)_4IEKKIAKMEKASSVFNVVNSKKKCa$,

SEQ ID NO:222: $GG(IEKKIEA)_4IEKKIAKMEKASSVFNVVNSKKKCa\text{-}NH_2$,

SEQ ID NO:223: $GG(IEKKIES)_4IEKKIAKMEKASSVFNVVNSKKKCa$,

SEQ ID NO:224: $GG(IEKKIES)_4IEKKIAKMEKASSVFNVVNSKKKCa\text{-}NH_2$,

SEQ ID NO:225: $GG(IEKKIEA)_4IEKKIAKMEKASSVFNVVNSKKKC$, and

SEQ ID NO:226: $GG(IEKKIES)_4IEKKIAKMEKASSVFNVVNSKKKC$.

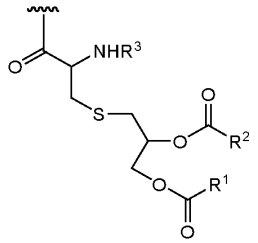
In another very preferred embodiment, said peptide moiety comprises or preferably consists of SEQ ID NO:223, SEQ ID NO:224 or SEQ ID NO:226.

In an even more preferred embodiment, said peptide moiety comprises or preferably consists of (SEQ ID NO:224): $GG(IEKKIES)_4IEKKIAKMEKASSVFNVVNSKKKCa\text{-}NH_2$.

Lipid moiety

The lipopeptide building blocks (LBBs) as referred herein comprises a lipid moiety. (which is herein also referred to and abbreviated as **LM**). Said lipid moieties can be same or can be different in the LBBs referred herein, be it for said first LBB, said second LBB, said third LBB, said bi-epitope lipopeptide building block (LBB) and/or for any other LBB. The below described embodiments are applicable for any LBB as used in the present invention, except where referred to and explicitly described differently.

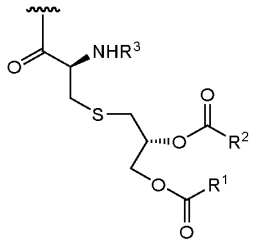
In a preferred embodiment, said lipid moiety comprises, preferably consisting of, the formula LM-I



LM-I

wherein R^1 and R^2 are independently C_{11-15}alkyl, preferably R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and further preferably R^1 and R^2 are $-C_{15}H_{31}$; and R^3 is hydrogen or $-C(O)C_{11-15}$alkyl, preferably R^3 is H or $-C(O)C_{15}H_{31}$, wherein the wavy line in formula **LM-I** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

In another preferred embodiment, said lipid moiety comprises preferably consists of, the formula LM-II



LM-II

wherein R^1 and R^2 are independently C_{11-15}alkyl, preferably R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and further preferably R^1 and R^2 are $-C_{15}H_{31}$; and R^3 is hydrogen or $-C(O)C_{11-15}$alkyl, preferably R^3 is H or $-C(O)C_{15}H_{31}$, wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety

Lipopeptide building blocks comprising Pam_2Cys or Pam_3Cys moieties with the (R)-

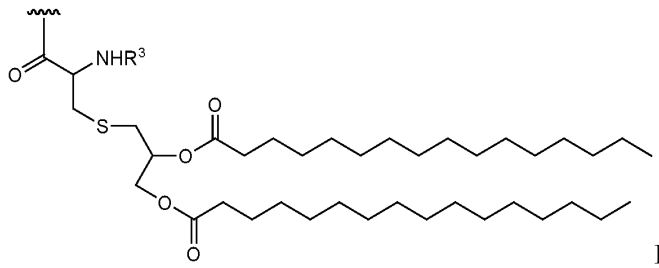
configuration at the 2-propyl carbon atom and further comprising as coiled coil peptide chain segment several units of the sequence IEKKIE-X0 are most preferred, with X0 being Gly, Ala or Ser, most preferably X0 is Ser.

In a preferred embodiment, said R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$. In a very preferred embodiment, said R^1 and R^2 are $-C_{15}H_{31}$. In a preferred embodiment, said R^3 is H or $-C(O)C_{15}H_{31}$. In a preferred embodiment, said R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl. In a very preferred embodiment, said R^1 and R^2 are $-C_{15}H_{31}$, and R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl. In a preferred embodiment, said R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and R^3 is H or $-C(O)C_{15}H_{31}$. In a very preferred embodiment, said R^1 and R^2 are $-C_{15}H_{31}$, and R^3 is H or $-C(O)C_{15}H_{31}$.

In a preferred embodiment, said lipid moiety is linked to the N-terminus of said peptide moiety. This conveniently allows that said linking can be performed on-resin after assembly of the peptide chain of said peptide moiety by solid phase peptide synthesis. Linking of said lipid moiety to the C-terminus of said peptide moiety is also encompassed within the present invention and is possible using linkage chemistry known by the skilled person in the art.

A preferred lipid moiety is di-palmitoyl-S-glycerylcysteinyl (Pam_2Cys) or tripalmitoyl-S-glyceryl cysteine (Pam_3Cys), more preferably, Pam_2Cys. More preferably, Pam_2Cys or Pam_3Cys are both with the *R*-configuration at the chiral 2-propyl carbon atom and the *R*-configuration of the chiral carbon of the cysteinyl moiety.

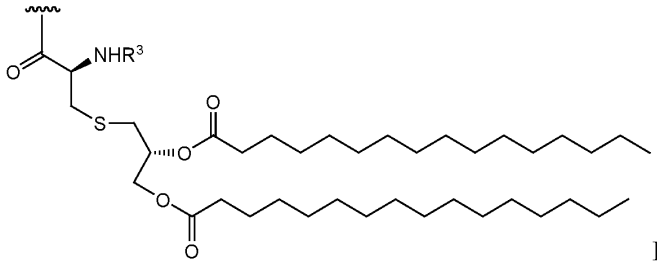
In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I***



LM-I*

wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably H or $-C(O)C_{15}H_{31}$; wherein the wavy line in formula **LM-I*** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-II***



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; wherein the wavy line in formula **LM-II*** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

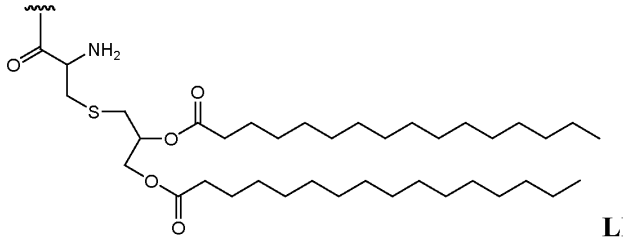
In a preferred embodiment, said lipid moiety consists of the formula **LM-I*** or **LM-II***, wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl. In a preferred embodiment, said lipid moiety consists of the formula **LM-I***, wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl. In a preferred embodiment, said lipid moiety consists of the formula **LM-I***, wherein R^3 is hydrogen. In a preferred embodiment, said lipid moiety consists of the formula **LM-I***, wherein R^3 is -C(O)C_{11-15}alkyl. In a preferred embodiment, said lipid moiety consists of the formula **LM-II***, wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl. In a preferred embodiment, said lipid moiety consists of the formula **LM-II***, wherein R^3 is hydrogen. In a preferred embodiment, said lipid moiety consists of the formula **LM-II***, wherein R^3 is -C(O)C_{11-15}alkyl.

In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I*** or **LM-II***, wherein R^3 is H or -C(O)$C_{15}H_{31}$. In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I*** or **LM-II***, wherein R^3 is H or -C(O)$C_{15}H_{31}$ and wherein said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-I*** or **LM-II***, wherein R^3 is H or -C(O)$C_{15}H_{31}$ and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-I***, wherein R^3 is H or -C(O)$C_{15}H_{31}$ and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-I***, wherein R^3 is H and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-I***, wherein R^3 is -C(O)$C_{15}H_{31}$ and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-II***, wherein R^3 is H or -C(O)$C_{15}H_{31}$ and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-II***, wherein R^3 is H and wherein

preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a preferred embodiment, said lipid moiety consists of, the formula **LM-II***, wherein R^3 is $-C(O)C_{15}H_{31}$ and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

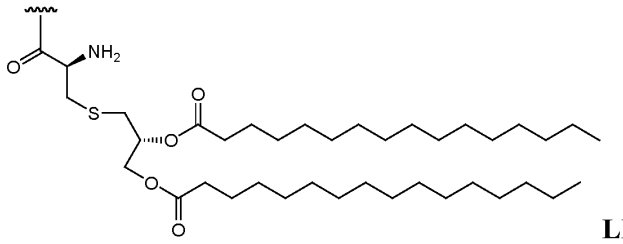
In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I*1**, **LM-I*2, LM-II*1** or **LM-II*2**. In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I*1**. In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-I*2**. In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-II*1**. In a preferred embodiment, said lipid moiety comprises, preferably consists of, the formula **LM-II*2**. In another preferred embodiment, said lipid moiety consists of the formula **LM-I*1**, **LM-I*2, LM-II*1** or **LM-II*2**.

In a preferred embodiment, said lipid moiety consists of the formula **LM-I*1**.



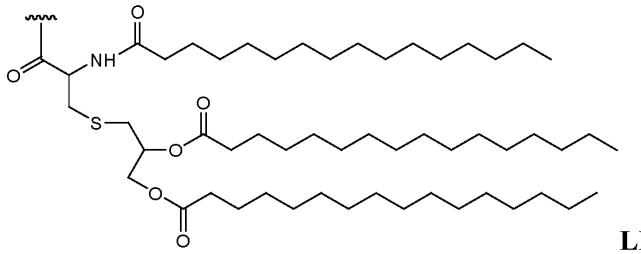
LM-I*1.

In a very preferred embodiment, said lipid moiety consists of the formula **LM-II*1**.



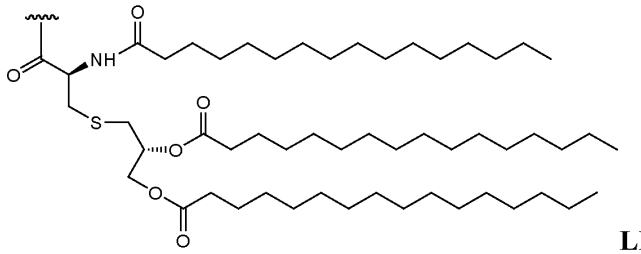
LM-II*1.

In a preferred embodiment, said lipid moiety consists of the formula **LM-I*2**.



LM-I*2.

In another preferred embodiment, said lipid moiety consists of the formula **LM-II*2**.



LM-II*2.

Thus, very preferred lipid moieties of the present invention are Pam_3Cys **LM-II*2**, i.e. tripalmitoyl-S-glyceryl cysteine (N-palmitoyl-S-[(2,3-bis-(O-palmitoyloxy)-(2-propyl)]-cysteinyl-) and Pam_2Cys **LM-II*1**, i.e. dipalmitoyl-S-glyceryl cysteine (S-[2,3-bis-(O-palmitoyloxy)-(2-propyl)]-cysteinyl-). In a very preferred embodiment, said lipid moiety is N-α-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2-propyl)]-cysteine or S-[2,3-bis(palmitoyloxy)-(2-propyl)]-cysteine, thus **LM-II*1**.

The lipid moiety is linked to the peptide moiety, either directly or via a coupling moiety. Preferably, the lipid moiety is linked to the peptide moiety at or near one terminus, i.e. the N-terminus or the C-terminus, preferably the N-terminus. In a preferred embodiment, the lipid moiety is linked to the first, second, third, fourth or fifth amino acid of the peptide moiety, calculated from the N-terminus or C-terminus of the peptide moiety. The lipid moiety may be linked, directly or through a coupling moiety, to the backbone or to the side chain of one of the amino acids of the peptide moiety, preferably said amino acid is near to the terminus, more preferably it is the first, second, third, fourth or fifth amino acid of the peptide moiety.

The lipid moiety is attached to the peptide moiety directly, or via a coupling moiety. If the peptide moiety and the lipid moiety 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 moiety. It will be apparent to the skilled person in the art that a large variety of suitable coupling moieties 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 alpha-, beta-, gamma-, omega-amino acids), or sugar (carbohydrate) units, or containing heterocyclic rings.

In a preferred embodiment, said lipid moiety and said peptide moiety are directly linked. In a preferred embodiment, said lipid moiety and said peptide moiety are linked via a coupling moiety. In a preferred embodiment, said lipid moiety and said peptide moiety are linked via a coupling moiety, wherein said coupling moiety is an amino acid linker consisting of 2-15 amino acids. Examples hereto include the amino acid linker sequences comprised by FSL-1, FSL-2, FSL-3, PAM2 or MALP-2 moieties. In a preferred embodiment, said lipid moiety and said

peptide moiety are linked via a coupling moiety, wherein said coupling moiety is an amino acid linker consisting of 2-10 amino acids. In a preferred embodiment, said lipid moiety and said peptide moiety are linked via a coupling moiety, wherein said coupling moiety is an amino acid linker consisting of 2-5 amino acids. In a preferred embodiment, said lipid moiety and said peptide moiety are linked via a coupling moiety, wherein said coupling moiety is an amino acid linker, wherein said amino acid linker is a Gly-Gly moiety. In a preferred embodiment, said lipid moiety and said peptide moiety are linked via a coupling moiety, wherein said coupling moiety is a Gly-Gly moiety.

In a preferred embodiment, said direct linking of said lipid moiety and said peptide moiety is by way of an amide bond between a carbonyl function of said lipid moiety and an amino function of said peptide moiety. In a preferred embodiment, said linking of said lipid moiety and said peptide moiety via said coupling moiety is by way of an amide bond between a carbonyl function of said lipid moiety and an amino function of said coupling moiety. In a preferred embodiment, said linking of said lipid moiety and said peptide moiety via said coupling moiety is by way of an amide bond between a carbonyl function of said lipid moiety and an amino function of said coupling moiety, wherein said coupling moiety is an amino acid linker, preferably consisting of 2-15 amino acids, preferably 2-10 amino acids, and wherein said amino function is the N-terminal amino function of said coupling moiety. In a preferred embodiment, said linking of said lipid moiety and said peptide moiety via said coupling moiety is by way of an amide bond between a carbonyl function of said lipid moiety and an amino function of said coupling moiety, wherein said coupling moiety is an amino acid linker, preferably consisting of 2-5 amino acids, and wherein said amino function is the N-terminal amino function of said coupling moiety. In a preferred embodiment, said direct linking of said lipid moiety and said peptide moiety is by way of an amide bond between a carbonyl function of said lipid moiety and an amino function of said peptide moiety, wherein said amino function is the N-terminal amino function of said peptide moiety.

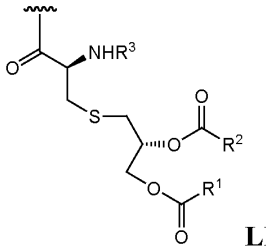
In a preferred embodiment, two Gly residues are included as linker between the lipid moiety, preferably said (*R,R*)-Pam$_2$Cys moiety **LM-I*1** of the present invention and the start of the coiled-coil heptad repeats, typically and preferably the coiled coil peptide chain segment comprising, preferably consisting of, the sequence (IEKKIES)$_4$ (SEQ ID NO:179).

The introduction of an amino acid linker, and preferably a short amino acid linker consisting of two amino acids, preferably glycine, allows during peptide synthesis that after each amino acid coupling, a capping step can be performed with acetic anhydride. This has the practical advantage that after completion of peptide assembly, and coupling of the lipid

moieties, preferably the lipid moieties consisting of the formula **LM-II*1** [(*R,R*)-Pam_2Cys moiety] or formula **LM-II*2** [(*R,R*)-Pam_3Cys moiety] to the free N-terminus, the HPLC retention time of the peptide is dramatically altered by lipidation, thus greatly facilitating HPLC purification of the desired lipopeptide building block.

LBB-4 lipopeptide building block contains a peptide moiety comprising a coiled-coil domain ($(IEKKIES)_4$) (SEQ ID NO:179) with four heptad repeats (IEKKIES) (SEQ ID NO:176). The C-terminus of the peptide moiety is a D-Ala (written "a" in the formula above), with an amide ($CONH_2$) instead of a free carboxyl terminus to improve solubility. The lipid moiety S-[2,3-bis(palmitoyloxy)-(2)-propyl]-cysteine (Pam_2Cys) is conjugated to the N-terminus of the peptide moiety via a GG-linker as coupling moiety.

In a preferred embodiment, said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently $C_{11\text{-}15}$alkyl, preferably wherein R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are $-C_{15}H_{31}$; and wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably wherein R^3 is H or $-C(O)C_{15}H_{31}$; and wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

Lipopeptide building block (LBB)

Specifically preferred lipopeptide building blocks (LBBs) are referred herein such said first LBB, said second LBB, said third LBB, or said bi-epitope lipopeptide building block (LBB). The below described embodiments are applicable for any LBB as used in the present invention, except where referred to and explicitly described differently. The lipopeptide

building blocks (LBBs) as referred herein can be same or can be different, be it for said first LBB, said second LBB, said third LBB, said bi-epitope lipopeptide building block (LBB) and/or for any other LBB. The below described embodiments are applicable for any LBB as used in the present invention, except where referred to and explicitly described.

Lipopeptide building blocks described for the present invention and used therefor have already been disclosed, in particular, in WO 2008/068017 and in WO 2020/127728. In particular, and with reference to the preparation it is hereby referred to Example 1 of WO 2020/127728.

In a preferred embodiment, the lipopeptide building block comprises, preferably consists of:

(i) a peptide moiety comprising a coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), and wherein further preferably said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176);

(ii) a lipid moiety comprising, preferably consisting of, the formula **LM-I** or **LM-II**, wherein R^1 and R^2 are independently $C_{11\text{-}15}$alkyl, wherein preferably R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and wherein R^3 is hydrogen or -C(O)$C_{11\text{-}15}$alkyl, and wherein preferably R^3 is H or -C(O)$C_{15}H_{31}$; and wherein said lipid moiety is linked to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

In a further preferred embodiment, the lipopeptide building block comprises, preferably consists of:

(i) a peptide moiety comprising a coiled coil peptide chain segment, and wherein said coiled coil peptide chain segment comprises, preferably consists of, the sequence of IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176);

(i) a lipid moiety comprising, preferably consisting of, the formula **LM-I** or **LM-II**, wherein R^1 and R^2 are independently $C_{11\text{-}15}$alkyl, wherein preferably R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and wherein R^3 is hydrogen or -C(O)$C_{11\text{-}15}$alkyl, and wherein preferably R^3 is H or -C(O)$C_{15}H_{31}$; and wherein said lipid moiety is linked to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

In a further preferred embodiment, said lipopeptide building block consists of

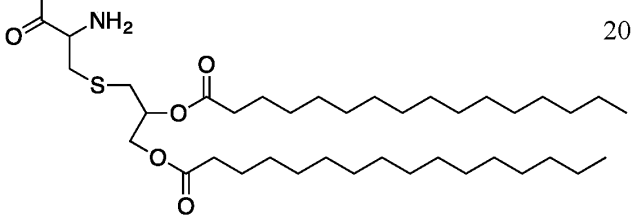
(i) a peptide moiety comprising a coiled coil peptide chain segment, and wherein said coiled coil peptide chain segment comprises, preferably consists of, the sequence of IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176)

(ii) a lipid moiety comprising, preferably consisting of, the formula **LM-II,** wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, wherein preferably R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, and wherein preferably R^3 is H or -C(O)$C_{15}H_{31}$;

and wherein said lipid moiety is linked to said peptide moiety, wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

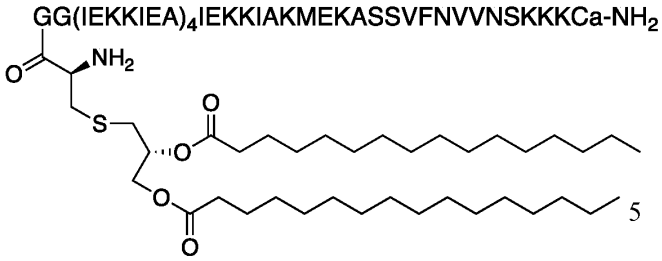
In a further very preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NOs:227-232), wherein preferably, said lipopeptide building block is of the formula **LBB-4**, **LBB-5** or **LBB-6**, again more preferably, wherein said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, and most preferably wherein said lipopeptide building block is of the formula **LBB-5**.

GG(IEKKIEA)$_4$IEKKIAKMEKASSVFNVVNSKKKCa-NH_2 SEQ ID NO:227



LBB-1

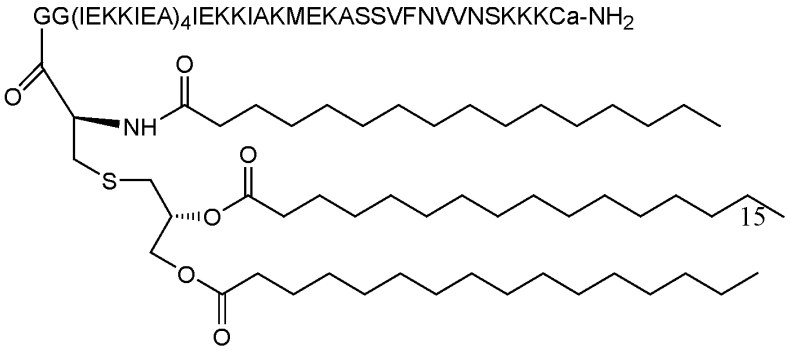
LBB-1 lipopeptide building block contains a peptide moiety comprising a coiled-coil domain ((IEKKIEA)$_4$) (SEQ ID NO:178) with four heptad repeats (IEKKIEA) (SEQ ID NO:175). The C-terminus of the peptide moiety is a D-Ala (written "a" in the formula above), with an amide ($CONH_2$) instead of a free carboxyl terminus to improve solubility. The lipid moiety S-[2,3-bis(palmitoyloxy)-(2)-propyl]-cysteine (Pam_2Cys) is conjugated to the N-terminus of the peptide moiety.



SEQ ID NO:228

LBB-2

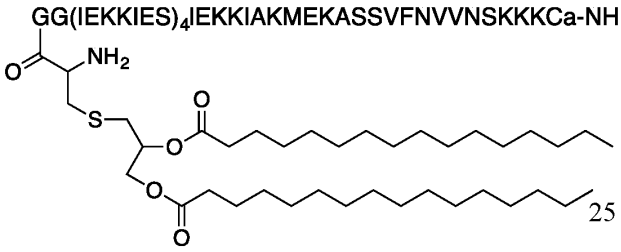
LBB-2 corresponds to **LBB-1** except that the lipid moiety Pam2-Cys has the *R*-configuration at the chiral 2-propyl carbon atom and the *R*-configuration of the chiral carbon of the cysteinyl moiety.



SEQ ID NO:229

LBB-3

LBB-3 corresponds to **LBB-2** except that Pam3-Cys instead of Pam2-Cys is used.

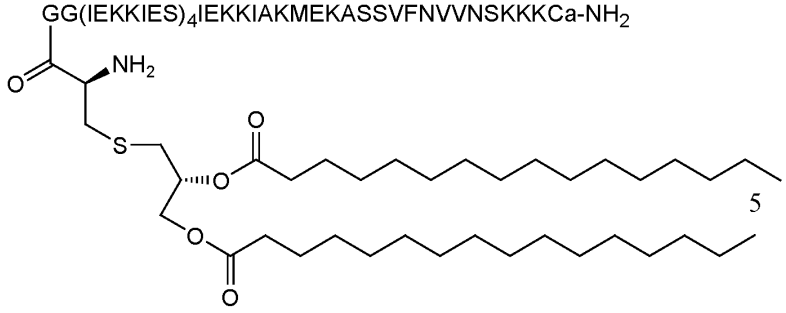


SEQ ID NO:230

LBB-4

LBB-4 lipopeptide building block contains a peptide moiety comprising a coiled-coil domain ((IEKKIES)$_4$) (SEQ ID NO:179) with four heptad repeats (IEKKIES) (SEQ ID NO:176). The C-terminus of the peptide moiety is a D-Ala (written "a" in the formula above), with an amide ($CONH_2$) instead of a free carboxyl terminus to improve solubility. The lipid moiety S-[2,3-bis(palmitoyloxy)-(2)-propyl]-cysteine (Pam_2Cys) is conjugated to the N-terminus of the peptide moiety.

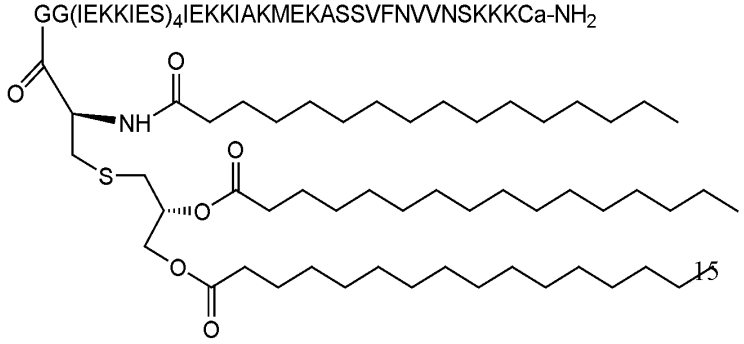
GG(IEKKIES)$_4$IEKKIAKMEKASSVFNVVNSKKKCa-NH$_2$ SEQ ID NO:231



LBB-5

LBB-5 corresponds to **LBB-4** except that the lipid moiety Pam2-Cys has the *R*-configuration at the chiral 2-propyl carbon atom and the *R*-configuration of the chiral carbon of the cysteinyl moiety.

GG(IEKKIES)$_4$IEKKIAKMEKASSVFNVVNSKKKCa-NH$_2$ SEQ ID NO:232



LBB-6

LBB-6 corresponds to **LBB-5** except that Pam3-Cys instead of Pam2-Cys is used.

In a further very preferred embodiment, said lipopeptide building block is of the formula **LBB-4** or **LBB-5**. In an even more preferred embodiment, said lipopeptide building block is of the formula **LBB-5**.

Linker

The said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen are linked to said at least one carrier, either directly or by way of a linker.

Conjugation procedures that may be used to attach the pneumococcal antigens to the lipopeptide building block are known to those skilled in the art (see for example Hermanson, G.T, Bioconjugate Techniques, 2nd edition, Academic Press, 2008). Further linkers suitable for the present invention are listed in the Pierce Catalog and Handbook, Pierce Chemical Company, Rockford (1997); Bioconjugate Techniques, Greg T. Hermanson, Pierce Biotechnology, Thermo Fisher Scientific, Rockford (2013); and are described in EP 1321466 A1, DE 19821859

A1, US 6875737, US 5456911, US 5612036, US 5965532, WO 2001004135, WO 2001070685, US 20140302001 A1, US 6800728, US 20140171619 A1, US 8168190, WO 2012/166594 A1 and WO 2015/082501.

By way of example, preferred embodiments for said linking of said first pneumococcal antigen, said second pneumococcal antigen and/or said third pneumococcal antigen including the bi-epitope or multi-epitope pneumococcal antigens, are described for linking said antigens to said LBB of a SVLP, and in particular to a peptide moiety thereof, either directly or via such said linker.

In a preferred embodiment, free amino groups in the side chains of amino acids in the peptide moiety of the lipopeptide building block are coupled to reactive esters of the pneumococcal antigen (e.g. N-hydroxysuccinimide esters prepared from carboxylic acids). In another preferred embodiment, thiols (as part of the side chain of amino acids such in cysteines) in the peptide moiety are coupled to the pneumococcal antigen by way of maleimide groups comprised in the linker. In another preferred embodiment, azides are incorporated into the side chains of amino acid residues in the peptide moiety and coupled to the pneumococcal antigen or said linker, which then typically and preferably contains acetylene groups using copper catalyzed cycloaddition reactions or non-copper based click chemistry reactions based on alkyne-comprising moieties, typically and preferably cyclooctyne derivatives. Other nucleophiles (e.g. hydrazino, hydroxyl amino, vic-amino thiol groups) in the peptide moiety may be coupled to electrophiles (e.g. aldehydes, ketones, active esters) in the pneumococcal antigen or said linker. It will be obvious that it is possible, in principle, to reverse the positions of the two reactive groups in the peptide moiety and pneumococcal antigen or the linker in order to achieve selective coupling.

The at least pneumococcal antigens are typically linked either to the N- or to the C-terminus of the peptide moiety of the LBB or to one or more amino acid side chains of the peptide moiety, such as the side chain of aspartic acid, glutamic acid, lysine, ornithine or the cysteine side chain.

In a preferred embodiment, said pneumococcal antigens are linked to the LBB via a linker. In a preferred embodiment, said linker is covalently attached to the N terminus of the pneumococcal antigen. In another preferred embodiment, said linker is covalently attached to the N-terminus, typically and preferably the N-terminal amino acid, of the pneumococcal antigen via an amide bond. In a preferred embodiment, said N-terminus of the pneumococcal antigen is connected via said amide bond to said linker which covalently attaches the pneumococcal antigen to an amino acid side chain of the peptide moiety, preferably to a

cysteine side chain via the free thiol of the cysteine. In a preferred embodiment, said linker is covalently attached to a cysteine side chain of the peptide moiety via thiosuccinimide binding.

In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety which comprises, or preferably consists of, -C(O)-$(CH_2)_w$-, wherein w is an integer from 1 to 10, preferably 1 to 5, more preferably 1 to 3, again more preferably 1 or 2, most preferably 1; -O-NH_2, -O-NH- (aminooxy moiety), -C(O)-CH_2-O-NH_2, -C(O)-CH_2-O-NH- (aminooxy acetyl moiety), -NH-NH_2, -NH-NH- (hydrazine moiety); -E(O)-NH-NH_2, or -E(O)-NH-NH- (hydrazide moiety), wherein E is C, S(O) or P. In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety which comprises, or preferably consists of, an -C(O)-$(CH_2)_w$, wherein w is an integer from 1 to 5, preferably 1 to 3, again more preferably 1 or 2, most preferably 1; -O-NH_2, -O-NH- (aminooxy moiety), -C(O)-CH_2-O-NH_2, -C(O)-CH_2-O-NH- (aminooxy acetyl moiety), -NH-NH_2, -NH-NH- (hydrazine moiety), or (-C(O)-NH-NH_2, -C(O)-NH-NH-(carbohydrazide moiety). In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety which comprises, or preferably consists of, -C(O)-$(CH_2)_2$, -O-NH_2 or -O-NH- (an aminooxy moiety). In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety which comprises, or preferably consists of ,an -C(O)-$(CH_2)_w$, wherein w is an integer from 1 to 5, preferably 1 to 3, again more preferably 1 or 2, most preferably 1.

In a preferred embodiment, said linker comprises at least one preferably exactly one second moiety which comprises, or preferably consists of, a polyethylenglycol (PEG) -(O-CH_2-CH_2)$_n$-, -(O-CH_2-CH_2)$_n$-NH-C(O))-$(CH_2)_w$, -(O-CH_2-CH_2)$_n$-$(CH_2)_w$-NH-C(O)-, -$(CH_2)_w$-(O-CH_2-CH_2)$_n$-NH-C(O)-$(CH_2)_w$-, -C(O)-$(CH_2)_w$-(O-CH_2-CH_2)$_n$-NH-C(O)-$(CH_2)_w$-, -$(CH_2)_w$-(O-CH_2-CH_2)$_n$-$(CH_2)_w$-NH-C(O)-$(CH_2)_w$-, -C(O)-CH_2-O-N-CH-CH_2-NH-C(O)-$(CH_2)_w$-(O-CH_2-CH_2)$_n$-NH-C(O)-$(CH_2)_w$-, NH_2-CH_2-CH_2-(O-CH_2-CH_2)$_n$-C(O)- or -NH-CH_2-CH_2-(O-CH_2-CH_2)$_n$-C(O)-, wherein n is an integer of 1 to 45, preferably 2 to 20, more preferably 4 to 8, most preferably 6, and w is an integer from 1 to 10, preferably 1 to 5, more preferably 1 to 3, most preferably 2; or NH_2-$(CH_2)_m$-C(O)- or -NH-$(CH_2)_m$-C(O)-, wherein m is an integer of 2 to 45, preferably 2 to 20, more preferably 2 to 8, most preferably 6. In a preferred embodiment, said linker comprises at least one preferably exactly one second moiety which comprises, or preferably consists of, PEG, -(O-CH_2-CH_2)$_n$-, wherein n is an integer of 1 to 45, preferably 2 to 20, more preferably 4 to 8, most preferably 6. In a preferred embodiment, said linker comprises at least one preferably exactly one second moiety which comprises, or preferably consists of, -(O-CH_2-CH_2)$_n$-NH-C(O))-$(CH_2)_w$, wherein n is an integer of 1 to 45, preferably 2 to 20, more

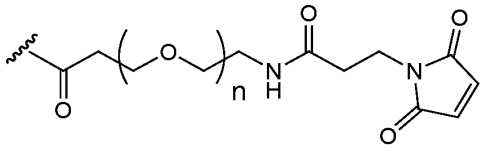
preferably 4 to 8, most preferably 6, and w is an integer from 1 to 10, preferably 1 to 5, more preferably 1 to 3, most preferably 2.

In a preferred embodiment, said linker comprises at least one, preferably exactly one third moiety which comprises, or preferably consists of, a maleimide moiety, an aldehyde moiety, such as a glutaraldehyde moiety, octanedialdehyde moiety, dialdehyde moiety, succinaldehyde moiety; carbodiimide moiety, such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride moiety; glyoxol moiety; N-hydroxy-sulphosuccinimidyl moiety, such as N-hydroxy-sulphosuccinimidyl moiety; a cationic linking moiety; polyethyleneglycol moiety; or benzoyl benzoic acid moiety. In a preferred embodiment, said linker comprises at least one, preferably exactly one third moiety which comprises, or preferably consists of, a maleimide moiety.

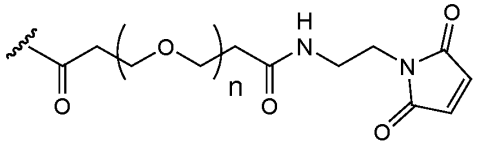
In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety, at least one, preferably exactly one second moiety, and at least one, preferably exactly one third moiety.

In a preferred embodiment, said linker comprises at least one, preferably exactly one first moiety which comprises, or preferably consists of ,an $-C(O)-(CH_2)_w$, wherein w is an integer from 1 to 5, preferably 1 to 3, again more preferably 1 or 2, most preferably 1; at least one preferably exactly one second moiety which comprises, or preferably consists of, a $-(O-CH_2-CH_2)_n-NH-C(O)-(CH_2)_w$, wherein n is an integer of 1 to 45, preferably 2 to 20, more preferably 4 to 8, most preferably 6, and w is an integer from 1 to 10, preferably 1 to 5, more preferably 1 to 3, most preferably 2; and at least one, preferably exactly one third moiety which comprises, or preferably consists of, a maleimide moiety.

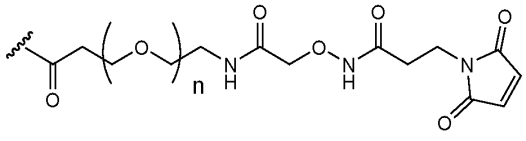
In a preferred embodiment, said linker is selected from the following formulas:



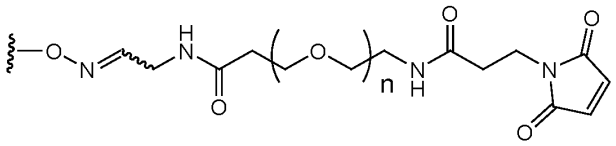
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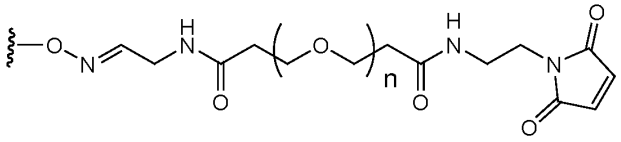
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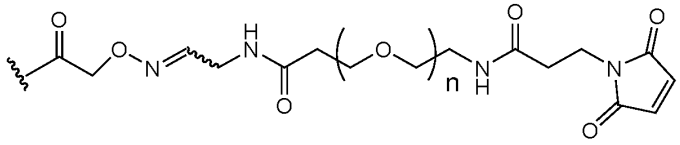
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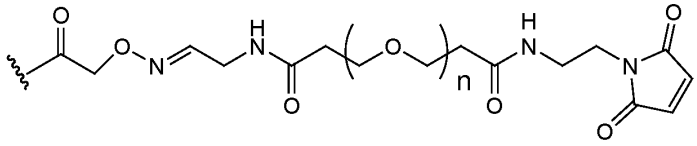
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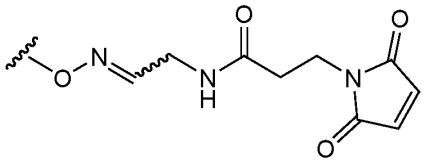
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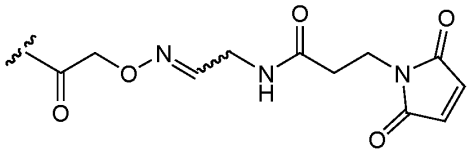
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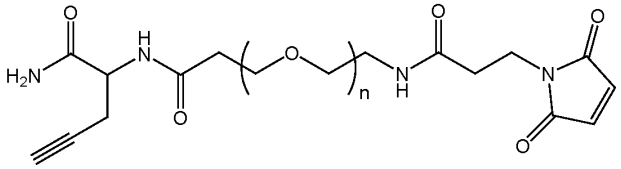
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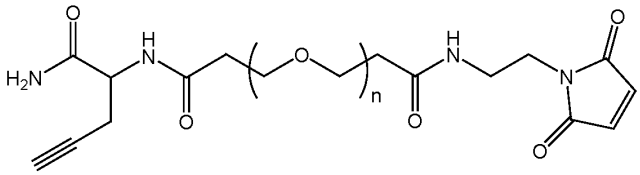
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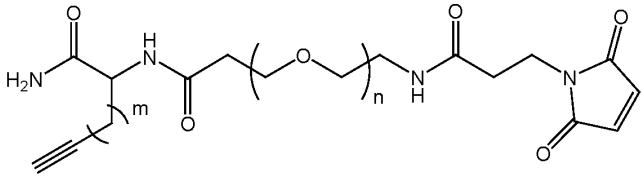
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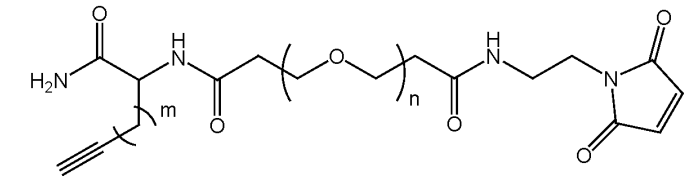
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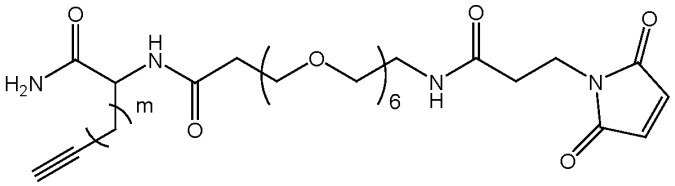
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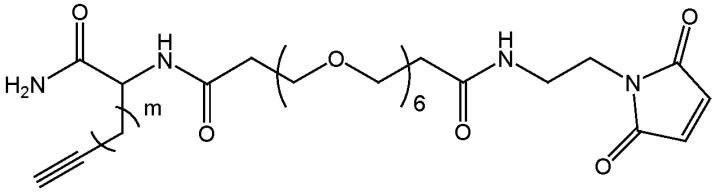
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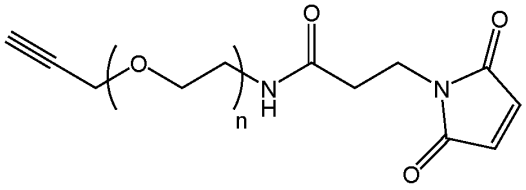
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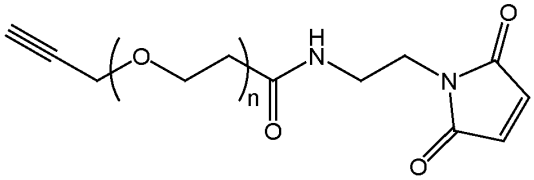
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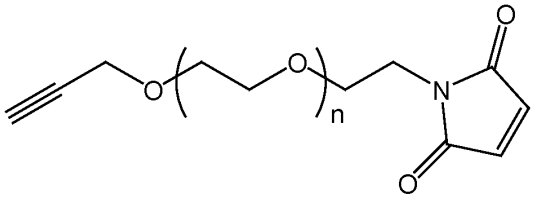
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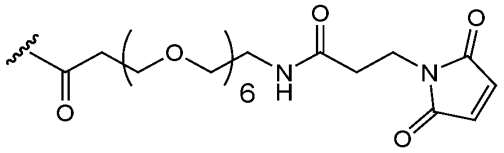
(linker 11)

wherein n is an integer of 1 to 45, preferably 2 to 20 or 4 to 20, further preferably 4 to 10 or 4 to 8, and again further preferably 6 to 8, and again further preferably n is an integer of 6, and wherein m is an integer of 1 to 6, preferably 1 to 4, further preferably 1, 2, 3 or 4, and again further preferably m is an integer of 1, and wherein the wavy line indicates the attachment site to said pneumococcal antigen, preferably to said pneumococcal peptide epitope, and wherein, if present, said alkyne functionality reacts with an azido functionality comprised by said linking moiety, typically and preferably, comprised by said bi-epitope or multi-epitope branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen. In a further

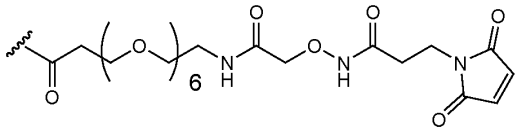
preferred embodiment, said linker is a linker 1, wherein n is an integer of 1 to 45, preferably 2 to 20 or 4 to 20, further preferably 4 to 10 or 4 to 8, and again further preferably 6 to 8, and again further preferably n is an integer of 6, and wherein m is an integer of 1 to 6, preferably 1 to 4, further preferably 1, 2, 3 or 4, and again further preferably m is an integer of 1. In a further preferred embodiment, said linker is a linker 7, wherein n is an integer of 1 to 45, preferably 2 to 20 or 4 to 20, further preferably 4 to 10 or 4 to 8, and again further preferably 6 to 8, and again further preferably n is an integer of 6, and wherein m is an integer of 1 to 6, preferably 1 to 4, further preferably 1, 2, 3 or 4, and again further preferably m is an integer of 1.

In case compounds of the present invention such as the conjugates, and hereby including said linkers, comprise one or more double bonds, said double bonds can be of either the (*E*)- or (*Z*)-configuration, or mixtures thereof in any ratio. The same applies for the preferred linker comprising an oxime moiety. Thus, the preferred linker comprising an oxime moiety thus may include either said linker with said oxime moiety in its syn-configuration (and thus as syn-isomer), said linker with said oxime moiety in its anti-configuration (and thus as anti-isomer) and mixtures thereof in any ratio. Within the chemical formulas presented herein for said double bond or said oxime moiety, this is typically and preferably represented by a wavy line.

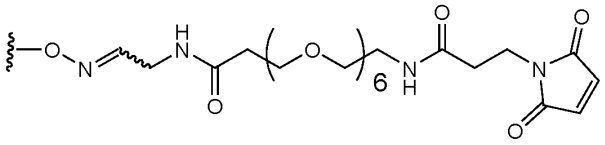
In a further very preferred embodiment, said linker is selected from the following formulas:



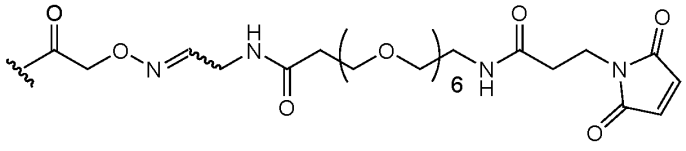
(linker 1-6)



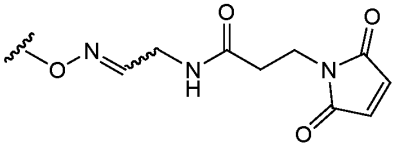
(linker 2-6)



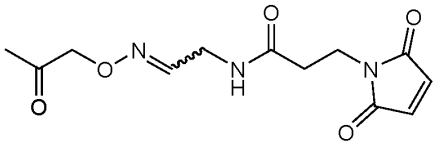
(linker 3-6)



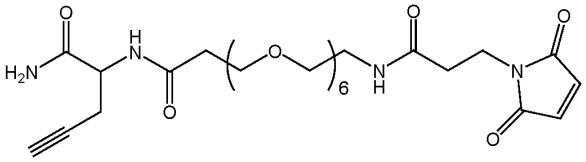
(linker 4-6)



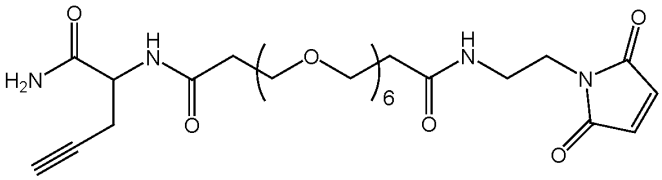
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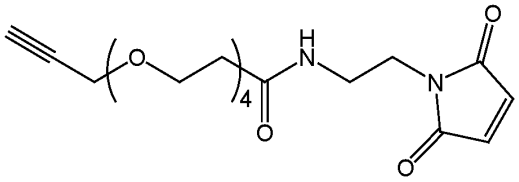
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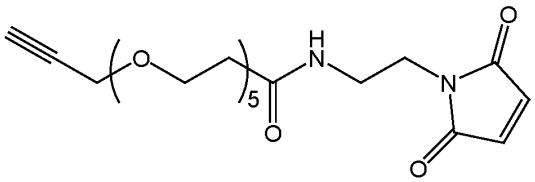
(linker 7-6)



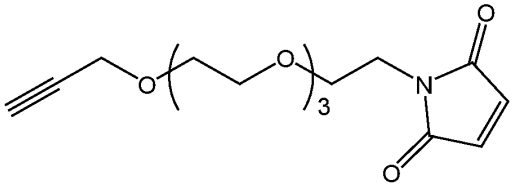
(linker 7R-6)



(linker 10R-4)



(linker 10R-5)



(linker 11-4),

and wherein the wavy line indicates the attachment site to said pneumococcal antigen, preferably to said pneumococcal peptide epitope, and wherein, if present, said alkyne functionality reacts with an azido functionality comprised by said linking moiety, typically and preferably, comprised by said bi-epitope or multi-epitope branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen.

Conjugate

The SVLPs typically and preferably bear multiple copies of the pneumococcal antigens and pneumococcal peptide epitopes, respectively. An SVLP comprises, preferably consists of, conjugates. In a further preferred embodiment, said SVLP comprises at least one conjugate.

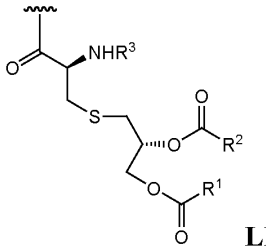
Conjugates, preferred and very preferred conjugates are referred herein also as a single epitope conjugate such as said first conjugate, said second conjugate, said third conjugate, or as said bi-epitope conjugate. The reference to a single epitope conjugate such as said first conjugate typically and preferably shall refer to conjugate containing said first pneumococcal antigen which comprises said first pneumococcal peptide epitope, while reference to said second conjugate typically and preferably shall refer to conjugate containing said second pneumococcal antigen which comprises said second pneumococcal peptide epitope, and reference to said third conjugate typically and preferably shall refer to conjugate containing said third pneumococcal antigen which comprises said third pneumococcal peptide epitope. The reference to a bi-epitope conjugate typically and preferably shall refer to a conjugate comprising two of said first, second and third pneumococcal antigens which comprises said first pneumococcal peptide epitope, said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. The below and herein described embodiments are therefore applicable for any conjugate as used in the present invention, except where explicitly described differently.

In a further preferred embodiment, said composition comprises

(a) a first synthetic virus-like particle (SVLP) as said first carrier, wherein said first SVLP comprises at least one first conjugate, wherein said first conjugate comprises a first lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said first lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

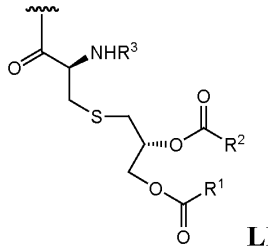
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker;

(b) a second synthetic virus-like particle (SVLP) as said second carrier, wherein said second SVLP comprises at least one second conjugate, wherein said second conjugate comprises a second lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said second lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

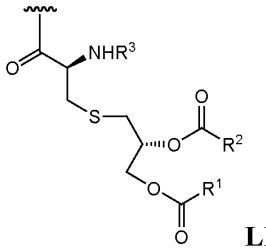
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said second pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker; and

(c) a third synthetic virus-like particle (SVLP) as said third carrier, wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

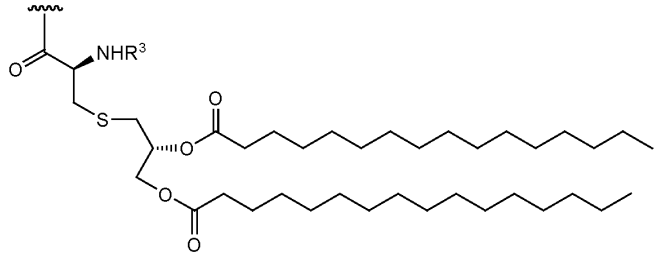
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said first lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said first lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said first lipopeptide building block is of the formula

LBB-5. In a further preferred embodiment, said second lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said second lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said second lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

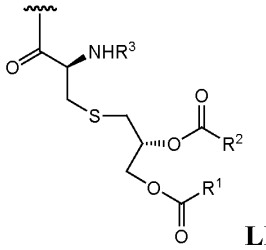
In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175)

or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

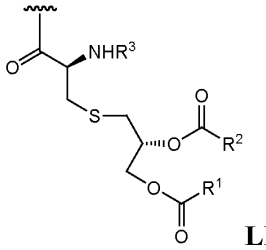
wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), preferably said third SVLP, wherein said single epitope SVLP carrier, preferably said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175)

or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

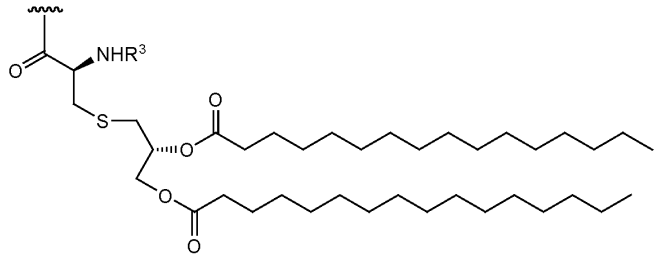
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide

building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group

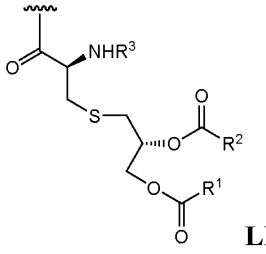
consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap "a", and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

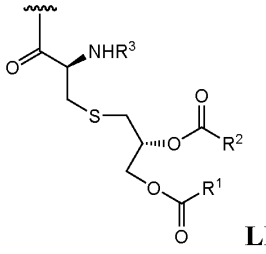
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one first, second or third conjugate, wherein said first conjugate comprises a lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said second conjugate comprises a lipopeptide building block (LBB) and said second pneumococcal antigen, and wherein said third conjugate comprises a lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

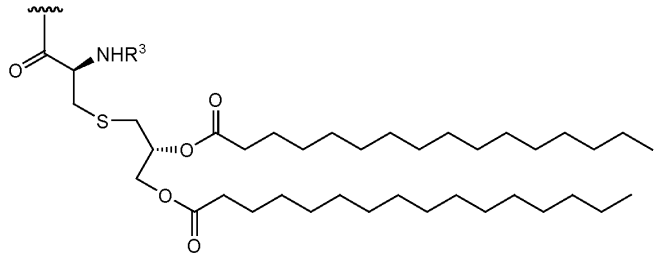
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first, said second or said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said first lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said first lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said first lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said second lipopeptide building block is of the formula **LBB-**

1, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said second lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said second lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**,

wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap "a", and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said first pneumococcal antigen comprises said first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21. In a further preferred embodiment, said first pneumococcal antigen comprises said first pneumococcal peptide epitope, and wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6. In a further preferred embodiment, said second pneumococcal antigen comprises said second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100. In a further preferred embodiment, said second pneumococcal antigen comprises said second pneumococcal peptide epitope, and wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25. In a further preferred embodiment, wherein said third pneumococcal antigen comprises said third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal antigen comprises said third pneumococcal peptide epitope, and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

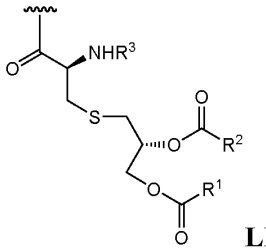
In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, and wherein said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide

epitope and said second pneumococcal peptide epitope, and wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11-15}alkyl, preferably wherein R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are $-C_{15}H_{31}$; and

wherein R^3 is hydrogen or $-C(O)C_{11-15}$alkyl, preferably wherein R^3 is H or $-C(O)C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

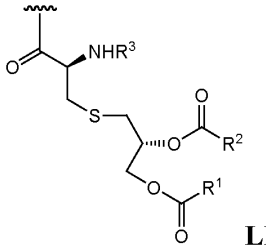
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one third conjugate, wherein said third conjugate comprises a lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

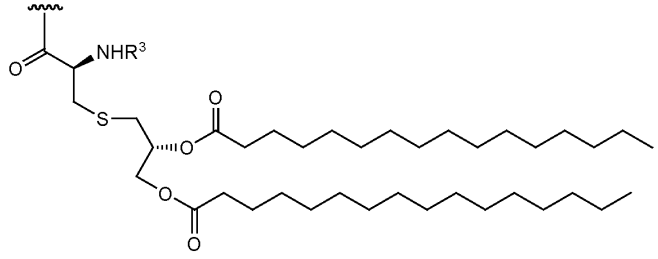
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first, said second or said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, and wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, wherein independently at each occurrence PG1, PG2 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap “a”, and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23. In a further preferred embodiment, said third pneumococcal antigen comprises said third pneumococcal peptide epitope, wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal antigen comprises said third pneumococcal peptide epitope, and wherein said third pneumococcal peptide epitope comprises, preferably consists

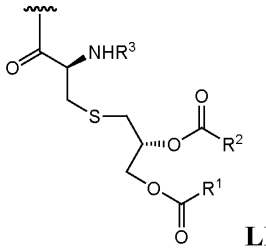
of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, and wherein said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, and wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11-15}alkyl, preferably wherein R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are $-C_{15}H_{31}$; and

wherein R^3 is hydrogen or $-C(O)C_{11-15}$alkyl, preferably wherein R^3 is H or $-C(O)C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

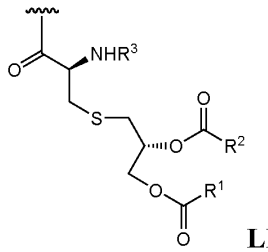
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one second conjugate, wherein said second conjugate comprises a lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

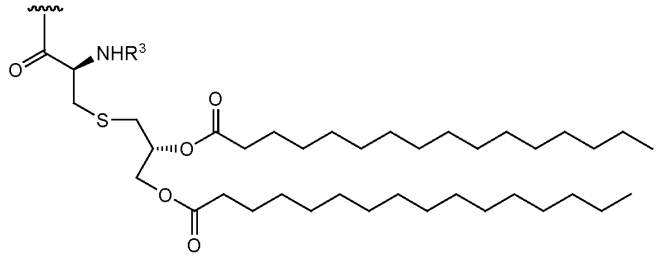
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first, said second or said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably H or $-C(O)C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, wherein independently at each occurrence

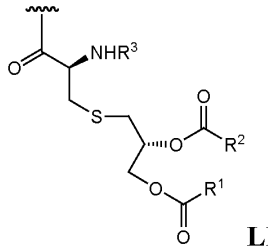
PG1, PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap "a", and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:113. In a further preferred embodiment, said second pneumococcal antigen comprises said second pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100. In a further preferred embodiment, said second pneumococcal antigen comprises said second pneumococcal peptide epitope, and wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25.

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, and wherein said bi-epitope branched pneumococcal antigen comprises (i) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, and wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

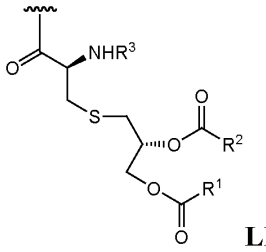
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one first conjugate, wherein said first conjugate comprises a lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

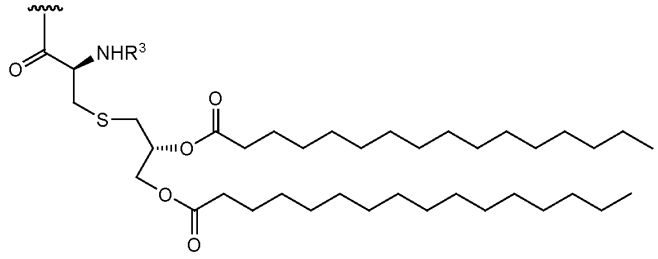
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first, said second or said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*

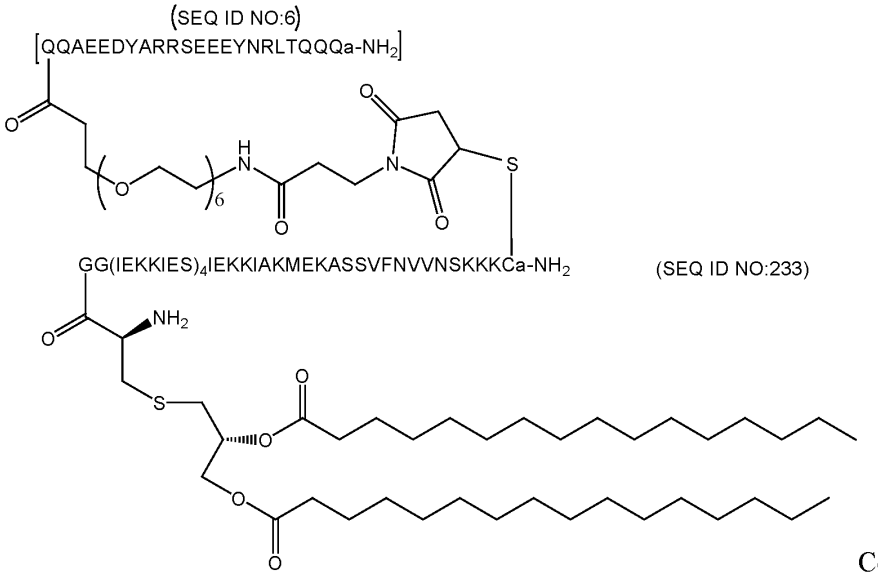


LM-II*

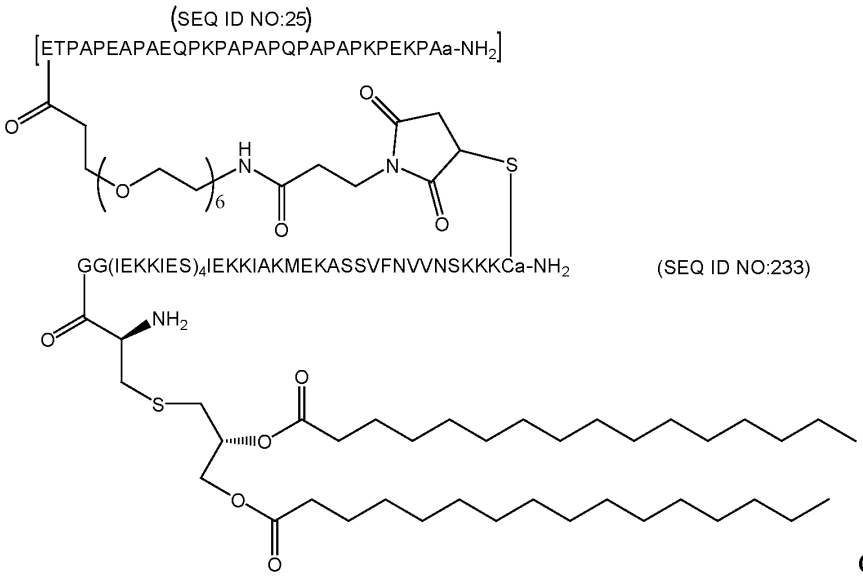
wherein R^3 is hydrogen or -C(O)C_{11-15}alkyl, preferably H or -C(O)$C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building

block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100, wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25, and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG2, PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap “a”, and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said first pneumococcal antigen comprises said first pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21. In a further preferred embodiment, said first pneumococcal antigen comprises said first pneumococcal peptide epitope, and wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6.

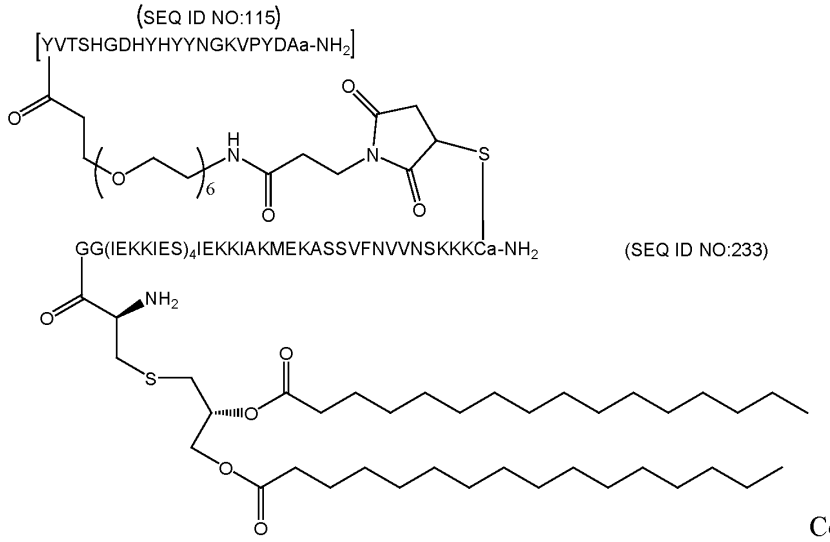
In a very preferred embodiment, said first conjugate is Conjugate **1**, said second conjugate is Conjugate **2**, and said third conjugate is Conjugate **3**



Conjugate **1**



Conjugate **2**



Conjugate **3.**

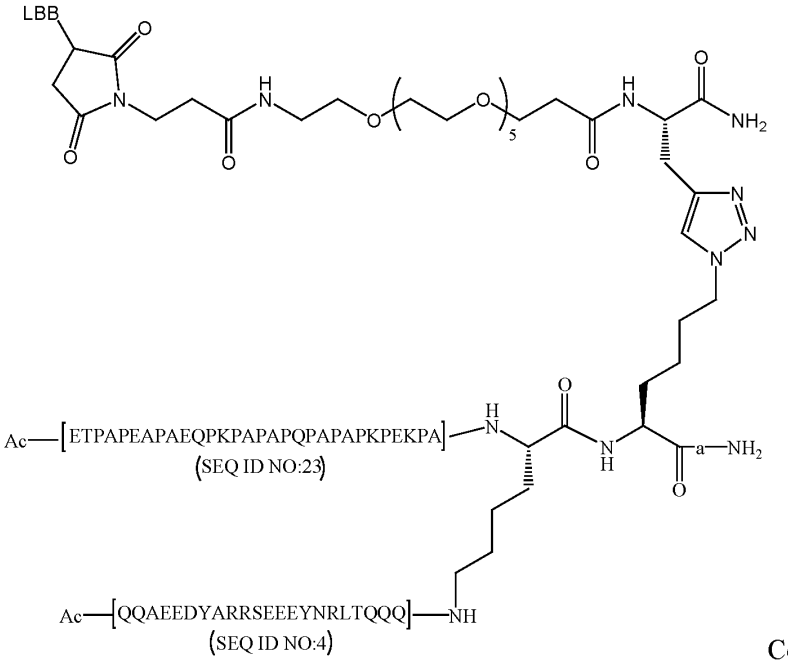
In a further preferred embodiment, said said bi-epitope conjugate is selected from Conjugate **21**, Conjugate **22**, Conjugate **23**, and Conjugate **24.**

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate is selected from Conjugate **21**, Conjugate **22**, Conjugate **23**, or Conjugate **24**, and

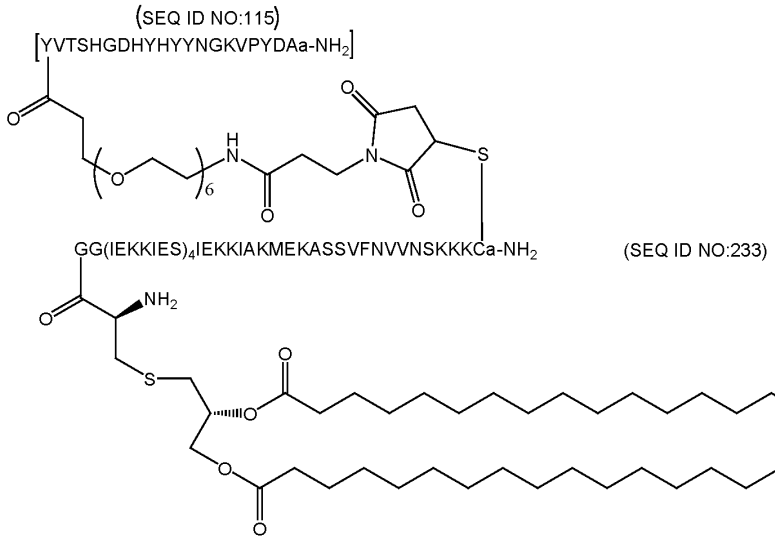
(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one conjugate selected from Conjugate **1**, Conjugate **2**, or Conjugate **3**.

In a further preferred embodiment, said bi-epitope conjugate is Conjugate **21**, and said conjugate is Conjugate **3**



Conjugate **21**,

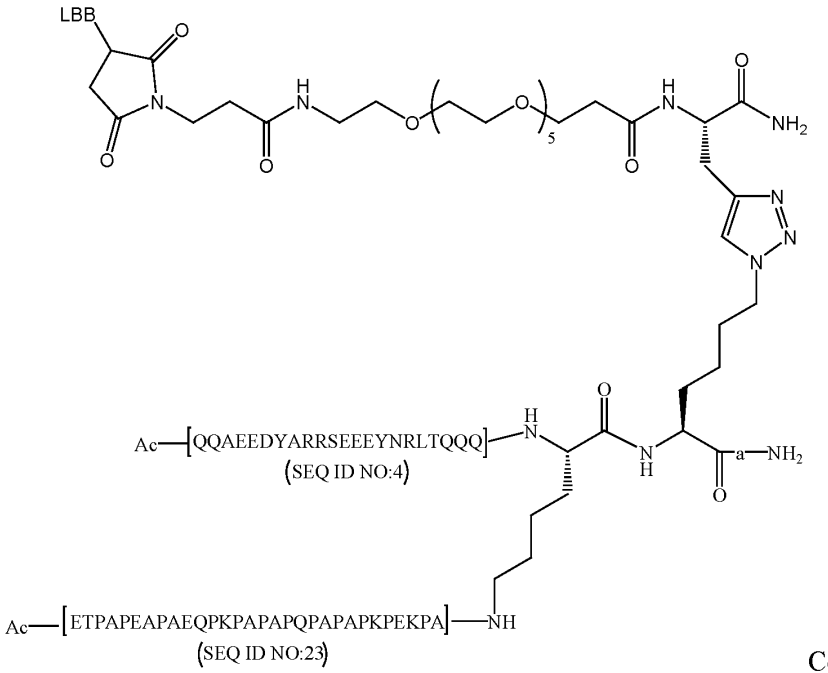
wherein said LBB is of the formula **LBB-5,**



Conjugate **3**,

or

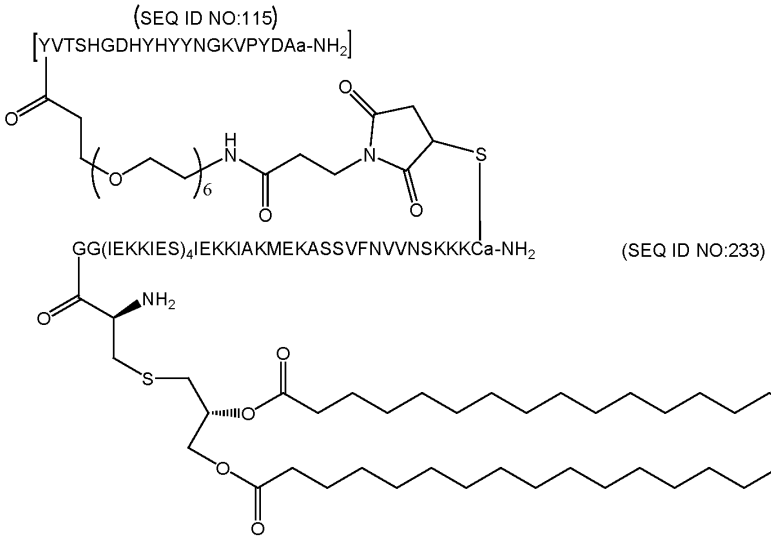
wherein said bi-epitope conjugate is Conjugate **22**, and said conjugate is Conjugate **3**



Conjugate **22,**

wherein said LBB is of the formula **LBB-5**,

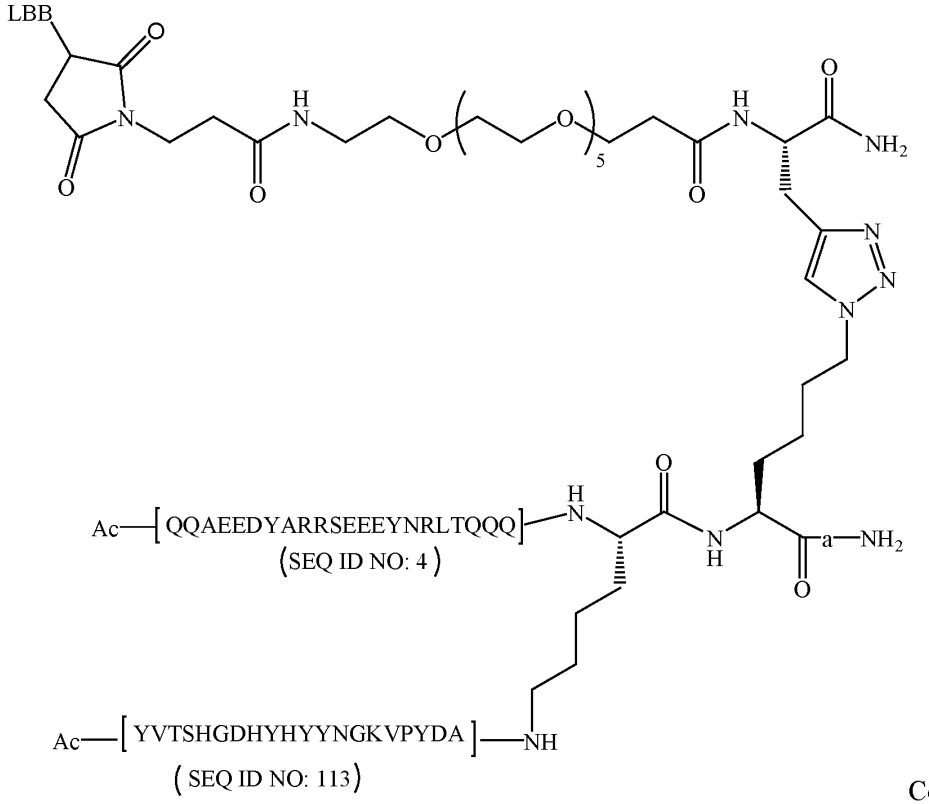
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Conjugate **3,**

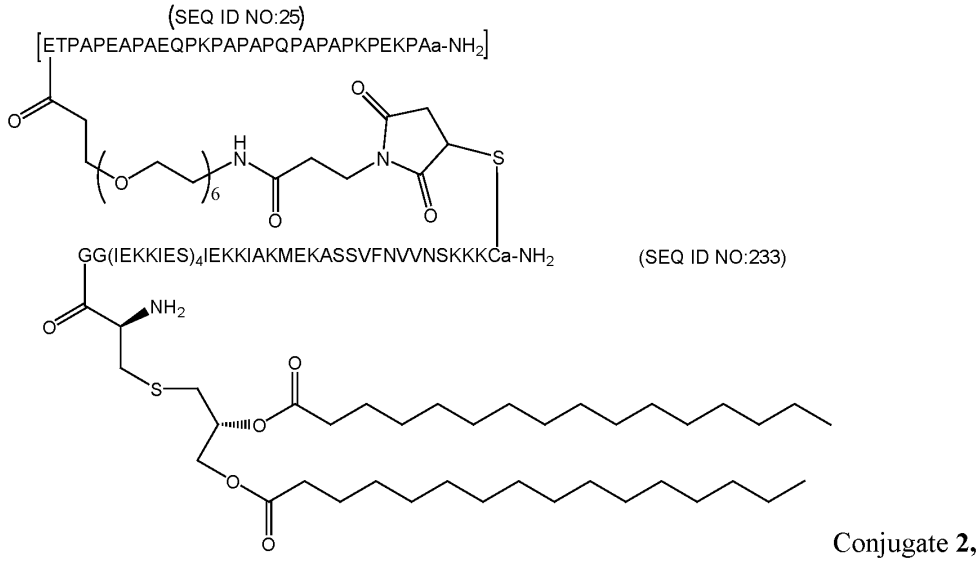
or

wherein said bi-epitope conjugate is Conjugate **23**, and said conjugate is Conjugate **2**



Conjugate **23**,

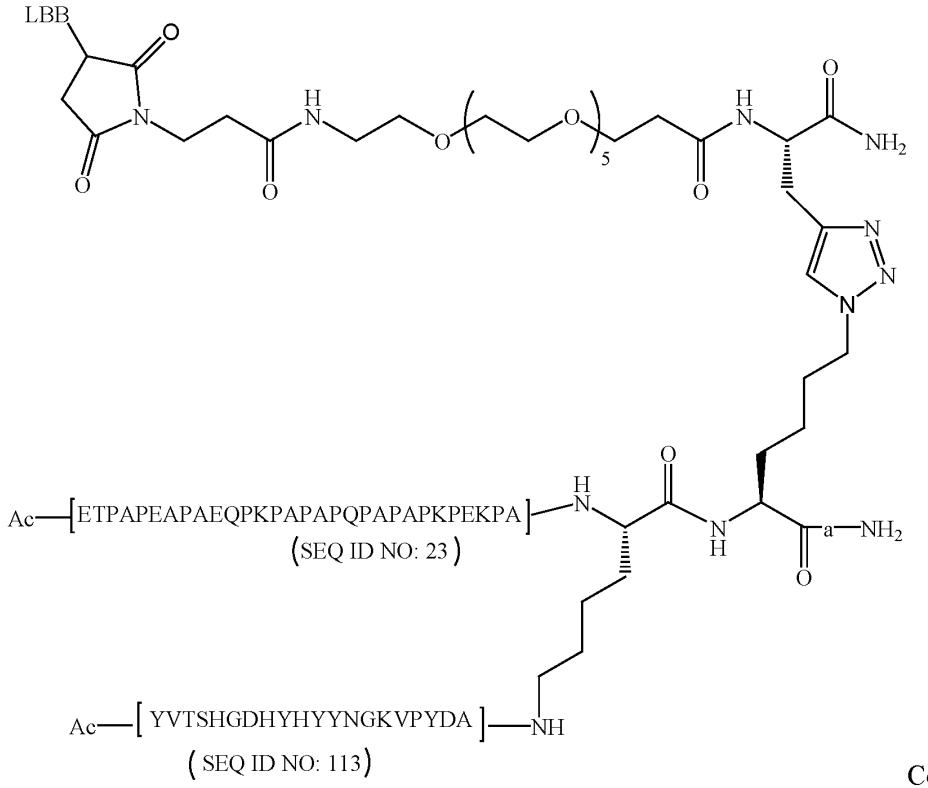
wherein said LBB is of the formula **LBB-5**,



Conjugate **2,**

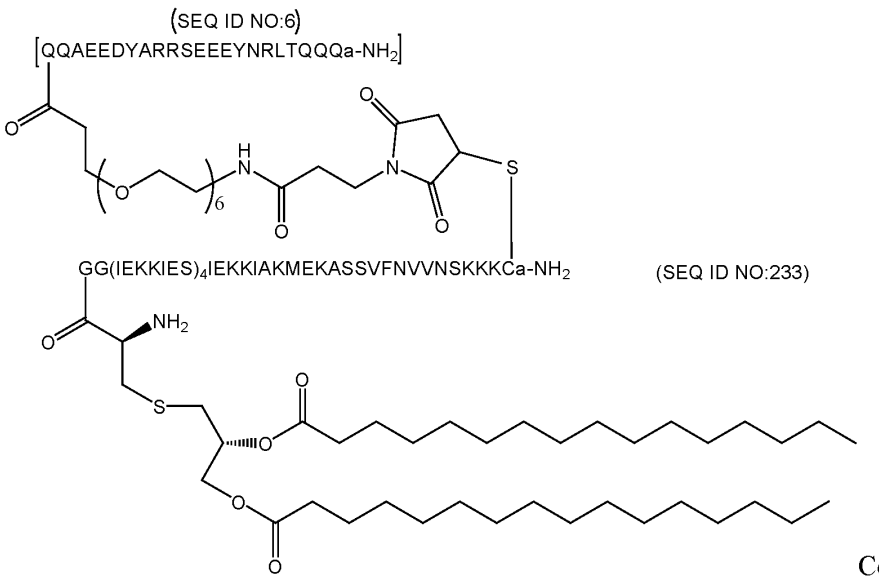
or

wherein said bi-epitope conjugate is Conjugate **24**, and said conjugate is Conjugate **1**



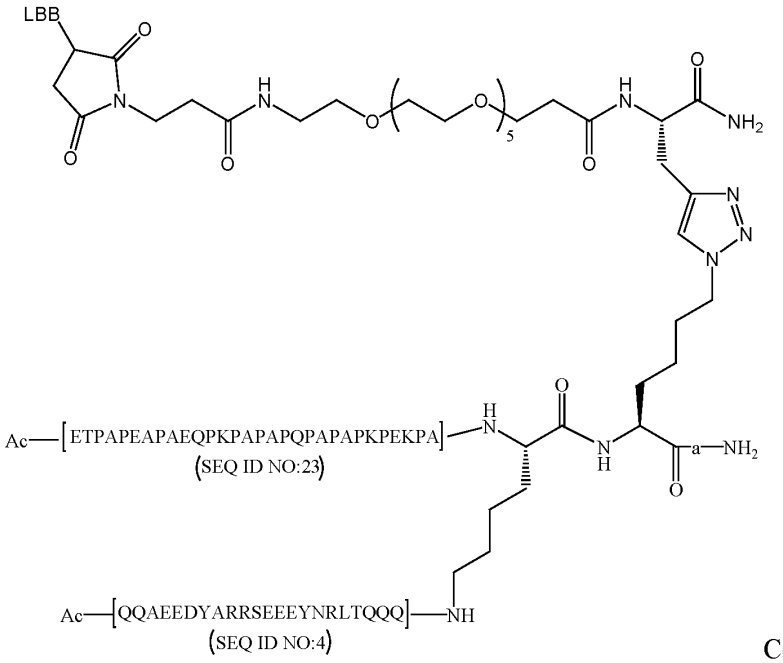
Conjugate **24**,

wherein said LBB is of the formula **LBB-5**,



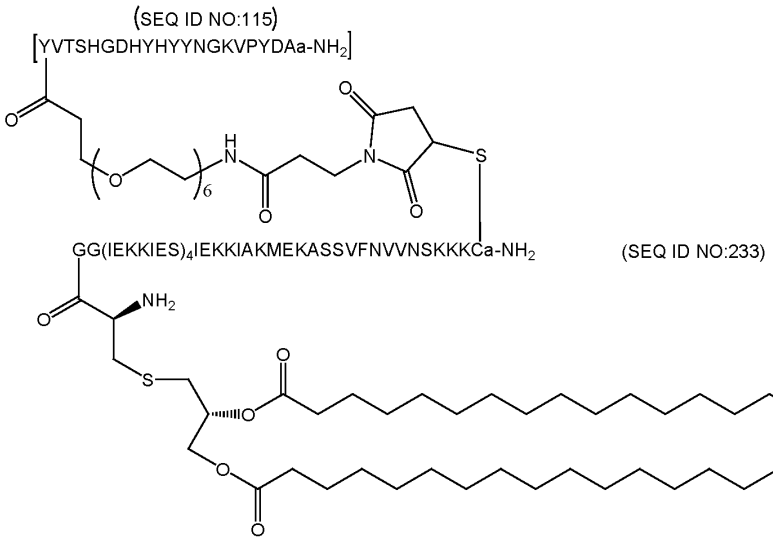
Conjugate **1**.

In a further very preferred embodiment, said bi-epitope conjugate is Conjugate **21**, and said third conjugate is Conjugate **3**



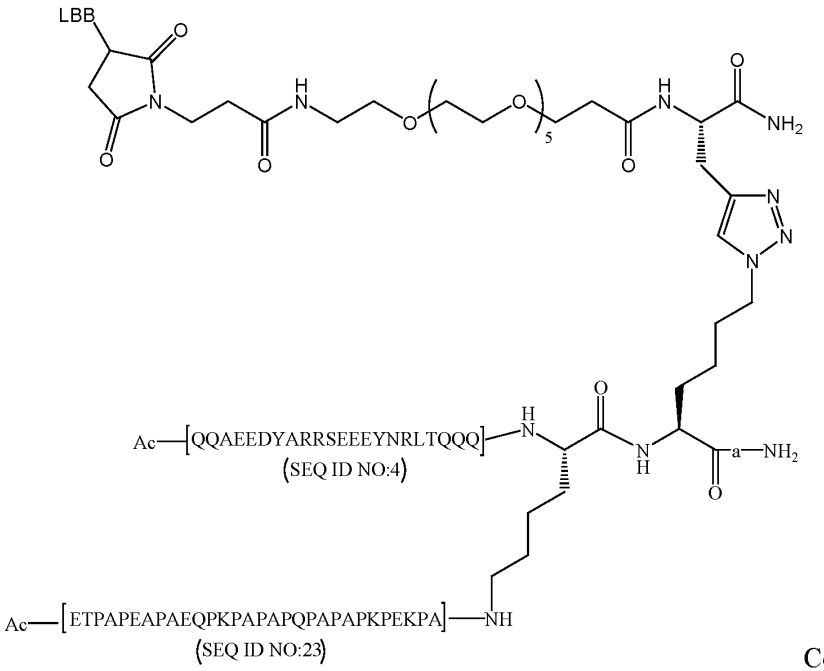
Conjugate **21**,

wherein said LBB is of the formula **LBB-5**,



Conjugate **3.**

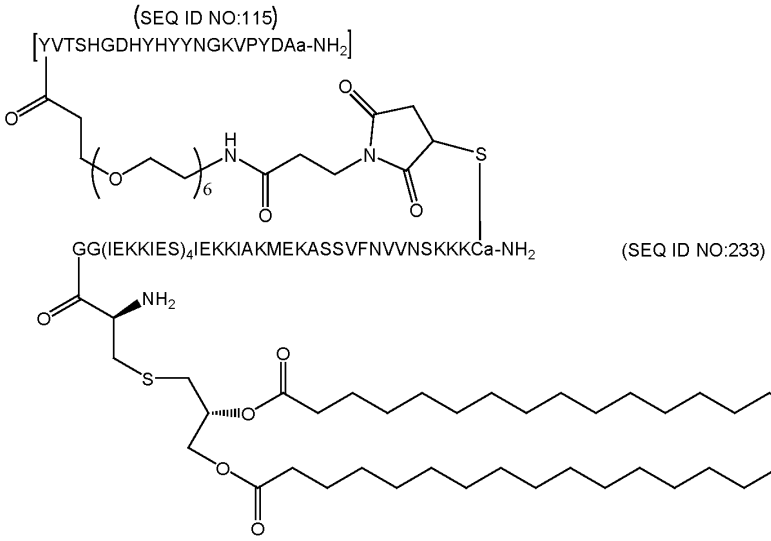
In a further very preferred embodiment, said bi-epitope conjugate is Conjugate **22**, and said third conjugate is Conjugate **3**



Conjugate **22**,

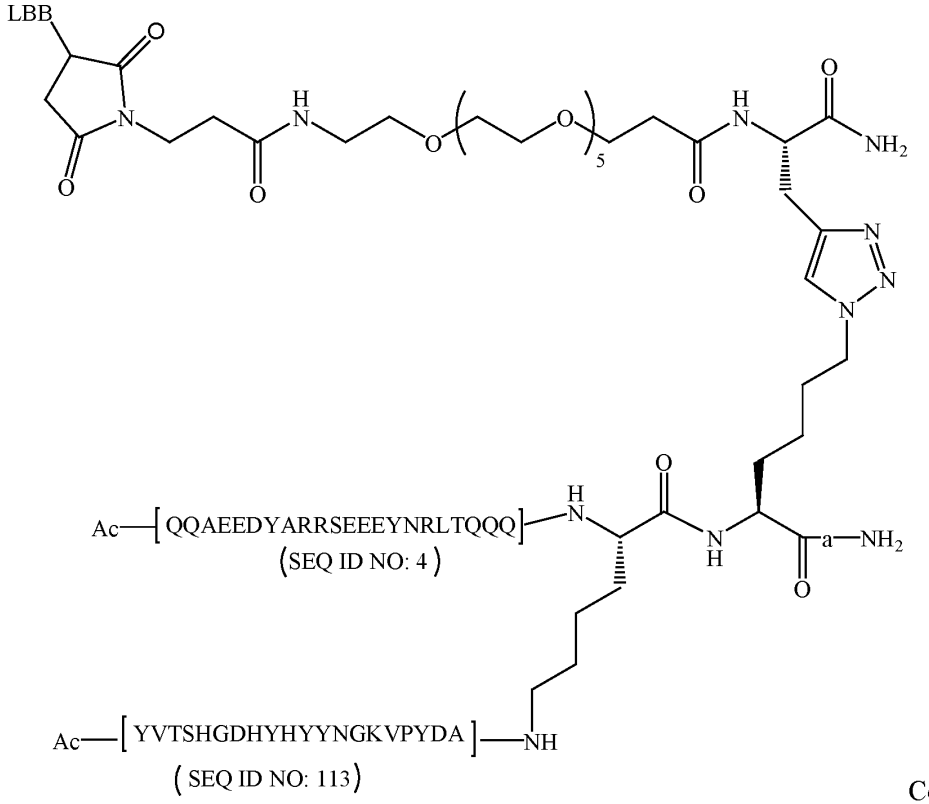
wherein said LBB is of the formula **LBB-5**,

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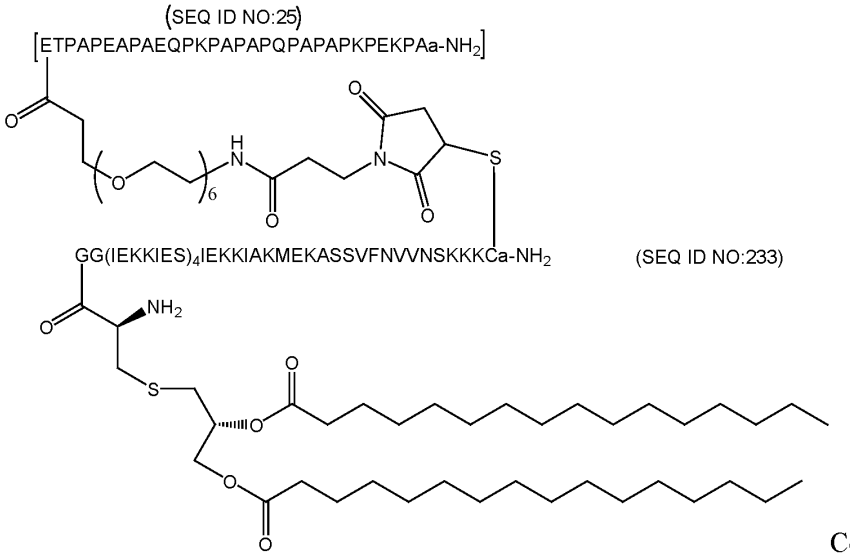
Conjugate **3.**

In a further preferred embodiment, said bi-epitope conjugate is Conjugate **23**, and said conjugate is Conjugate **2**



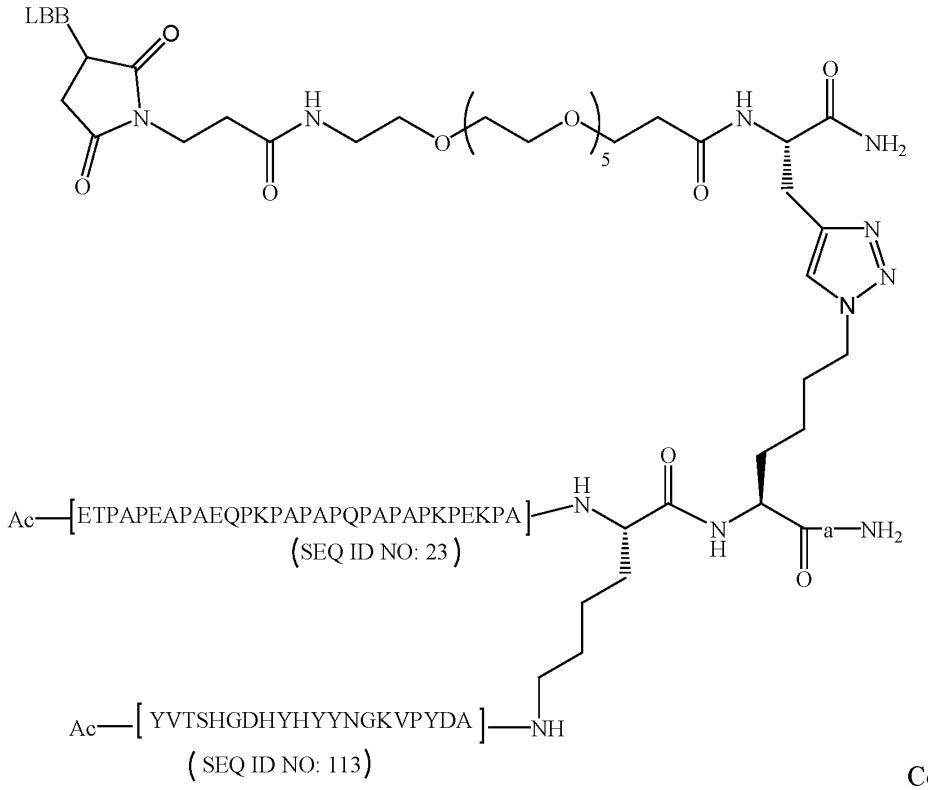
Conjugate **23**,

wherein said LBB is of the formula **LBB-5**,



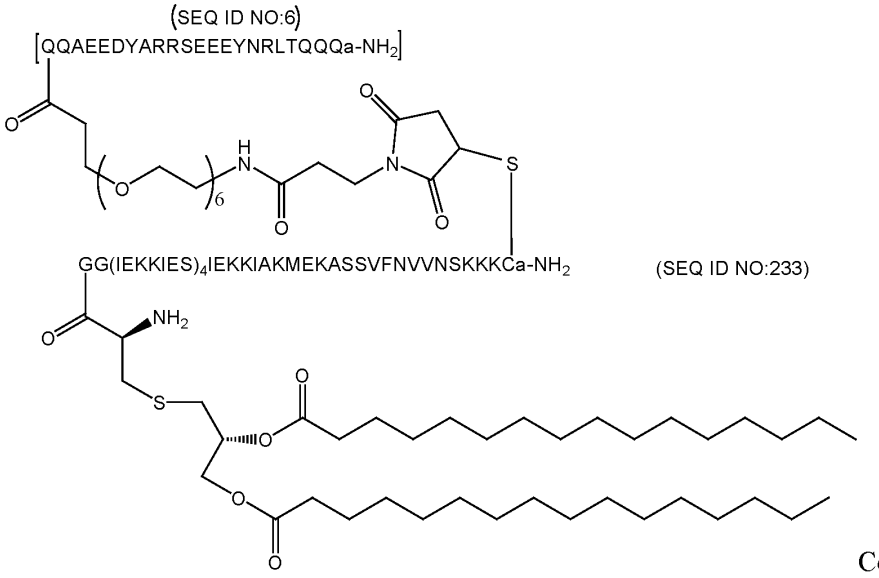
Conjugate **2.**

In a further preferred embodiment, said bi-epitope conjugate is Conjugate **24**, and said conjugate is Conjugate **1**



Conjugate **24**,

wherein said LBB is of the formula **LBB-5**,



Conjugate **1**.

In another aspect, the present invention provides for a bundle of 2, 3, 4, 5, 6 or 7 conjugates, more preferably 2, 3, 4 or 5 conjugates, most preferably 3 conjugates. In a further preferred embodiment, in said bundle, the coiled coil peptide chain segments of said peptide moieties comprised by said conjugates are coiled together, wherein preferably said coiled coil peptide chain segments are helically coiled together, more preferably said coiled coil peptide chain segments are alpha-helically coiled together. In a preferred embodiment, said coiled coil peptide chain segments of said peptide moieties are coiled together left-handed or right-handed. More preferably said coiled coil peptide chain segments of said peptide moieties form an alpha-helical left-handed coil. In one embodiment, said coiled coil peptide chain segments have a parallel orientation, i.e. they run in the same direction; or they have an anti-parallel orientation, i.e. they run in directions opposite to each other; wherein the first option is preferred. The term "direction" is based on the direction of a peptide chain having on one side an N-terminus and on the other side a C-terminus. In a preferred embodiment in said bundle, said coiled coil peptide chain segments of said peptide moieties form a left-handed alpha-helical coiled coil, wherein the coiled coil peptide chain segments have a parallel orientation in said coiled coil, and bundle comprises 3 helically twisted coiled coil peptide chain segments. Preferably, said coiled coil peptide chain segments have the same sequence (homo-) or different sequences (hetero-).

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate is selected from Conjugate **21**, and

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one Conjugate **3**.

In a further preferred embodiment, said composition comprises at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate is selected from Conjugate **22**, and

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), wherein said SVLP comprises at least one Conjugate **3**.

In a further preferred embodiment, said composition further comprise a pneumococcal conjugate vaccine (PCV). Pneumococcal conjugate vaccines (PCV) are known to the skilled person in the art. In a further preferred embodiment, said composition further comprise a pneumococcal conjugate vaccine (PCV), wherein said pneumococcal conjugate vaccine (PCV) is PCV13.

Methods of Use

In a further aspect, the present invention provides a pharmaceutical composition comprising the composition of the invention. In another aspect, the present invention provides a pharmaceutical composition comprising said kit-of-parts.

In another aspect, the present invention provides for a pharmaceutical composition comprising an immunologically effective amount of the combination of the pneumococcal antigens of the present invention or the synthetic virus like particles comprising said combination of the pneumococcal antigens of the present invention, together with a pharmaceutically acceptable diluent, carrier or excipient, wherein preferably said pharmaceutical composition is a vaccine. Said SVLPs and respective pharmaceutical compositions and vaccines are prepared in a manner known by the skilled person and as described herein, including 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, in particular pharmaceutically acceptable salts and/or buffering substances.

As used herein, the term “effective amount” refers to an amount necessary or sufficient to realize a desired biologic effect. Preferably, the term “effective amount” refers to an amount of the antigen(s) and antigen combination of the present invention, the nucleic acids preferably mRNAs encoding said pneumococcal antigens, or the synthetic virus like particle(s) of the present invention comprising said combination of the pneumococcal antigens of the present invention that (i) treats or prevents the particular disease, medical condition, or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, medical condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, medical condition, or disorder described herein. An immunogenically effective amount, as herein understood, is an amount that is capable of modulating, preferably enhancing the response of the immune system of a subject to an antigen or pathogen, and thus said pneumococcal antigens and/or the combination of the pneumococcal antigens of the present invention.

In a preferred embodiment, the composition comprises an effective amount to protect a patient from infection by *Streptococcus pneumoniae*.

In another aspect, the present invention provides an immunogenic composition comprising the composition of the invention.

In another aspect, the present invention provides a vaccine composition comprising the composition of the invention.

In another aspect, the present invention provides the composition of the invention for use in a method of immunizing a subject.

In another aspect, the present invention provides the composition of the invention for use as a vaccine against an infection caused by *Streptococcus pneumoniae*

In another aspect, the present invention provides the composition of the invention for use in preventing or treating an infection caused by *Streptococcus pneumoniae*.

A vaccine, as used herein, is a pharmaceutical composition that is used to modulate, preferably to stimulate the response of the body's immune system to a particular antigen or pathogen, and thus to said pneumococcal antigens and/or the combination of the pneumococcal antigens of the present invention. In a preferred embodiment, the pharmaceutical composition or preferably the vaccine is used for preventing or reducing the risk of an infection caused by *Streptococcus pneumoniae* in a subject, preferably a human

Preferably, said infection caused by *Streptococcus pneumoniae* is selected from the group consisting of otitis, pneumonia, meningitis, sepsis, sinusitis, rhinitis, ulcus serpens corneae, appendicitis, peritonitis and combinations thereof. More preferably, said infection caused by *Streptococcus pneumoniae* is selected from the group consisting of pneumonia, otitis, rhinitis meningitis, sepsis and combinations thereof. Again more preferably, said infection caused by *Streptococcus pneumoniae* is pneumonia and/or sepsis. In another embodiment, said *Streptococcus pneumoniae* infection is selected from the group consisting of an infection of the upper and lower respiratory tract, sinus, ear and meniges; sepsis and combinations thereof.

In another aspect, the present invention provides a method of immunizing a subject comprising administering the composition of the invention to an animal, preferably to a human.

In another aspect, the present invention provides a method of vaccinating a subject against an infection caused by *Streptococcus pneumoniae* comprising administering the composition of the invention to an animal, preferably to a human.

In another aspect, the present invention provides a method of preventing or treating an infection caused by *Streptococcus pneumoniae*, via administration of the composition of the invention to said subject. Said subject is preferably an animal, more preferably a mammalian,

again more preferably a human.

In another aspect, the present invention provides an use of the composition of the invention for the manufacture of a medicament for immunizing a subject. In another aspect, the present invention provides an use of the composition of the invention for the manufacture of a vaccine for preventing or treating an infection caused by *Streptococcus pneumoniae*.

In a preferred embodiment, said composition is preferably administered orally or parenterally, more preferably parenterally. In a preferred embodiment, said composition is preferably administered parenterally, more preferably said composition is injected, administered mucosally, transdermally, epidermally or epicutaneously. Again more preferably, said composition is injected, administered mucosally, or epidermally. Mucosal administration relates preferably to administration to the eye, upper and/or lower respiratory tract. Preferably mucosal administration relates to oral administration including inhalation, sublingual, intranasal, ocular, rectal and/or vaginal administration. The term "administration" includes intramuscular, intravenous, and subcutaneous administration. In another preferred embodiment, said composition is administered using an epicutaneous patch, preferably an epicutaneous microneedle patch.

In a further aspect, the present invention provides for kit-of-parts. In a further aspect, the present invention provides for kit-of-parts comprising the composition of the present invention.

A kit-of-parts may comprise multiple different pneumococcal antigens. A kit may comprise any of a number of additional components or reagents in any combination. According to certain embodiments of the invention, a kit may include, for example, (i) single pneumococcal antigen or a combination thereof; (ii) an adjuvant; and (iii) instructions for administering a composition including the pneumococcal antigen and the adjuvant to a subject in need thereof.

The term "kit-of-parts" as used herein preferably refers to a combination of at least two separate parts, typically and preferably two or three parts, namely said first pneumococcal antigen comprising a first pneumococcal peptide epitope or said nucleic acid encoding said first pneumococcal antigen; said second pneumococcal antigen comprising said second pneumococcal peptide epitope or a nucleic acid encoding said second pneumococcal antigen; said third pneumococcal antigen comprising said third pneumococcal peptide epitope or a nucleic acid encoding said third pneumococcal antigen; and/or said multi-epitope pneumococcal antigen, preferably said bi-epitope pneumococcal antigen.

The arrangement and construction of such kit-of-parts is conventionally known to one skilled in the art. In a particularly preferred embodiment, said kit-of-parts of the invention or

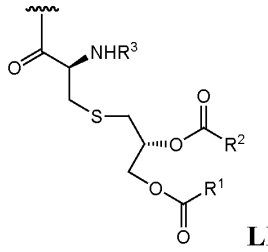
said parts of the kit-of-parts of the invention, i.e. one or several of the pneumococcal antigens and/or the one or more of the other pneumococcal antigens forming together the inventive composition comprise independently of each other at least one pharmaceutically acceptable diluent, excipient or carrier. In another preferred embodiment, said kit-of-parts of the invention or said parts of the kit-of-parts of the invention, comprise independently of each other one or more adjuvants.

Thus, in a further aspect, the present invention provides a kit of parts comprising

(a) a first synthetic virus-like particle (SVLP) as said first carrier, wherein said first SVLP comprises at least one first conjugate, wherein said first conjugate comprises a first lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said first lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11-15}alkyl, preferably wherein R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are $-C_{15}H_{31}$; and

wherein R^3 is hydrogen or $-C(O)C_{11-15}$alkyl, preferably wherein R^3 is H or $-C(O)C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

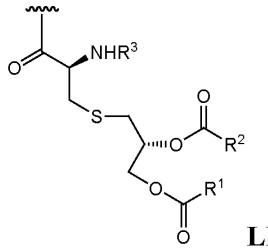
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker;

(b) a second synthetic virus-like particle (SVLP) as said second carrier, wherein said second SVLP comprises at least one second conjugate, wherein said second conjugate comprises a second lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said second lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently $-C_{11}H_{23}$, $-C_{13}H_{27}$ or $-C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are $-C_{15}H_{31}$; and

wherein R^3 is hydrogen or $-C(O)C_{11}$-$_{15}$alkyl, preferably wherein R^3 is H or $-C(O)C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

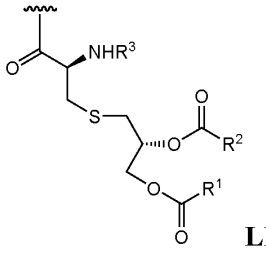
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said second pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker; and

(c) a third synthetic virus-like particle (SVLP) as said third carrier, wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

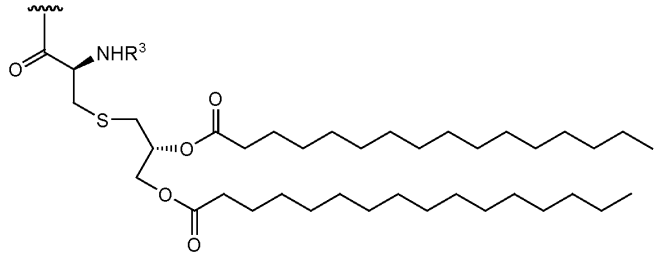
wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain

segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably H or $-C(O)C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said first lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said first lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said first lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said second lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said second lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said second lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5.** In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, said second pneumococcal peptide epitope comprises, preferably

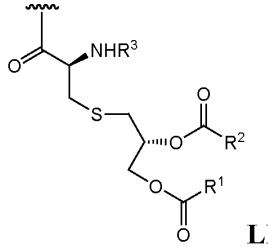
consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25, and said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

In another aspect, the present invention provides a kit of parts comprising at least one carrier, preferably two carriers, wherein

(a) one of said two carriers is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

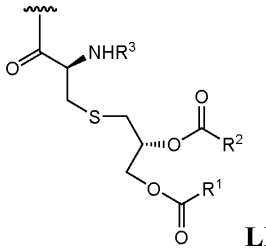
wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) the other of said two carriers is single epitope carrier, wherein said single epitope carrier is a synthetic virus-like particle (SVLP), preferably said third SVLP, wherein said single epitope SVLP carrier, preferably said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, wherein said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains, wherein said lipid moiety comprises formula **LM-II**



LM-II

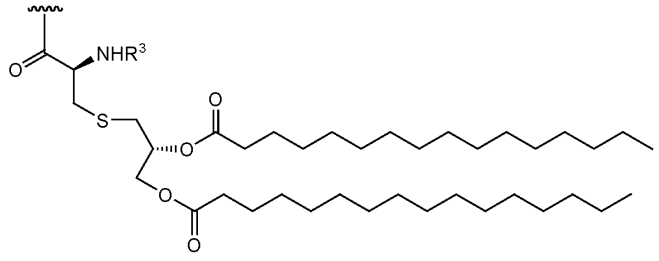
wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker. In a further preferred embodiment, said coiled coil peptide chain segment consists of the sequence $(IEKKIES)_4$ (SEQ ID NO:179) and said lipid moiety consists of formula LM-II*



LM-II*

wherein R^3 is hydrogen or $-C(O)C_{11\text{-}15}$alkyl, preferably H or $-C(O)C_{15}H_{31}$; and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety. In a further preferred embodiment, said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said bi-epitope lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said bi-epitope lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said third lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said third lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said third lipopeptide building block is of the formula **LBB-5**. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an

amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:21, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:100 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen comprises (i) said first pneumococcal peptide epitope and said second pneumococcal peptide epitope; (ii) said first pneumococcal peptide epitope and said third pneumococcal peptide epitope; or (iii) said second pneumococcal peptide epitope and said third pneumococcal peptide epitope, wherein said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:4 to SEQ ID NO:6, wherein said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:23 to SEQ ID NO:25 and wherein said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115. In a further preferred embodiment, said bi-epitope branched pneumococcal antigen is selected from the group consisting of **BPA-1***, **BPA-1**, **BPA-2***, **BPA-2**, **BPA-3***, **BPA-3**, **BPA-4***, **BPA-4**, **BPA-5***, **BPA-5**, **BPA-6***, **BPA-6**, **BPA-7***, **BPA-7**, **BPA-8***, **BPA-8**, wherein independently at each occurrence PG1, PG2 and PG3 represents an amino protecting group, and wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and wherein D-Ala is represented as a small cap “a”, and wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the corresponding sequences, i.e. SEQ ID NO:4 and SEQ ID NO:23 or SEQ ID NO:4 and SEQ ID NO:113 or SEQ ID NO:23 and SEQ ID NO:113. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:172. In a further preferred embodiment, said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to SEQ ID NO:115.

EXAMPLES

MATERIALS AND METHODS

General Peptide Syntheses. The pneumococcal peptides were synthesized manually or employing a PurePep® Chorus synthesizer (Gyros Protein Technologies). The 9-fluoromethyl (Fmoc) amino acids (AA) used for Solid Phase Peptide Synthesis (SPPS) as well as the building blocks Fmoc-D-Ala-OH, Fmoc-Lys(Mmt)-OH and Fmoc-L-Lys(N_3)-OH, Mal-PEG6-COOH and Fmoc-L-Pra-OH were acquired from Iris Biotech. The resins employed for the syntheses was Rink Amide ChemMatrix® resin (Biotage), Fmoc-Rink-Amide-TentaGel XV (Iris Biotech) or Fmoc-Rink Amide aminomethyl-polystyrene Resin (Iris Biotech).

Couplings were performed by using Fmoc-AA of *N,N'*-diisopropylcarbodiimide (DIC, Iris Biotech) and ethyl cyano(hydroxyimino)acetate (Oxyma Pure®, Iris Biotech) or by using (1H-7-azabenzotriazole-1-yl)-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyAOP, Iris Biotech) with of *N,N*-diisopropylethylamine (DIPEA, Sigma Aldrich). Fmoc deprotection was performed using a 20% solution of piperidine (Thermo Fischer) in *N,N*-dimethylformamide (DMF, Iris Biotech). Peptide acetylation was performed by using acetic anhydride (Sigma Aldrich) and DIPEA (Sigma Aldrich) in DMF (Iris Biotech). Washes during the syntheses to remove excess of reagent were performed by DMF (Iris Biotech) and dichloromethane (DCM, Iris Biotech). Selective 4-methoxytrityl (Mmt) deprotection for epitope branching was performed with a deprotection cocktail consisting of 95% DCM, 3% Triisopropylsilane (TIPS, Sigma Aldrich) and 2% TFA (Iris Biotech).

For peptide cleavage from the resins and final global deprotection a cleavage cocktail that consists of TFA (Iris Biotech), TIPS (Sigma Aldrich) and H_2O for HPLC (Sigma Aldrich) was used. Peptide precipitation was done with cold diethyl ether (Sigma Aldrich).

Peptide purification was performed with a High-Performance Liquid Chromatography (HPLC) with a TFA system on a reversed-phase column with acetonitrile (ACN, Biosolve) and and H_2O for HPLC.

Animals, immunization and challenge. Female BALB/c mice, (Charles River Laboratories, France), were housed under SPF conditions. At 8-weeks of age mice were immunized subcutaneously (s.c.) with three doses in 2 weeks interval, with 30 µL solution containing 2.105 nmol of each pneumococcal peptide epitopes conjugated to SVLPs or with SVLP alone as a negative control. Subsequently mice were infected intranasally (i.n.) with 5x10^5 CFUs of strain ATCC6303 (serotype 3) two weeks after the last immunization, and survival was monitored for 10 or 14 days, along with other parameters (bodyweight, clinical

signs). Sera were collected by retro-orbital bleeding at 4 different timepoints (on D0, D14, D 28 and D42) and analyzed for the presence of antibodies against the peptide epitopes or for binding onto the surface of various bacterial serotypes.

Bacterial colonization. At days 3 and 6 post-infection of the three times immunized animals, blood was collected for bacterial enumeration. Additionally, lungs, livers and spleens were collected at the study endpoint (D52) or when mice were moribund for bacterial enumeration.

Serology. Antibody titers were evaluated using anti-peptide ELISA assays with the pneumococcal peptide epitope as coating antigens to detect serum IgG. Mice sera were incubated with each corresponding pneumococcal peptide epitope at different concentrations, and antibody levels were assessed using an anti-mouse IgG (HRP) secondary antibody, according to standard ELISA protocols.

Whole cell ELISA assay. Serotypes were grown overnight in Todd-Hewitt broth with 5% yeast extract (THY) at 37°C, till OD600 0.3-0.6 was reached. The culture was spun down and 100 µL bacterial suspensions (OD600 0.1) in PBS were used to coat high-binding microtiter ELISA plates in triplicates for 2 h at 37°C and under UV light for bacterial inactivation. Sera from three times immunized mice with a mixture of the three antigens as NPB/PR and PhtE, each presented on a SVLP, empty SVLP as a control or PCV13, diluted at 1:500, were applied onto the plates and incubated for 2 h at room temperature. After washing, plates were incubated with an anti-mouse IgG-HRP antibody for 1h. Signal was detected at OD450 after development with orthophenylenediamine (OPD) substrate at a concentration of 0.4 mg/mL in citrate-phosphate buffer (pH 5.0) with 0.012% hydrogen peroxide.

EXAMPLE 1

Synthesis of pneumococcal peptide epitopes

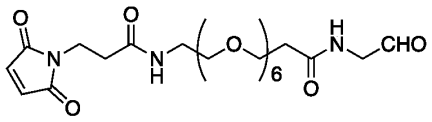
The pneumococcal peptide epitopes were synthesized using SPPS based on the Fmoc/tBu strategy employing a PurePep® Chorus synthesizer (Gyros Protein Technologies) or manual synthesis. AA couplings were performed using 10eq of DIC (Iris Biotech) and 5eq OxymaPure® (Iris Biotech) with 5 eq of Fmoc protected amino acids (Iris Biotech) according to the synthesis scale. Couplings were performed over 1h at room temperature (RT) with shaking (350 rpm). The resin used was Fmoc-Rink-Amide AM PS resin. The deprotection of the Fmoc groups was performed with 20% piperidine (Sigma Aldrich) solution in DMF (Iris Biotech), 2 times for 5 min.

Cleavage from the resin and simultaneous cleavage of the peptide sidechain protecting groups was accomplished by treatment of the dried resin with a deprotection cocktail of 95% TFA, 2.5% TIPS (Sigma Aldrich) and 2.5% H_2O for 180 min. A D-Ala (herein typically denoted as "a") was typically and preferably coupled to the C-terminus as the amide, in order to confer stability to exoproteases . The peptide was collected in a tube filled with cold diethyl ether (Sigma Aldrich) and precipitated dropwise. Centrifugation at 3000 rpm for 2 min followed. The pellet was then washed again 2 more times with diethyl ether. The crude product was purified by preparative High-Performance Liquid Chromatography (HPLC) with a TFA (Sigma Aldrich) system on a reversed-phase column with ACN (Biosolve) gradient elution and UV detection at 220 nm.

EXAMPLE 2

Synthesis of linker Mal-PEG6-aldehyde

Maleimide PEG6 aldehyde linker was synthesized by reacting SM-PEG_6 (Thermo Fisher Scientific; Mal-PEG6-NHS) with aminoacetaldehyde dimethyl acetal in H_2O.



SM-PEG_6 (7.6 mg, 12.6 µmol) was suspended in 0.3 ml H_2O and 17 µl of a 1:10 (v/v) solution of aminoacetaldehyde dimethyl acetal in H_2O was added. The mixture was stirred for 90 min. at RT. The linker was purified by RP-HPLC on a C8 column and lyophilized. For hydrolysis of the dimethyl acetal the linker (20 mg) was treated with 95% TFA, 5% H_2O for 5 min. The TFA was removed *in vacuo*.

EXAMPLE 3

Synthesis of pneumococcal peptide epitopes with linker

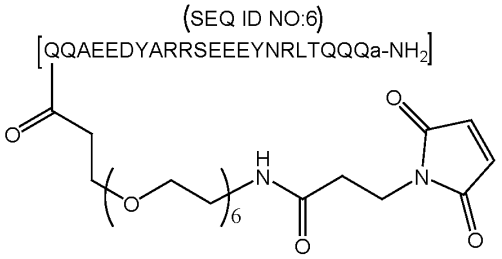
The syntheses of the pneumococcal peptide epitopes with linker allowing to link the pneumococcal peptide epitopes to a carrier such as to an SVLP, and hereby to a LBB of an SVLP, said linker is coupled to the pneumococcal peptide epitopes, preferably to the N-terminus of the pneumococcal peptide epitopes.

The synthesis of the pneumococcal peptide epitopes was effected as described above in Example 1. Once the pneumococcal peptide epitopes were fully synthesized, the N-terminal Fmoc protecting group were removed and the linker, for example, Mal-PEG6-COOH (2.5eq) using a solution of PyAOP (10eq, Iris Biotech) and DIPEA (10eq) in DMF was added for 3h at

RT with continuous shaking (350 rpm). After this period the resin was washed 4 times with DMF and 4 times with DCM. The resin was then dried under N_2 flow for 10 min.

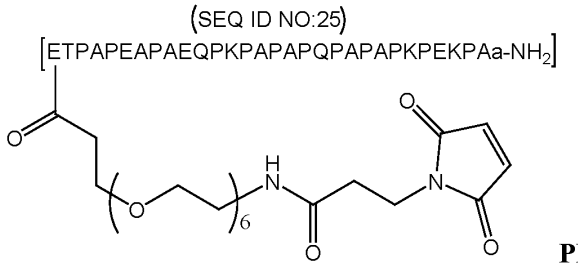
The cleavage of the pneumococcal peptide epitopes with linker from the resin and simultaneous cleavage of the peptide sidechain protecting groups was accomplished in a similar way to the pneumococcal peptide epitopes, by treatment of the dried resin with a deprotection cocktail of 95% TFA, 2.5% TIPS (Sigma Aldrich) and 2.5% H_2O for 180 min. The peptide was collected in a tube filled with cold diethyl ether (Sigma Aldrich) and precipitated dropwise. Centrifugation at 3000 rpm for 2 min followed. The pellet was then washed again 2 more times with diethyl ether. The crude product was purified by preparative High-Performance Liquid Chromatography (HPLC) with a TFA (Sigma Aldrich) system on a reversed-phase column with ACN (Biosolve) gradient elution and UV detection at 220 nm.

Representative and very preferred examples of pneumococcal peptide epitopes with preferred linker comprising a maleimide functionality for further conjugation to a carrier, preferably to a LBB of a SVLP are **NPB1-Mal**, **PR2-Mal** and **PhtE3-Mal** having the formula as depicted in the following:



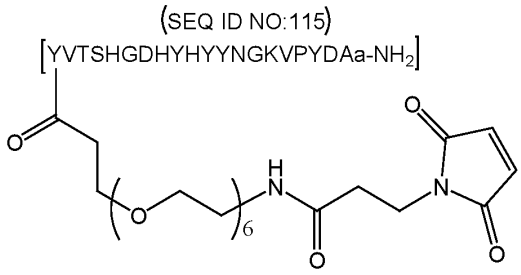
NPB1-Mal comprising the very preferred first pneumococcal peptide epitope of SEQ ID NO:6.

NPB1-Mal was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) at 40°C using a gradient of 5 to 95% B over 20 min [A: H2O/TFA 99.97/0.03 and B: MeCN/TFA 99.97/0.03] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **NPB1-Mal** was calculated for $C_{138}H_{215}N_{41}O_{55}$, MW: 3328.5 with an expected ESI $[M+3H]^{3+}$:1110.5, observed $[M+3H]^{3+}$:1110.3.



PR2-Mal comprising the very preferred second pneumococcal peptide epitope of SEQ ID NO:25.

PR2-Mal was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) at 40 °C using a gradient of 5 to 95% B over 20 min [A: H2O/TFA 99.97/0.03 and B: MeCN/TFA 99.97/0.03] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **PR2-Mal** was calculated for $C_{161}H_{253}N_{39}O_{52}$, MW: 3567.0 with an expected ESI $[M+3H]^{3+}$:1190.0, observed $[M+3H]^{3+}$:1189.7.



PhtE3-Mal comprising the very preferred third pneumococcal peptide epitope of SEQ ID NO:115.

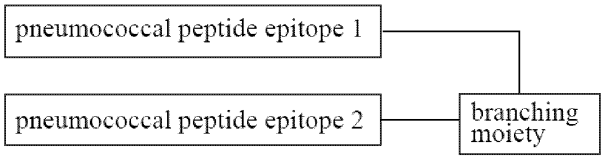
PhtE3-Mal was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Hypersil GOLD C18 column (150 mm x 2.1 mm, 1.9 µm) (Thermo Fisher, USA) at 60 °C using a gradient of 10 to 30% B over 20 min [A: H2O/TFA 99.85/0.15 and B: MeCN/TFA 99.85/0.15] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **PhtE3-Mal** was calculated for $C_{135}H_{184}N_{32}O_{43}$, MW: 2943.1 with an expected ESI $[M+3H]^{3+}$:982.1, observed $[M+3H]^{3+}$: 982.8.

EXAMPLE 4

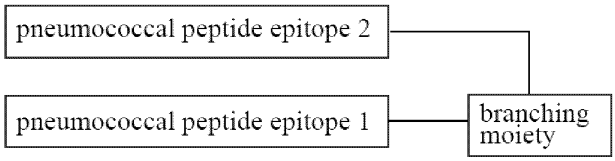
Synthesis of branched pneumococcal antigens

The structure of a branched pneumococcal antigen comprising a first and a second pneumococcal peptide epitope is schematically illustrated below, wherein said branched

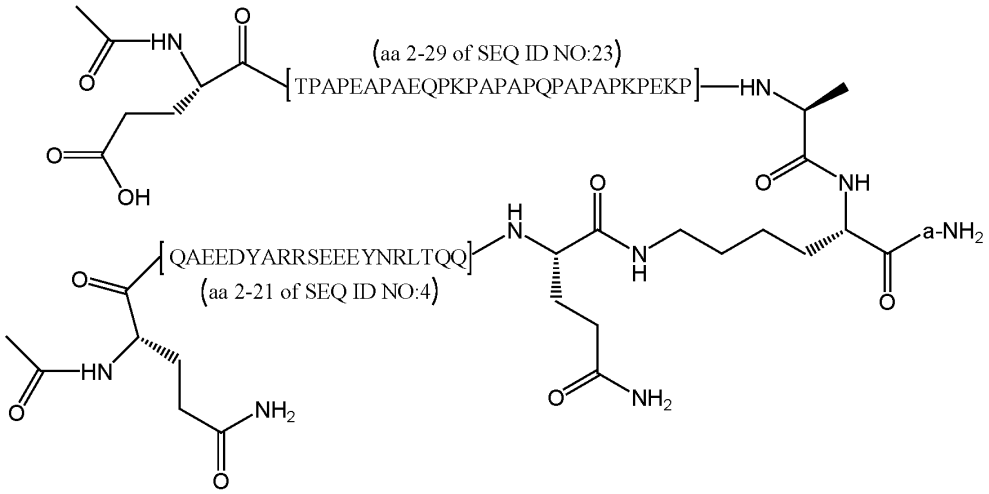
pneumococcal antigen comprises a branching moiety allowing to covalently link two or more, preferably the represented two pneumococcal peptide epitopes leading to a bi-epitope branched pneumococcal antigen.



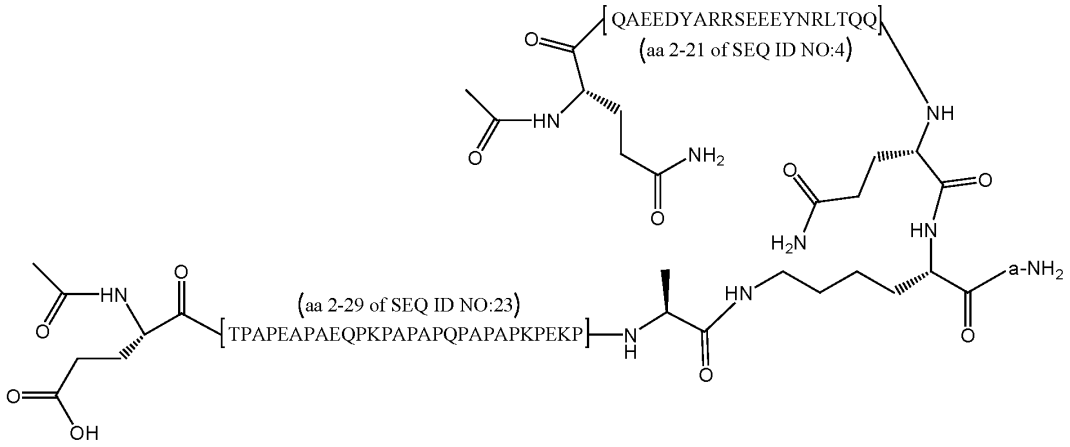
It is of note that the present invention also provides branched pneumococcal antigens, preferably bi-epitope branched pneumococcal antigens, having the opposite branching with respect to the main peptide chain as referred below.



The chemical structure of preferred bi-epitope branched pneumococcal antigens **BPA-1** and **BPA-3** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, and D-Ala is represented as a small cap "a".

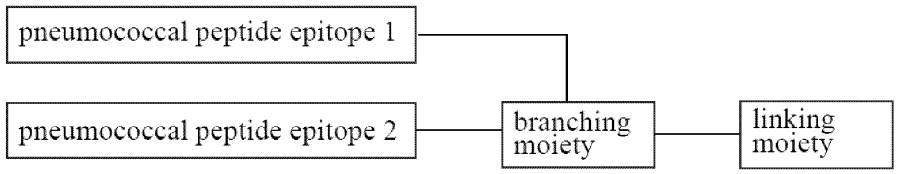


BPA-1

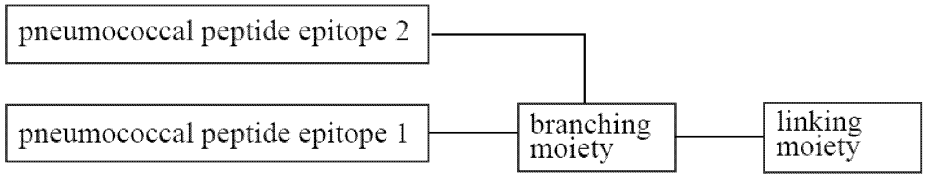


BPA-3

If said branched pneumococcal antigen, preferably said branched bi-epitope pneumococcal antigen, is to be further connected to a carrier such as, preferably, to a SVLP, and hereby to a LBB of a SVLP, said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, typically and preferably further comprises a linking moiety allowing to covalently link said branched pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, to said carrier such as, preferably, to said SVLP, and hereby to a LBB of a SVLP as illustrated below.



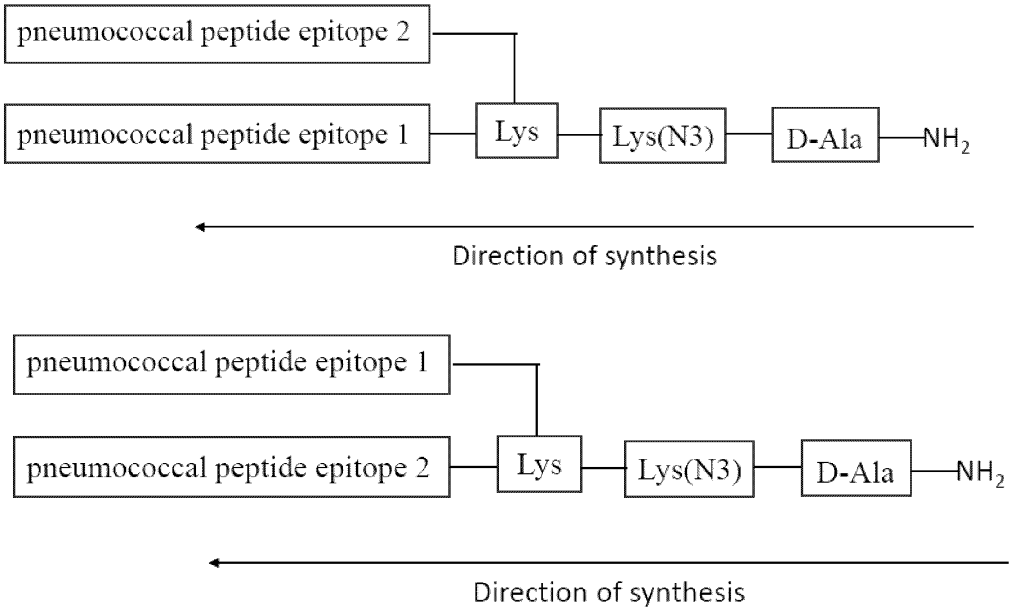
It is of note that the present invention also provides branched pneumococcal antigens, preferably bi-epitope branched pneumococcal antigens, having the opposite branching with respect to the main chain as depicted below.



The synthesis of said branched bi-epitope pneumococcal antigens are described in the following by way of the syntheses of the preferred bi-epitope branched pneumococcal antigens **BPA-2** and **BPA-4**, both comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the second pneumococcal peptide epitope of SEQ ID NO:23 but having the opposite

branching with respect to the main chain. Said preferred bi-epitope branched pneumococcal antigens **BPA-2** and **BPA-4** comprising the first pneumococcal peptide epitope of SEQ ID NO:4 and the second pneumococcal peptide epitope of SEQ ID NO:23 further comprises a lysine residue as branching moiety and an additional lysine residue as linking moiety which linking moiety is modified to contain a terminal azide group allowing for linking to a carrier, preferably, as further described below, to a SVLP, and hereby to a LBB of a SVLP. Said preferred branched bi-epitope pneumococcal antigens **BPA-2** and **BPA-4** further comprise N-terminal amine protecting groups such as acetyl groups as well as an amidated C-terminal D-alanine residue which is believed to increase the stability and protect the C-terminus of said branched bi-epitope pneumococcal antigen.

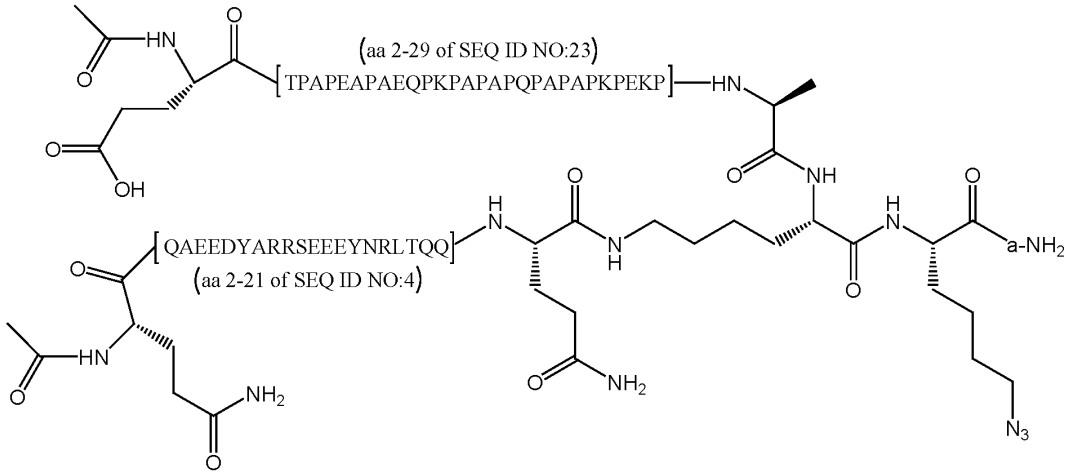
The schematic structures of preferred branched bi-epitope pneumococcal antigens is shown below.



It is of note and as shown in the illustrations, said synthesis is, preferably, effected starting from the C-terminus, and the synthesis of pneumococcal peptide epitope 1 or pneumococcal peptide epitope 2 will be completed before the synthesis of the pneumococcal peptide epitope 2 or pneumococcal peptide epitope 1 is initiated. Hereto, the lysine residue representing the branching moiety is, preferably, protected with a protecting group that can be released by 1% TFA after synthesis of the pneumococcal peptide epitope 1 or pneumococcal peptide epitope 2.

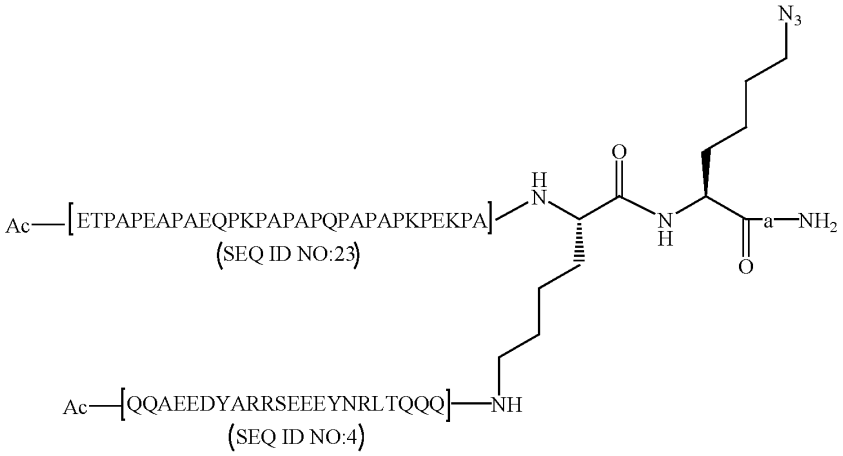
The chemical structure of said preferred branched bi-epitope pneumococcal antigen **BPA-2** is shown below, wherein amino acids are represented as their chemical structure or their one-

letter abbreviations, while D-Ala is represented as a small cap “a”.



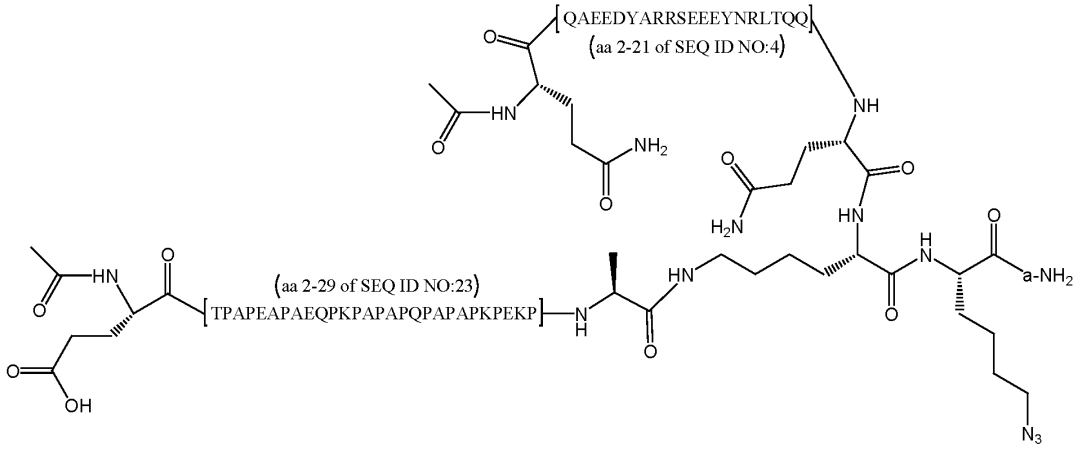
BPA-2

The more simplified representation of **BPA-2** is as follows:



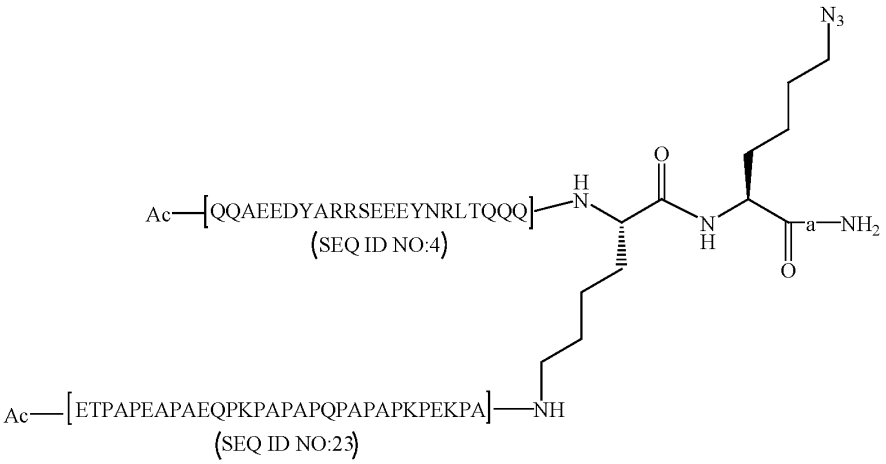
wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23.

The chemical structure of the preferred branched bi-epitope pneumococcal antigen **BPA-4** is shown below, wherein amino acids are represented as their chemical structure or their one-letter abbreviations, while D-Ala is represented as a small cap “a”.



BPA-4

The more simplified representation of **BPA-4** is as follows:



wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23.

In detail, the syntheses of said preferred branched bi-epitope pneumococcal antigens **BPA-2** and **BPA-4** are described. The syntheses of **BPA-2** and **BPA-4** were divided in two parts due to its branched character. The main chain represents the peptide chain that is elongated from the initiating resin. The branch represents the peptide chain that is elongated through the quasi orthogonal protected Lys sidechain (Fmoc-L-Lys(Mmt)-OH). Thus, the present invention also allows branched pneumococcal antigens, preferably branched bi-epitope pneumococcal antigens, having the opposite branching with respect to the main chain.

The syntheses of **BPA-2** and **BPA-4** were performed using solid phase peptide synthesis (SPPS) based on the Fmoc/*t*Bu strategy employing a PurePep® Chorus synthesizer (Gyros Protein Technologies). Amino acid (AA) couplings were performed using *N,N'*-

Diisopropylcarbodiimide (DIC, Iris Biotech) and OxymaPure® (Ethyl cyanoglyoxylate-2-oxime; Iris Biotech) with an excess of 4eq of fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (AAs) (Iris Biotech) according to the synthesis scale. Couplings were performed for 1h at room temperature (RT) with shaking (350 rpm). The resin used for this synthesis was Rink Amide ChemMatrix® resin (0.42 mmol/g) (Biotage). Alternatively, Fmoc-Rink-Amide-TentaGel XV was used providing the same results. The deprotection of the Fmoc groups was performed with 20% piperidine (Sigma Aldrich) solution in dimethylformamide (DMF, Iris Biotech), 2 times for 5 min.

To branch the peptide the quasi orthogonal building block Fmoc-L-Lys(Mmt)-OH (N-alpha-(9-Fluorenylmethyloxycarbonyl)-N-epsilon-4-methoxytrityl-L-lysine; Iris Biotech) was used. The 4-methoxytrityl (Mmt) group can be selectively deprotected with a solution containing a 1-2% of trifluoroacetic acid (TFA). Finally, to enable a linker insertion through copper-azide alkyne cycloaddition (CuAAC) (click chemistry) the Fmoc-L-Lys(N_3)-OH (N-alpha-(9-Fluorenylmethyloxycarbonyl)-epsilon-azido-L-lysine, Iris Biotech) was introduced. A Fmoc-D-Ala-OH (Iris Biotech) amino acid was used at the C-terminus.

The main chain was synthesized according to the procedures described above. Once the main chain was completed, the peptide was N-terminally deprotected from its Fmoc group and capped with a solution of 25eq of acetic acid anhydride and 25eq of *N*,*N*-Diisopropylethylamine (DIPEA, Sigma Aldrich) in DMF for 30 min. After capping, the peptide was washed with DMF and dichloromethane (DCM, Iris Biotech). The Lys(Mmt) group was selectively deprotected with a solution of 2% TFA, 3% TIPS, 95% DCM. To fully deprotect this 4-methoxytrityl (Mmt) group this step was repeated 4 times for 30 min each time. Once the (Mmt) group was selectively deprotected, the resin was washed 4 times with DCM and 4 times with DMF, leaving the sidechain of this Lys unprotected and ready for the synthesis of the branch of **BPA-2** and **BPA-4**.

The branch was synthesized following the procedures described above. When the branch synthesis was completed, the N-terminal Fmoc protecting group was removed and capped with a solution of 25eq of acetic acid anhydride and 25eq of DIPEA in DMF for 30 min. Following capping, the resin was washed 4x with DMF and 4x with DCM. The resin was then dried under N_2 flow for 10 min.

Cleavage from the resin and simultaneous cleavage of the peptide sidechain protecting groups was accomplished by treatment of the dried resin with a deprotection cocktail of 95% TFA, 2.5% Triisopropylsilane (TIPS, Sigma Aldrich) and 2.5% H_2O for 180 min. The peptide was collected in a tube filled with cold diethyl ether (Sigma Aldrich) and precipitated dropwise.

Centrifugation at 3000 rpm for 2 min followed. The pellet was then washed again 2 more times with diethyl ether. The crude product was purified by preparative High-Performance Liquid Chromatography (HPLC) with a TFA system on a reversed-phase column with acetonitrile (Acn) gradient elution and UV detection at 220 nm.

BPA-2 was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, a Hypersil GOLD C18 column (150 mm x 2.1 mm, 1.9 µm) (Thermo Fisher, USA) at 60 °C using a linear gradient of 10 to 34% B over 32 min [A: H2O/TFA 99.85/0.15 and B: MeCN/TFA 99.85/0.15] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **BPA-2** was calculated for $C_{268}H_{418}N_{80}O_{90}$, MW: 6200.76 with an expected ESI $[M+5H]^{5+}$:1241.2, observed $[M+5H]^{5+}$: 1241.9.

BPA-4 was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, a Hypersil GOLD C18 column (150 mm x 2.1 mm, 1.9 µm) (Thermo Fisher, USA) at 60 °C using a linear gradient of 10 to 34% B over 32 min [A: H2O/TFA 99.85/0.15 and B: MeCN/TFA 99.85/0.15] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **BPA-4** was calculated for $C_{268}H_{418}N_{80}O_{90}$, MW: 6200.76 with an expected ESI $[M+5H]^{5+}$:1241.2, observed $[M+5H]^{5+}$: 1241.9.

In analogous manner and following the same synthesis strategy as for **BPA-2** and **BPA-4**, the syntheses of **BPA-6** and **BPA-8** has been effected. Thus, the syntheses of **BPA-6** and **BPA-8** were divided in two parts due to its branched character. The main chain represents the peptide chain that is elongated from the initiating resin. The branch represents the peptide chain that is elongated through the quasi orthogonal protected Lys sidechain (Fmoc-L-Lys(Mmt)-OH). Thus, the present invention also allows branched pneumococcal antigens, preferably branched bi-epitope pneumococcal antigens, having the opposite branching with respect to the main chain.

The syntheses of **BPA-6** and **BPA-8** were performed using solid phase peptide synthesis (SPPS) based on the Fmoc/*t*Bu strategy employing a PurePep® Chorus synthesizer (Gyros Protein Technologies). Amino acid (AA) couplings were performed using *N,N'*-Diisopropylcarbodiimide (DIC, Iris Biotech) and OxymaPure® (Ethyl cyanoglyoxylate-2-oxime; Iris Biotech) with an excess of 10eq of fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (AAs) (Iris Biotech) according to the synthesis scale. Couplings were performed for 1h at room temperature (RT) with shaking (350 rpm). The resin used for this synthesis was TentaGel XV (0.24 mmol/g). The deprotection of the Fmoc groups was performed with 20%

piperidine (Sigma Aldrich) solution in dimethylformamide (DMF, Iris Biotech), 2 times for 5 min.

To branch the peptide the quasi orthogonal building block Fmoc-L-Lys(Mmt)-OH (N-alpha-(9-Fluorenylmethyloxycarbonyl)-N-epsilon-4-methoxytrityl-L-lysine; Iris Biotech) was used. The 4-methoxytrityl (Mmt) group can be selectively deprotected with a solution containing a 1-2% of trifluoroacetic acid (TFA). Finally, to enable a linker insertion through copper-azide alkyne cycloaddition (CuAAC) (click chemistry) the Fmoc-L-Lys(N_3)-OH (N-alpha-(9-Fluorenylmethyloxycarbonyl)-epsilon-azido-L-lysine, Iris Biotech) was introduced. A Fmoc-D-Ala-OH (Iris Biotech) amino acid was used at the C-terminus.

The main chain was synthesized according to the procedures described above. Once the main chain was completed, the peptide was N-terminally deprotected from its Fmoc group and capped with a solution of 25eq of acetic acid anhydride and 25eq of *N*,*N*-Diisopropylethylamine (DIPEA, Sigma Aldrich) in DMF for 30 min. After capping, the peptide was washed with DMF and dichloromethane (DCM, Iris Biotech). The Lys(Mmt) group was selectively deprotected with a solution of 2% TFA, 3% TIPS, 95% DCM. To fully deprotect this 4-methoxytrityl (Mmt) group this step was repeated 4 times for 30 min each time. Once the (Mmt) group was selectively deprotected, the resin was washed 4 times with DCM and 4 times with DMF, leaving the sidechain of this Lys unprotected and ready for the synthesis of the branch of **BPA-6** and **BPA-8**

The branch was synthesized following the procedures described above. When the branch synthesis was completed, the N-terminal Fmoc protecting group was removed and capped with a solution of 25eq of acetic acid anhydride and 25eq of DIPEA in DMF for 30 min. Following capping, the resin was washed 4x with DMF and 4x with DCM. The resin was then dried under N_2 flow for 10 min.

Cleavage from the resin and simultaneous cleavage of the peptide sidechain protecting groups was accomplished by treatment of the dried resin with a deprotection cocktail of 95% TFA, 2.5% Triisopropylsilane (TIPS, Sigma Aldrich) and 2.5% H_2O for 180 min. The peptide was collected in a tube filled with cold diethyl ether (Sigma Aldrich) and precipitated dropwise. Centrifugation at 3000 rpm for 2 min followed. The pellet was then washed again 2 more times with diethyl ether. The crude product was purified by preparative High-Performance Liquid Chromatography (HPLC) with a TFA system on a reversed-phase column with acetonitrile (Acn) gradient elution and UV detection at 220 nm.

BPA-6 was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module

(MS) and a TUV detector (UV) set at 210 nm, a ACQUITY BEH C8 column (150 mm x 2.1 mm, 1.7 μm) (Waters Corporation, USA) at 40 °C using a linear gradient of 5 to 60% B over 18 min [A: H_2O/FA 99.97/0.03 and B: MeCN/FA 99.97/0.03] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **BPA-6** was calculated for $C_{242}H_{349}N_{73}O_{81}$, MW: 5576.88 with an expected ESI $[M+7H]^{7+}$:797.7, observed $[M+7H]^{7+}$: 798.9.

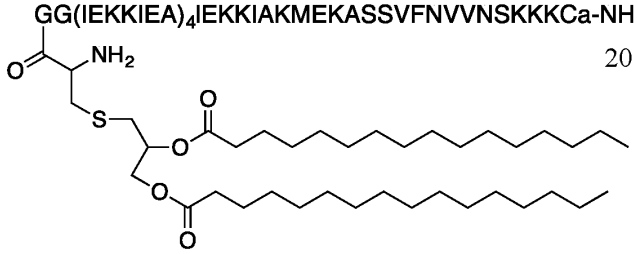
BPA-8 was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, a ACQUITY BEH C8 column (150 mm x 2.1 mm, 1.7 μm) (Waters Corporation, USA) at 40 °C using a linear gradient of 5 to 60% B over 18 min [A: H2O/FA 99.97/0.03 and B: MeCN/FA 99.97/0.03] with a flow of 0.4 mL min^{-1}. The expected molecular weight for **BPA-8** was calculated for $C_{265}H_{387}N_{71}O_{78}$, MW: 5815.43 with an expected ESI $[M+7H]^{7+}$:831.8, observed $[M+7H]^{7+}$: 832.7.

EXAMPLE 5

Synthesis of lipopeptide building blocks (LBBs)

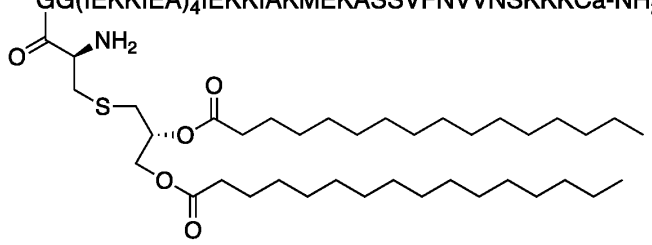
Lipopeptide building blocks described and used herein were synthesized, purified and characterized as described in WO 2008/068017 and, in particular, in WO 2020/127728. Specifically, it is hereby referred to Example 1 of WO 2020/127728 for the synthesis and preparation of **LBB-1**, **LBB-2**, **LBB-4** and **LBB-5:**

GG(IEKKIEA)$_4$IEKKIAKMEKASSVFNVVNSKKKCa-NH_2 SEQ ID NO:227



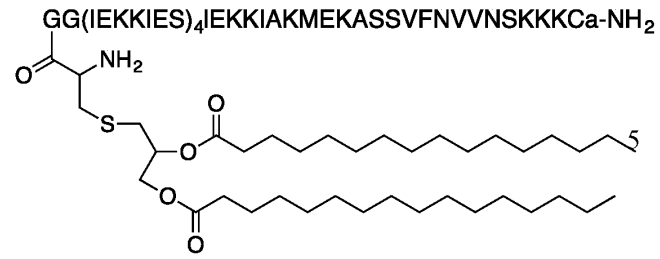
LBB-1

GG(IEKKIEA)$_4$IEKKIAKMEKASSVFNVVNSKKKCa-NH_2 SEQ ID NO:228



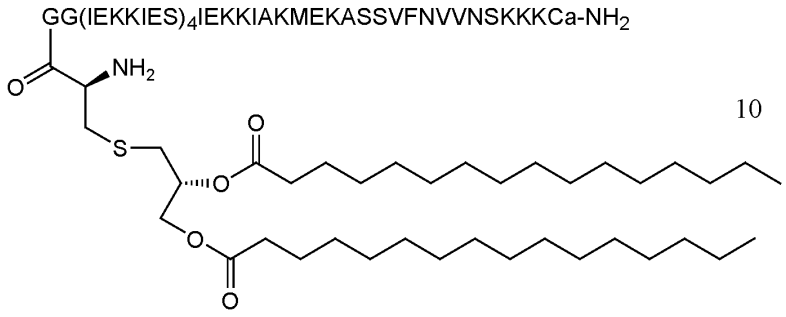
LBB-2

SEQ ID NO:229



LBB-4

SEQ ID NO:230

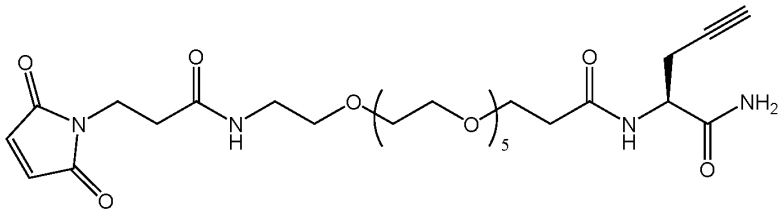


LBB-5

EXAMPLE 6

Synthesis of linker Mal-PEG6-Pra

The linker **Mal-PEG6-Pra** containing an alkyne maleimide moiety was synthesized using SPPS employing a PurePep® Chorus synthesizer (Gyros Protein Technologies) or manual synthesis.



Mal-PEG6-Pra

The Fmoc-L-Pra-OH coupling was performed using 10eq of DIC (Iris biotech) and 5eq OxymaPure® (Iris biotech) with 5 eq of Fmoc protected amino acid (Iris Biotech) according to the synthesis scale. The coupling was performed for 1h at RT with shaking (350 rpm). The resin used was Fmoc-Rink-Amide AM PS resin. The deprotection of the Fmoc group was performed with 20% piperidine (Sigma Aldrich) solution in DMF (Iris Biotech), 2 times for 5 min.

Once the first AA was attached to the resin and the N-terminal Fmoc protecting group removed, the linker Mal-PEG6-COOH (2.5eq) was added using a solution of PyAOP (10 eq, Iris Biotech) and DIPEA(10 eq) in DMF over 3h at RT with continuous shaking (350 rpm).

After this period the resin was washed 4 times with DMF and 4 times with DCM. The resin was then dried under N2 flow during 10 min.

Cleavage from the resin was accomplished by treatment of the dried resin with a deprotection cocktail of 95% TFA, 2.5% TIPS (Sigma Aldrich) and 2.5% H_2O for 180 min. The cleavage cocktail was collected on a falcon and the TFA evaporated under N_2 flow. Once the TFA was evaporated the crude linker was dissolved and purified by preparative High-Performance Liquid Chromatography (HPLC) with a TFA (Sigma Aldrich) system on a reversed-phase column with ACN (Biosolve) gradient elution and UV detection at 220 nm.

EXAMPLE 7

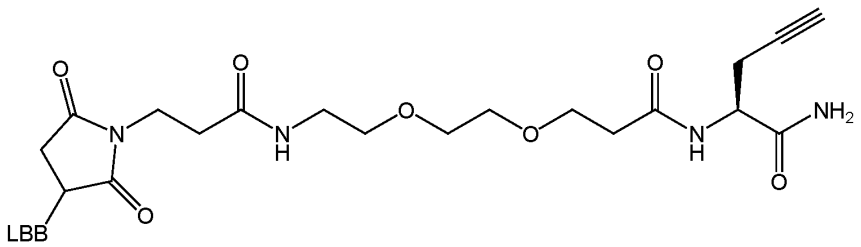
Synthesis of Conjugate Intermediates

In accordance with the present invention, pneumococcal antigens and peptide epitopes were presented on LBBs forming conjugates leading to the SLVPs as described herein. Further, in accordance with the present invention, the generation of conjugates, and hereby the linking of the LBB with the pneumococcal antigens and peptide epitopes by way of a linker, can either be effected by, first, attaching the linker to LBB and then couple the resulting construct with the pneumococcal antigens and peptide epitopes, or by, first, attaching the linker to the pneumococcal antigens and peptide epitopes and then the couple the resulting construct to the LBB. Conjugate preparation or the syntheses of the linker comprising intermediates are exemplarily described herein for preferred conjugates and linker comprising intermediates. Analogous procedures can be applied for the synthesis of further conjugates and linker comprising intermediates in accordance with the present invention.

EXAMPLE 8

Conjugation of Mal-PEG6-Pra to LBB

The preferred conjugate intermediate Mal-PEG6-Pra-LBB was synthesized as indicated below.



Mal-PEG6-Pra-LBB

In detail, the synthesis specifically refers to the synthesis of the preferred Mal-PEG6-Pra-**LBB-5**. Thus, the Mal-PEG6-Pra linker was added in a 1.2 eq excess to a solution of **LBB-5** in MilliQ® H_2O (MQ H_2O). The solution was vortexed and sonicated until everything was homogenously dissolved, and its pH was adjusted to pH 5.5-6. The conjugation reaction was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters). Once the **LBB-5** was exhausted, the conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm.

Mal-PEG6-Pra-LBB-5 was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) at 26 °C using a gradient of 20 to 80% B over 60 min [A: H_2O/TFA 99.97/0.03 and B: MeCN/TFA 99.97/0.03] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for **Mal-PEG6-Pra-LBB-5** was calculated for $C_{339}H_{594}N_{78}O_{100}S_3$, MW: 7459.11 with an expected ESI $[M+6H]^{6+}$:1244.2, observed $[M+10H]^{10+}$:1244.9.

EXAMPLE 9

Synthesis of Conjugates

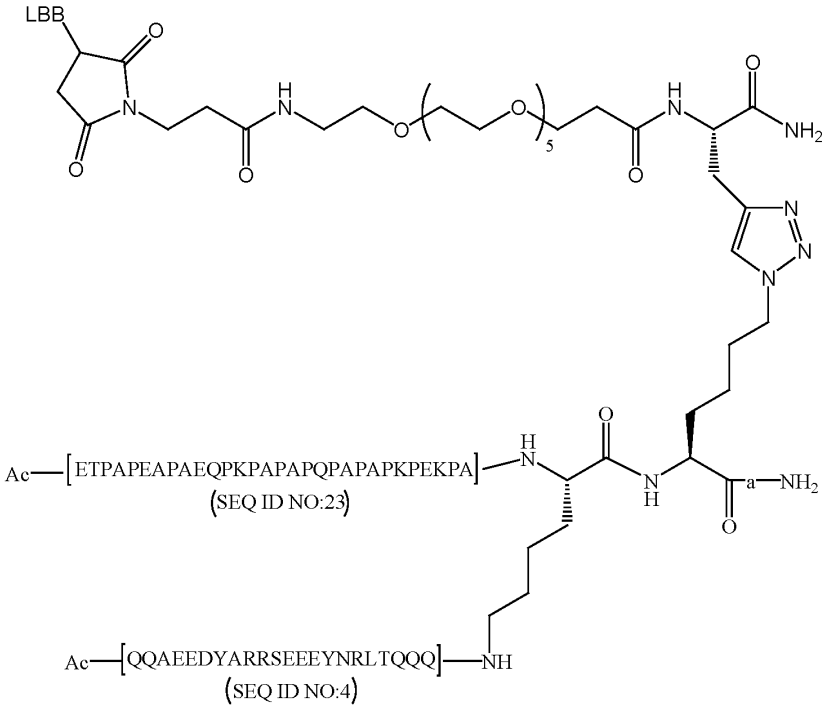
In accordance with the present invention, pneumococcal antigens and peptide epitopes can be presented on a carrier, preferably on a LBB forming conjugates leading to the SLVPs as described herein. As further described below, inventive pneumococcal antigens and peptide epitopes were presented on a synthetic virus-like particle (SVLP) platform for evaluation as vaccine candidates. Conjugate preparation is exemplarily described herein for preferred conjugates leading to preferred SVLPs. Analogous procedures can be applied for the synthesis of further conjugates in accordance with the present invention.

The pH for conjugations forming inventive conjugates involving either maleimide-thiol reaction or Copper Catalyzed Alkyne Azide Cycloaddition (CuAAC) was adjusted with 0.1M NaOH (Sigma Aldrich) aqueous solution. Copper Catalyzed Alkyne Azide Cycloaddition (CuAAC) was performed by mixing $CuSO_4$ (Sigma Aldrich) and tris(benzyltriazolylmethyl)amine (THPTA, Click Chemistry Tools) and the posterior addition of sodium ascorbate (Sigma Aldrich).

EXAMPLE 10

Synthesis of Conjugate 21, Conjugate 22, Conjugate 23 and Conjugate 24

The formula of the very preferred Conjugate **21** is depicted below.



Conjugate **21**,

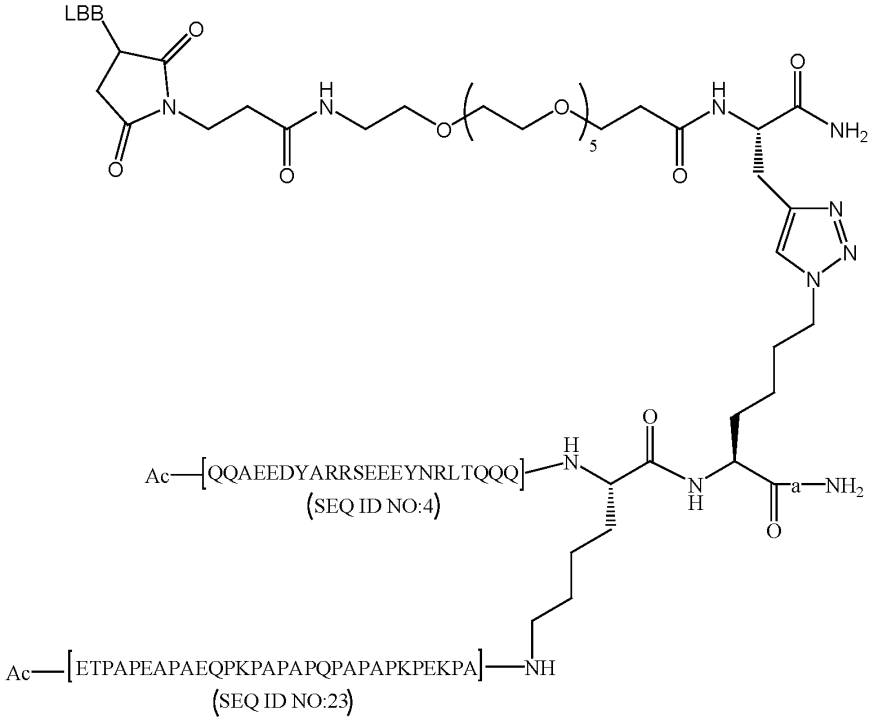
wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23, and LBB refers to the preferred **LBB-5**.

To generate the preferred Conjugate **21**, 1.2eq of the azide containing branched bi-epitope pneumococcal antigen **BPA-2** and the alkyne containing LBB moiety **Mal-PEG6-Pra-LBB-5** were dissolved in MQ H_2O, vortexed and sonicated until a homogenous solution was obtained. The pH was adjusted to 7.4, and 3eq of copper (II) sulfate and Tris(benzyltriazolylmethyl)amine (THPTA) were added. After 30 seconds of mixing, 30eq of sodium ascorbate solution were added. The conjugation reaction was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters) until **Mal-PEG6-Pra-LBB-5** was exhausted. The conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm. At the final step, the salt exchange from trifluoroacetate to hydrochloride was performed using a Dowex chloride resin or by dissolving and freeze drying the sample 3 times in a 5mM HCl solution.

Conjugate **21** was analyzed with Waters Acquity ultrahigh-pressure liquid

chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) with a BEH C8 column at 50°C, using a linear gradient from 10 to 85% B during 75 min, using a binary solvent system [A: H2O/TFA 99.9/0.1 (v/v) and B:MeCN/TFA 99.9/0.1 (v/v)] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **21** was calculated for $C_{607}H_{1012}N_{158}O_{190}S_3$, MW: 13651.4 with an expected ESI $[M+16H]^{16+}$:854.7, observed $[M+16H]^{16+}$:855.7.

The formula of the very preferred Conjugate **22** is depicted below.



Conjugate **22**,

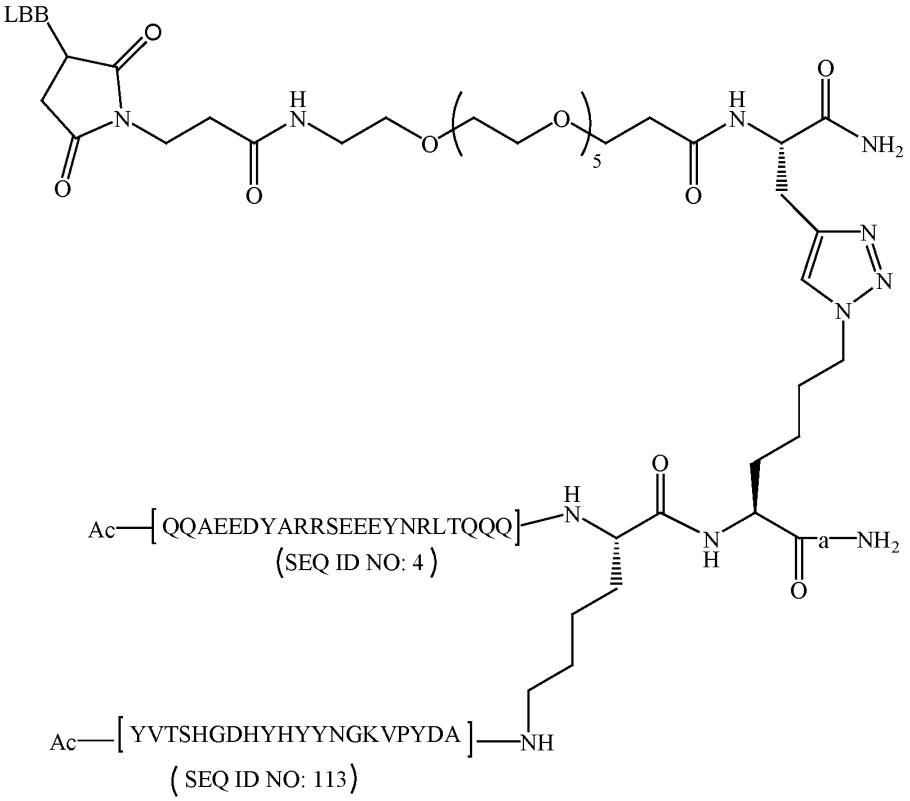
wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:23, and LBB refers to the preferred **LBB-5**.

To generate the preferred Conjugate **22**, 1.2eq of the azide containing branched bi-epitope pneumococcal antigen **BPA-4** and the alkyne containing LBB moiety **Mal-PEG6-Pra-LBB-5** were dissolved in MQ H_2O, vortexed and sonicated until a homogenous solution was obtained. The pH was adjusted to 7.4, and 3eq of copper (II) sulfate and Tris(benzyltriazolylmethyl)amine (THPTA) were added. After 30 seconds of mixing, 30eq of sodium ascorbate solution were added. The conjugation reaction was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters) until **Mal-PEG6-Pra-LBB-5** was exhausted.

The conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm. At the final step, the salt exchange from trifluoroacetate to hydrochloride was performed using a Dowex chloride resin or by dissolving and freeze drying the sample 3 times in a 5mM HCl solution.

Conjugate **22** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) with a BEH C8 column at 50°C, using a linear gradient from 10 to 85% B during 75 min, using a binary solvent system [A: H2O/TFA 99.9/0.1 (v/v) and B:MeCN/TFA 99.9/0.1 (v/v)] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **22** was calculated for $C_{607}H_{1012}N_{158}O_{190}S_3$, MW: 13651.4 with an expected ESI $[M+16H]^{16+}$:854.7, observed $[M+16H]^{16+}$:855.8.

The formula of Conjugate **23** is depicted below.



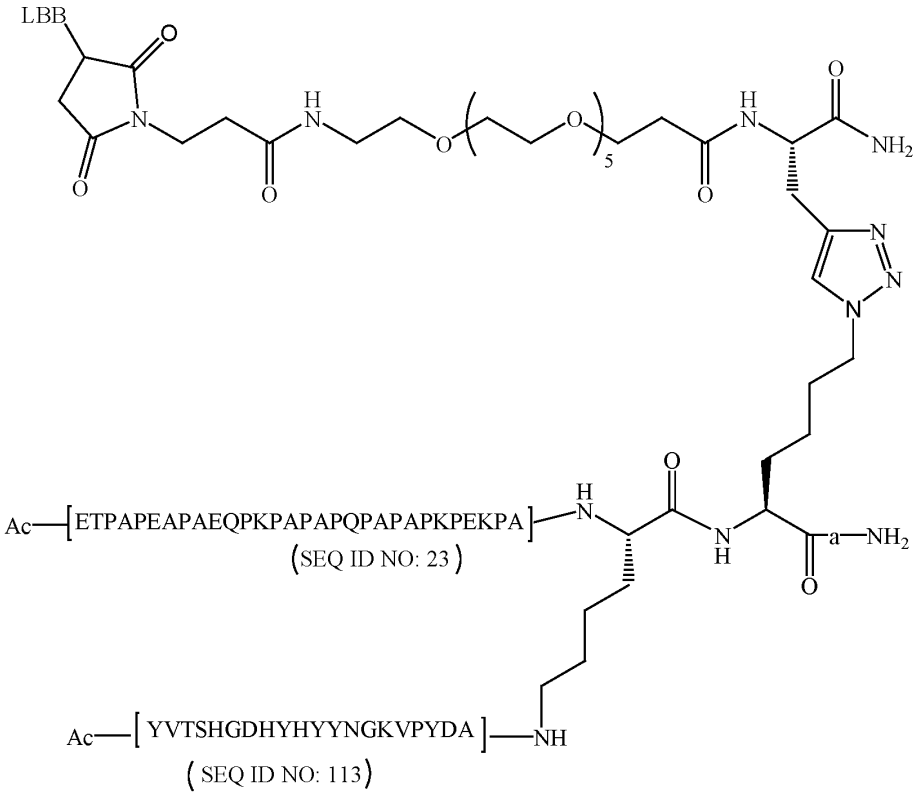
Conjugate **23**,

wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:4 and SEQ ID NO:113, and LBB refers to the preferred **LBB-5**.

To generate the preferred Conjugate **23**, 1.2eq of the azide containing branched bi-epitope pneumococcal antigen **BPA-6** and the alkyne containing LBB moiety **Mal-PEG6-Pra-LBB-5** were dissolved in MQ H_2O, vortexed and sonicated until a homogenous solution was obtained. The pH was adjusted to 7.4, and 3eq of copper (II) sulfate and Tris(benzyltriazolylmethyl)amine (THPTA) were added. After 30 seconds of mixing, 30eq of sodium ascorbate solution were added. The conjugation reaction was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters) until **Mal-PEG6-Pra-LBB-5** was exhausted. The conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm. At the final step, the salt exchange from trifluoroacetate to hydrochloride was performed using a Dowex chloride resin or by dissolving and freeze drying the sample 3 times in a 5mM HCl solution.

Conjugate **23** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) with a BEH C8 column at 50°C, using a linear gradient from 10 to 85% B during 75 min, using a binary solvent system [A: H_2O/TFA 99.9/0.1 (v/v) and B:MeCN/TFA 99.9/0.1 (v/v)] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **23** was calculated for $C_{581}H_{943}N_{151}O_{181}S_3$, MW: 13035.99 with an expected ESI $[M+16H]^{16+}$:815.8, observed $[M+16H]^{16+}$:816.9.

The formula of Conjugate **24** is depicted below.



Conjugate **24**,

wherein Ac represents acetyl residues as amino protecting group protecting the N-terminus of the SEQ ID NO:23 and SEQ ID NO:113, and LBB refers to the preferred **LBB-5**.

To generate the preferred Conjugate **24**, 1.2eq of the azide containing branched bi-epitope pneumococcal antigen **BPA-8** and the alkyne containing LBB moiety **Mal-PEG6-Pra-LBB-5** were dissolved in MQ H_2O, vortexed and sonicated until a homogenous solution was obtained. The pH was adjusted to 7.4, and 3eq of copper (II) sulfate and Tris(benzyltriazolylmethyl)amine (THPTA) were added. After 30 seconds of mixing, 30eq of sodium ascorbate solution were added. The conjugation reaction was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters) until **Mal-PEG6-Pra-LBB-5** was exhausted. The conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm. At the final step, the salt exchange from trifluoroacetate to hydrochloride was performed using a Dowex chloride resin or by dissolving and freeze drying the sample 3 times in a 5mM HCl solution.

Conjugate **24** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH

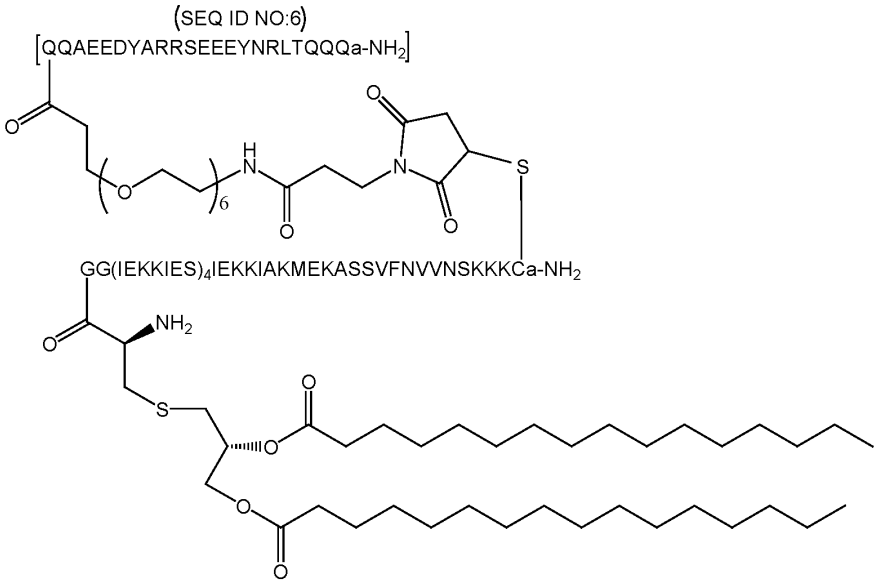
C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) with a BEH C8 column at 50°C, using a linear gradient from 10 to 85% B during 75 min, using a binary solvent system [A: H_2O/TFA 99.9/0.1 (v/v) and B:MeCN/TFA 99.9/0.1 (v/v)] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **24** was calculated for $C_{604}H_{981}N_{149}O_{178}S_3$, MW: 13274.54 with an expected ESI $[M+16H]^{16+}$:830.7, observed $[M+16H]^{16+}$:832.0.

EXAMPLE 11

Synthesis of linear Conjugates

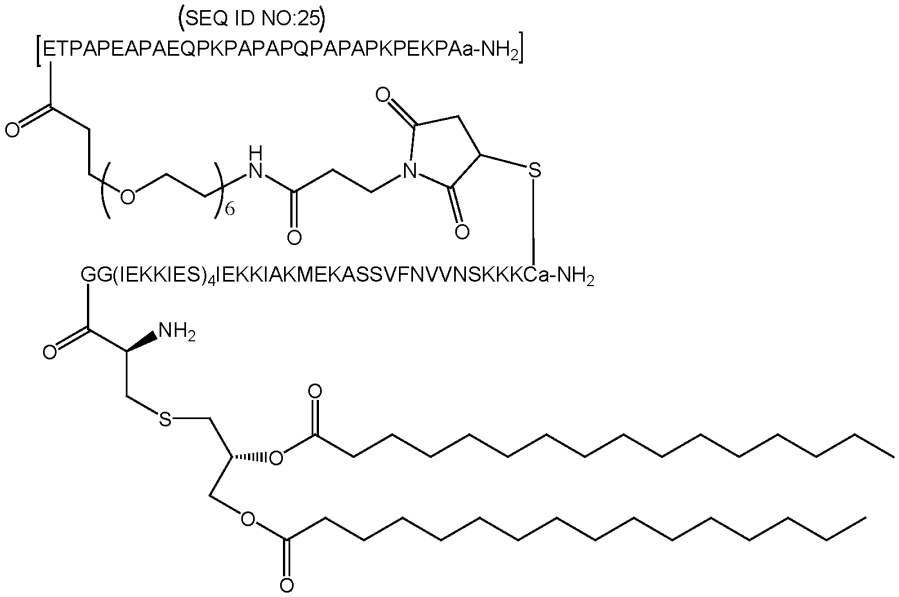
The linear pneumococcal peptide epitopes containing a maleimide moiety, in particular the very preferred examples of pneumococcal peptide epitopes with preferred linker comprising a maleimide functionality, were added in a 1.5 eq excess to a solution of a LBB, such as the very preferred **LBB-5**, in MQ H_2O. The solution was vortexed and sonicated until everything is homogenously dissolved. Afterwards the solution's pH was adjusted dropwise to pH 5.5-6 and the conjugation between the maleimide and the thiol functionality of the cysteine side chain of the peptide moiety of the LBB, preferably of **LBB-5,** was followed using a H-Class UPLC system (Waters) coupled to a Waters Acquity QDa mass detector (Waters). Once the LBB, preferably the **LBB-5,** was exhausted, the conjugated product was purified by preparative HPLC with a TFA system on a reversed-phase column with ACN gradient elution and UV detection at 220 nm. Finally, the salt exchange from trifluoroacetate to hydrochloride was performed using a Dowex chloride resin or by dissolving and freeze drying the sample 3 times in a 5mM HCl solution.

Representative and very preferred examples of linear Conjugates are **NPB1-Mal-LBB-5** (Conjugate **1**), **PR2-Mal-LBB-5** (Conjugate **2**) and **PhtE3-Mal-LBB-5** (Conjugate **3**) having the formula as depicted in the following:



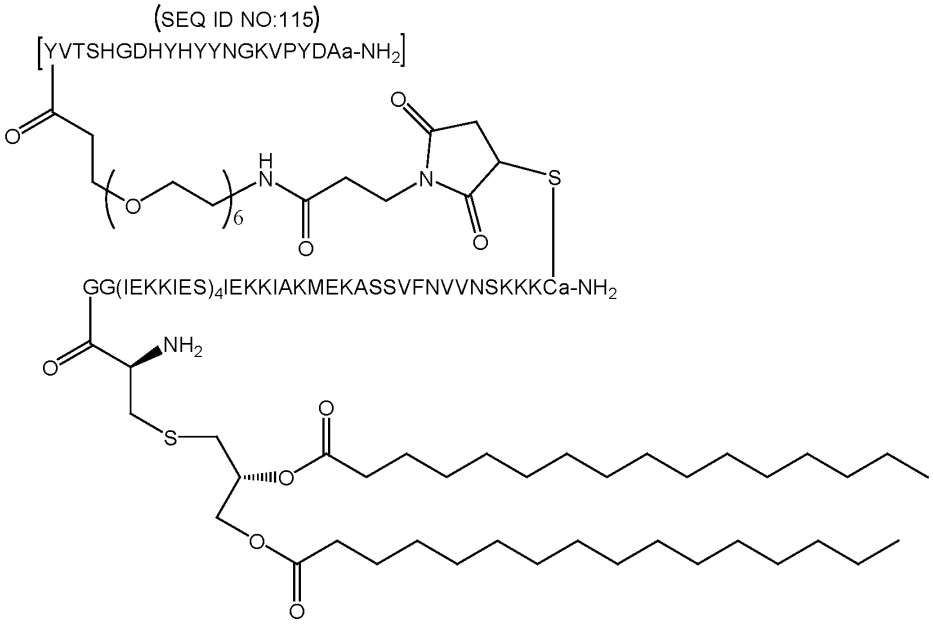
Conjugate **1**

Conjugate **1** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) at 26 °C using a gradient of 20 to 80% B over 60 min [A: H2O/TFA 99.97/0.03 and B: MeCN/TFA 99.97/0.03] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **1** was calculated for $C_{450}H_{767}N_{115}O_{144}S_3$, MW: 10188.9 with an expected ESI $[M+10H]^{10+}$:1019.9, observed $[M+10H]^{10+}$:1020.0.



Conjugate **2**

Conjugate **2** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) at 26 °C using a gradient of 20 to 80% B over 60 min [A: H2O/TFA 99.97/0.03 and B: MeCN/TFA 99.97/0.03] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **2** was calculated for $C_{473}H_{805}N_{113}O_{141}S_3$, MW: 10427.5 with an expected ESI $[M+10H]^{10+}$:1043.8, observed $[M+10H]^{10+}$:1044.1.



Conjugate **3**

Conjugate **3** was analyzed with Waters Acquity ultrahigh-pressure liquid chromatography (UPLC) system (Waters Corporation, USA) equipped with a quadrupole mass detector module (MS) and a TUV detector (UV) set at 210 nm, using a Acquity UPLC® BEH C8 column (150 mm x 2.1 mm, 1.7 µm) (Waters) with a BEH C8 column at 40°C, using a linear gradient from 10 to 85% B during 75 min, using a binary solvent system [A: H2O/TFA 99.9/0.1 (v/v) and B:MeCN/TFA 99.9/0.1 (v/v)] with a flow rate of 0.1 mL min^{-1}. The expected molecular weight for Conjugate **3** was calculated for $C_{447}H_{736}N_{106}O_{132}S_3$, MW: 9803.6 with an expected ESI $[M+10H]^{10+}$:981.4, observed $[M+10H]^{10+}$:981.5.

EXAMPLE 12

Preparation of SVLPs comprising the inventive pneumococcal antigens

After purification, the respective conjugate was lyophilized. MQ H_2O was added to the vial containing the lyophilized conjugate followed by vortexing for about 1 min until a clear solution containing the self-assembled SVLPs is obtained. Subsequently, a 9% NaCl solution is added to reach a final concentration of 0.9%. A 0.9% NaCl solution can be directly used to dissolve the lyophilized conjugate. Alternatively, a 5-30mM sodium acetate solution containing 0.9% NaCl is added to the lyophilized conjugate, followed by vortexing until a clear solution containing the self-assembled SVLPs is obtained. In a further alternative manner, PBS is added to the vial containing the lyophilized conjugate and the vial is placed on a roller/shaker adjusted to 35 rpm for 1-5 min till the conjugate is dissolved completely, leading to a clear colorless solution containing the self-assembled SVLPs. All these preparations are mentioned herein as SVLP composition or preparation and contain the self-assembled SVLPs. If the lyophilized conjugate is to be used for injection in animals, ion exchange is performed to remove TFA salts and replace them with HCl salts before lyophilization and the procedure described above to obtain self-assembled SVLPs.

The conjugates and SVLPs were analyzed by dynamic light scattering (DLS) using a Malvern Zetasizer® Ultra (Malvern Panalytical, Worcestershire, United Kingdom). Size distribution measurements were performed in triplicates at 25°C. The correlation functions were fitted by the cumulant approach to measure the Hydrodynamic diameter (Dh) and the polydispersity index (PI). By way of example: Conjugate **21** was reconstituted in 20 mM acetate buffer pH 4.3 containing 0.9% NaCl to a final concentration of 0.73 mg/mL of SVLP. Dh: 33.01 ± 0.16 nm, PI: 0.12 ± 0.02. Conjugate **22** was reconstituted in 20 mM acetate buffer pH 4.3 containing 0.9% NaCl to a final concentration of 0.73 mg/mL of SVLP. Dh: 33.08 ± 0.28 nm, PI: 0.1 ± 0.01. Conjugate **3** was reconstituted in 20mM acetate buffer pH 4.51 containing 0.9% NaCl to a final concentration of 0.3 mg/mL of SVLP. Dh: 26.73 ± 0.83 nm, PI: 0.20 ± 0.02. Conjugate **24** was reconstituted in 20 mM acetate buffer pH 4.3 containing 0.9% NaCl to a final concentration of 1.0 mg/mL of SVLP. Dh: 33.28 ± 0.21 nm, PI: 0.08 ± 0.01.

SVLPs preparations comprising a mixture of pneumococcal antigens

Lyophilized preparations of the inventive conjugates such as conjugates **1**, **2**, **3**, **21** or **22** (see Examples 10 and 11), if previously stored at -20°C, are brought to room temperature.

For the preparation of inventive compositions comprising a mixture of equal molarities of the inventive pneumococcal antigens presented on SVLPs, one selected dose of e.g. 2.105 nmol of each conjugate preparation is reconstituted with one of the ways described above, in a final equal volume of e.g. 25-100 µL. To obtain an inventive mixture of different pneumococcal

peptide epitope SVLPs of equal molarities with respect to the selected different pneumococcal peptide epitopes, equal amounts or volumes from each of the SVLP preparations are combined. For example, 25 µL of a SVLP preparation of conjugate **21** are combined with 25 µL of a SVLP preparation of conjugate **3** to result in a solution of a volume of 50 µL as injectable, typically and preferably intramuscularly or subcutaneously, to an animal.

The mix of SVLPs were analyzed by dynamic light scattering (DLS) using a Malvern Zetasizer® Ultra (Malvern Panalytical, Worcestershire, United Kingdom). Size distribution measurements were performed in triplicates at 25°C. The correlation functions were fitted by the cumulant approach to measure the Hydrodynamic diameter (Dh) and the polydispersity index (PI). By way of examples: The reconstituted SVLPs were mixed in a tube to obtain an equimolar mixture of 0.7mg/mL of Conjugate **21** and 0.5mg/mL of Conjugate **3** (molar ratio conjugate **21**: conjugate **3**, 1:1). This mixture was then analysed. Dh: 31.41 ± 0.24 nm; PI: 0.16 ± 0.01. Analogously, the reconstituted SVLPs were mixed in a tube to obtain an equimolar mixture of 0.365 mg/mL of Conjugate **22** and 0.26 mg/mL of Conjugate **3** (molar ratio conjugate **22**: conjugate **3**, 1:1). This mixture was then analysed. Dh: 30.85 ± 0.80 nm; PI: 0.22 ± 0.05.

In another procedure, the conjugates comprising the desired pneumococcal peptide epitopes are selected and provided, typically and preferably, in equal molarities, and SVLP reconstitution and preparation is effected thereafter. For example, one dose of e.g. 2.105 nmol of conjugate **21** and one dose of 2.105 nmol of conjugate **3** are transferred in a vial. The two conjugates are reconstituted with one of the ways described above, in a final volume of e.g. 50-100 µL. The composition containing the self-assembled SVLPs comprising the desired pneumococcal peptide epitopes is usable e.g. to be injected into an animal via the intramuscular or the subcutaneous route.

The mix of SVLPs were analyzed by dynamic light scattering (DLS) using a Malvern Zetasizer® Ultra (Malvern Panalytical, Worcestershire, United Kingdom). Size distribution measurements were performed in triplicates at 25°C. The correlation functions were fitted by the cumulant approach to measure the Hydrodynamic diameter (Dh) and the polydispersity index (PI). By way of example: A tube containing lyophilized material of Conjugate **21** was reconstituted in 20mM acetate buffer pH 4.51 containing 0.9% NaCl to a final concentration of 1 mg/mL. Another tube containing lyophilized material of Conjugate **3** was reconstituted in 20mM acetate buffer pH 4.51 containing 0.9% NaCl to a final concentration of 1 mg/mL. The reconstituted SVLPs were then mixed in a third tube to obtain a mixture of 0.5mg/mL of

Conjugate **21** and 0.5mg/mL of Conjugate **3** (molar ratio conjugate **21**: conjugate **3**, 1:1.4). This mixture was then analysed. Dh: 30.12 ± 0.26 nm, PI: 0.16 ± 0.04.

EXAMPLE 13

Vaccine efficacy against lethal pneumococcal challenge

Methods: BALB/c mice were immunized three times s.c. on days 0, 14 and 28, and challenged intranasally with *S. pneumoniae* serotype 3 or serotype 8, two weeks after the last immunization. Survival rates (Table below) and Kaplan-Meier survival curves (Figure 1A) after challenge with serotype 3 in animals immunized with single epitopes or epitope combinations on SVLPs, and thus with SVLPs formed from Conjugate **1,** Conjugate **2**, and/or Conjugate **3**, and/or Conjugate **21** (see Examples 10 and 11), and Kaplan-Meier survival curves (Figure 1B) after challenge with serotype 8 in animals immunized with inventive epitope combinations on SVLPs, namely with SVLPs formed from Conjugate **3** and Conjugate **21**. In Fig. 1A and FIG. 1B and the Table below, NPB/PR refers to the NPB and PR epitopes which are covalently linked leading to a branched pneumococcal antigen (see Example 4) and which are displayed on a single SVLP, whereas NPB + PR refers to the two epitopes each separately presented on SVLPs.

Animals, immunization and challenge. Female BALB/c mice, (Charles River Laboratories, France), were housed under SPF conditions. At 8-weeks of age mice were immunized subcutaneously (s.c.) with three doses in 2 weeks interval, with 30 µL solution containing 2.105 nmol of each pneumococcal peptide epitope conjugated to SVLPs or with SVLP alone as a negative control. Subsequently mice were infected intranasally (i.n.) with either 5x10^5 CFUs of strain ATCC6303 (serotype 3) or with 5x10^9 CFUs of strain W3531 (serotype 8; National Meningitis Reference Laboratory (NMRL) in Greece) two weeks after the last immunization, and survival was monitored for 10 or 14 days, along with other parameters (bodyweight, clinical signs). Sera were collected by retro-orbital bleeding at 4 different timepoints (on D0, D14, D28 and D42) and analyzed for the presence of antibodies against the peptide epitopes or for binding onto the surface of various bacterial serotypes.

Results: Vaccination with the single individual pneumococcal antigens protected animals only partially against serotype 3, with 17%, 33% and 50% protection, respectively (see Figure 1A and Table below). Surprisingly, a synergistic effect leading to 100% protection was achieved with the combination of the three pneumococcal peptide epitopes (NPB/PR+PhtE). Vaccination with the three pneumococcal peptide antigens conferred 70% protection against

the highly virulent serotype 8. This is particularly important, since serotype 8 is not part of conjugate vaccines, yet causes frequent outbreaks of invasive disease and is broadly resistant to antibiotics. These results demonstrate that vaccination with the inventive combination of the pneumococcal peptide epitopes NPB, PR and PhtE can protect against multiple pneumococcal serotypes.

Epitopes in SVLPs	10-day survival
Control	0%
PR	17%
NPB	33%
PhtE	50%
NPB/PR + PhtE	100%

EXAMPLE 14

Vaccine efficacy against bacteremia and organs colonization

Methods**:** Bacterial colonization: At days 3 and 6 post-infection with serotype 3 of the three times immunized animals (see Example 13), blood was collected for bacterial enumeration. Additionally, lungs, livers and spleens were collected at the study endpoint (D52) or when mice were moribund for bacterial enumeration.

Results**:** Bacterial counts in tissues and blood, and bacterial clearance - The combination of the three pneumococcal antigens in accordance with the present invention significantly reduces *S. pneumoniae*.

S. pneumoniae counts in blood**:**

Immunization with the single individual pneumococcal antigens (NPB, PR or PhtE) failed to reduce bacterial counts in the blood at days 3 and 6 post *S. pneumoniae* infection (Figure 2A). Again and surprisingly, complete and durable elimination of bacterial colonization of blood on days 3 and 6 post-infection was achieved with the combination of the three pneumococcal antigens (NPB/PR+PhtE) in accordance with the present invention, supporting the observation of complete (100%) protection of animals from death by *S. pneumoniae* serotype 3 infection when vaccinated with the combination of these 3 pneumococcal antigens as described in Example 13.

S. pneumoniae counts in lung, spleen and liver

Similar to the blood colonization results described, immunization with the single individual pneumococcal antigens (NPB, PR or PhtE) failed to reduce bacterial counts in the lungs, liver and spleen of immunized animals (Figure 2B). A marked reduction of bacterial

counts in lungs and near complete elimination of *S. pneumoniae* serotype 3 in liver and spleen was achieved when the combination of the three pneumococcal antigens (NPB/PR+PhtE) was used in accordance with the present invention.

In conclusion, vaccination with the combination of the three pneumococcal antigens (NPB/PR+PhtE) in accordance with the present invention led not only to increased animal survival but is further accompanied by low or cleared bacterial counts in blood and organs. In particular, the dramatic drop in serotype 3 bacterial counts when the combination of the three pneumococcal antigens was used (Figure 2A and Figure 2B) as compared when the single pneumococcal antigens were used supports the synergistic effect of the inventive combination.

EXAMPLE 15

Vaccine efficacy against lethal pneumococcal challenge

Methods**:** BALB/c mice were immunized three times s.c. on days 0, 14 and 28, and challenged with *S. pneumoniae* serotype 3 (see Example 13), two weeks after the last immunization. A control mice group received the polysaccharide-based PCV13 vaccine (Prevnar 13). Kaplan-Meier survival curves were measured after challenge with serotype 3 in animals immunized with single epitopes or epitope combinations on SVLPs, and thus with SVLPs formed by Conjugate **1,** Conjugate **2**, and/or Conjugate **3**, and/or Conjugate **21** (see Examples 10 and 11). In Figure 3, NPB/PR refers to the NPB and PR epitopes which are covalently linked leading to a branched pneumococcal antigen (see Example 4) and which are displayed on a single SVLP, whereas NPB + PR refers to the two epitopes each separately presented on SVLPs.

Results**:** In this confirmatory study, mice were immunized with the combination of the three pneumococcal antigens (NPB, PR, PhtE), be it with the combination of the three individual pneumococcal antigens each on an SVLP, or be it by using a bi-epitope pneumococcal antigen and a single epitope pneumococcal antigen presented on a carrier such as the SVLP in accordance with the present invention.

100% survival was observed by day 14 post-infection with *S. pneumoniae* by immunizing the mice either with said combinations, thus with single epitopes or with epitope combinations (NPB+PR+PhtE or NPB/PR+PhtE).

EXAMPLE 16

Functionality of antibodies tested with a whole cell ELISA assay

Methods: IgG antibodies from mice immunized three times (see Example 13) with empty SVLP as a control, a mixture of the three pneumococcal antigen in accordance with the present invention and applied as mixture of a bi-epitope pneumococcal antigen and a single epitope pneumococcal antigen each presented on a SVLP and comprising Conjugate **22** and Conjugate **3** as described, or with PCV13 were tested for their ability to bind onto the surface of various bacterial serotypes using a whole cell ELISA assay. In Figure 4, NPB/PR refers to the NPB and PR epitopes which are covalently linked leading to a branched pneumococcal antigen (see Example 4) and which are displayed on a single SVLP, whereas NPB + PR refers to the two epitopes each separately presented on SVLPs. Serotypes included in the PCV13 vaccine (Figure 4A) as well as non-PCV13 vaccine serotypes (Figure 4B) were tested for binding.

Whole cell ELISA assay. Serotypes were grown overnight in Todd-Hewitt broth with 5% yeast extract (THY) at 37°C, till OD600 0.3-0.6 was reached. The culture was spun down and 100 µL bacterial suspensions (OD600 0.1) in PBS were used to coat high-binding microtiter ELISA plates in triplicates for 2 h at 37°C and under UV light for bacterial inactivation. Sera from three times immunized mice with a mixture of the three antigens as NPB/PR1 and PhtE each presented on a SVLP, empty SVLP as a control or PCV13, diluted at 1:500, were applied onto the plates and incubated for 2 h at room temperature. After washing, plates were incubated with an anti-mouse IgG-HRP antibody for 1h. Signal was detected at OD450 after development with orthophenylenediamine (OPD) substrate at a concentration of 0.4 mg/mL in citrate-phosphate buffer (pH 5.0) with 0.012% hydrogen peroxide

Results: Immunization with the combination of NPB, PR and PhtE pneumococcal antigens produced antibodies which recognized all serotypes of *S. pneumoniae* tested, demonstrating that a vaccine combining these 3 pneumococcal antigens has the potential to provide broad and universal protection against *S. pneumoniae* infection regardless of serotype (Figure 4A and 4B). In contrast, PCV13 (Prevnar 13), which is based on carbohydrates of 13 *S. pneumoniae* serotypes, produced antibodies that could recognize some but not all of the tested serotypes contained in PCV13. Additionally, PCV13 antibodies did not recognize tested serotypes not contained in PCV13, highlighting the limitation of PCV13 in providing broad protection against *S. pneumoniae* infection and the advantage of a vaccine based on the inventive combination of pneumococcal antigens derived from the non-proline block (NPB) of the Pneumococcal surface protein A, the proline-rich region (PR) of the Pneumococcal surface protein A, and the surface-exposed pneumococcal protein PhtE.

EXAMPLE 17

Generation of antibodies using differently branched pneumococcal antigens

The different configurations of branched pneumococcal antigens as described and prepared in Example 4 were used within the respective inventive epitope combinations on SVLPs and led to similar strong antibody responses. In detail, SVLPs formed from the branched pneumococcal antigens **BPA-2** and **BPA-4**, in particular from the respective Conjugate **21** and Conjugate **22** each in combination with Conjugate **3** as described and prepared in Examples 10-12, produce similar strong antibody responses.

Methods: Balb/c mice were immunized i.m. three times (day 0, 14, 28) with two different SVLP-based vaccines at three different doses (200, 50 and 12.5 µg), namely with a first SVLP-based vaccine containing Conjugate **21** comprising the branched pneumococcal antigens **BPA-2** and Conjugate **3** (referred to as BPA-2 within the figures 5, i.e. FIG. 5A, FIG. 5B and FIG. 5C), and with a second SVLP-based vaccine containing Conjugate **22** comprising the branched pneumococcal antigens **BPA-4** and Conjugate **3** (referred to as BPA-4 within the figures 5, i.e. FIG. 5A, FIG. 5B and FIG. 5C). Anti-epitope-specific IgG antibodies were measured using an indirect ELISA protocol. Briefly, the respective peptide epitopes diluted in PBS at an optimal concentration were used to coat microtiter 96-well plates overnight at 4°C. Following blocking with 3% BSA in PBS, the plates were incubated for 1.5h at RT with mouse sera isolated on day 49 and diluted 1:100 in assay buffer. Following incubation with a secondary anti-mouse IgG antibody conjugated with horseradish peroxidase (HRP), the plate was developed using TMB as substrate, and the optical density was measured at 450 nm with an ELISA plate reader.

Results: Mice immunized with either of the two differently branched SVLP-based vaccines produced IgG responses to all three epitopes contained in the vaccines at similar levels. These results demonstrate that the inventive compositions and vaccines can be prepared comprising the three pneumococcal peptide epitopes and antigens in different configurations and successfully produce an antibody response to all three antigens contained in the inventive compositions and vaccines.

EXAMPLE 18

Combination vaccines of inventive compositions and PCVs

The preferred inventive vaccines comprising Conjugate **22** and Conjugate **3** as described and prepared in Examples 10-12 and referred to as NPB/PR + PhtE, can be administered with a pneumococcal conjugate vaccine (PCV13) concurrently leading to the generation and

detection of PCV13-induced immune responses.

Methods: Balb/c mice were immunized i.m. three times (day 0, 14, 28) concurrently with the NPB/PR + PhtE vaccine (50μg) and PCV13 (50 μl). Sera were isolated from the mice 14 days after the last immunization and diluted 1:100 in assay buffer. IgG antibody levels against the respective NPB, PR and PhtE epitopes were detected by epitope-specific ELISA. Type-specific IgG anti-*S. pneumoniae* capsular polysaccharides (CPS) were measured in the same sera using an indirect ELISA protocol adapted from the WHO training manual (https://www.vaccine.uab.edu/uploads/mdocs/ELISAProtocol(007sp).pdf).

Briefly, lyophilized CPS derived from various serotypes were diluted at a 1 mg/mL stock solution and were used to coat microtiter 96-well plates with an optimized concentration (2.5 μM for CPS from Ser3, Ser4, Ser9V and Ser14; 10 μM for CPS from Ser19A and Ser19F), at optimized conditions for each CPS (2h at RT for Ser3 and 37°C over 5h for the remaining serotypes). A blocking buffer was prepared using polysaccharide 22F and pneumococcal cell wall polysaccharide (CWPS), each at a final concentration of 5 μg/mL. The plates were incubated overnight at 4°C with mouse sera derived from Balb/c mice immunized three times (day 0, 14, 28) concurrently with the SVLP-based composition (50 μg) and vaccine, and PCV13 (50 μl), diluted 1:100 in assay buffer. Following incubation with a secondary anti-mouse IgG antibody conjugated with alkaline phosphatase (AP), the plate was developed using p-nitrophenyl phosphate (NP) as substrate, and the optical density was measured at 405 nm with an ELISA plate reader (FIG. 6).

Results: Concurrent immunization of mice with a vaccine containing preferred inventive compositions and a pneumococcal conjugate vaccine (PCV13) resulted in the generation of anti-polysaccharide-specific antibodies in mice, suggesting that the inventive compositions and vaccines can be combined with known PCV vaccines to provide broader serotype coverage while maintaining their immunogenicity.

EXAMPLE 19

IFN-gamma responses upon immunization with inventive compositions and vaccines

C57/BL6 mice (n=3) were immunized once on day 0 with the preferred inventive SVLP based composition and vaccine comprising Conjugate **22** and Conjugate **3** as described and prepared in Examples 10-12 (referred to as NPB/PR1 + PhtE in FIG. 7), or the SVLP vehicle control (referred to as Control in FIG. 7). Two mice remained unvaccinated and were used as naïve mice in the ELISPOT assay (referred to as Naïve in FIG. 7). On day 16, splenocytes were

isolated from all mice and the presence of interferon-gamma producing T cells was measured using a murine IFN-γ Single-Color Enzymatic (SCE) IMMUNOSPOT Kit (CTL) in a 96-well plate. In brief, 500,000 splenocytes were plated per well and incubated overnight at 37°C in a humidified incubator, supplemented with 5-9% CO_2 with either the preferred inventive SVLP-based composition and vaccine comprising Conjugate **22** and Conjugate **3**, or the respective control SVLP at a concentration of 10 microgram/ml. Splenocytes were also stimulated with serotype 3 *streptococcus pneumoniae* at a concentration of 2µg/ml. Plates were washed with 0.05% Tween-PBS then incubated a biotinylated detection antibody for 2 hours. Following additional washes with 0.05% Tween-PBS, the streptavidin-alkaline phosphatase solution was added for 30 minutes. After 2 more washes with 0.05% Tween-PBS and a wash with water, the blue developer solution was added for 15 minutes and the reaction stopped by rinsing the plate with water and airdrying it for 24 hours before counting the spots.

Results: Immunization of mice with a vaccine containing the preferred inventive SVLP based composition led to the generation of T-cell responses in form of IFN-γ release. Splenocytes from vaccinated mice, but not from empty SVLP vaccinated or naïve mice, recognized serotype 3 *streptococcus pneumoniae* further demonstrating the specificity of the vaccine. Antigen-specific T-cell immunity is important as T-cells can assist in producing a stronger antibody response to the antigens contained in the vaccine, as well as help mount a stronger recall response to the vaccine antigens upon exposure to the disease agent.

EXAMPLE 20

Generation of antibodies upon immunization with inventive mRNA compositions and vaccines

Single mRNA constructs encoding for each one of the preferred epitopes NPB, PR and PhtE of SEQ ID NO:4, SEQ ID NO:23 and SEQ ID NO:113 were generated by cloning the respective nucleic acid sequences of SEQ ID NO:234 (encoding for SEQ ID NO:4), SEQ ID NO:235 (encoding for SEQ ID NO:23) and SEQ ID NO:236 (encoding for SEQ ID NO:113) into respective *E. coli* vectors suitable for *in vitro* transcription with modified N(1)-methylpseudouridine (m1Psi-incorporated mRNA). The transcripts were mixed at a 1:1:1 molar ratio and encapsulated into lipid nanoparticles (LNP) consisting of phospholipids, cholesterol, PEGylated lipids and the SM-102 ionizable lipid using standard protocols. In summary, lipids (ionizable cationic lipid SM-102, helper lipid DSPC, cholesterol and PEGylated lipid PEG2000-DMG) were dissolved in ethanol. The mRNA was diluted in an aqueous citrate buffer

and LNPs were produced by mixing lipids with mRNA using a microfluidic mixer. This method is commonly used to produce lipid nanoparticles (Li, B., Dong, Y. (2017). Preparation and Optimization of Lipid-Like Nanoparticles for mRNA Delivery. In: Bindewald, E., Shapiro, B. (eds) RNA Nanostructures. Methods in Molecular Biology, vol 1632. Humana Press, New York, NY). Subsequently, LNPs were dialyzed against Tris-HCl buffer. After dialysis, LNPs were passed through a 0.22 μm filter and stored at -80°C. RiboGreen assay was used to quantify mRNA in LNPs. Encapsulation efficiency was 90-96%. Particle size was determined by dynamic light scattering (PDI, polydispersity index; ≤0.2) and surface charge was determined by the zeta potential using a Zetasizer device (Zeta potential: -4 to -8 mV). Particles of a diameter of 95-105 nm were produced. Endotoxin level was below the limit of detection. The particles were stored in a Tris-based buffer, pH 7.4 till further use.

Balb/c mice 6 weeks of age (n=6) were immunized twice on days 0 and 21 with the inventive composition comprising the mRNA's encoding for each one of the preferred epitopes NPB, PR and PhtE of SEQ ID NO:4, SEQ ID NO:23 and SEQ ID NO:113, and thus encoding the very preferred first pneumococcal antigen, second pneumococcal antigen and third pneumococcal antigen, encapsulated in said lipid nanoparticle (LNP). Said mRNA-LNP vaccine contains equal amounts of the three mRNA antigens in a total of 0.5, 2, or 10 μg of mRNA-LNP. Serum was collected on day 35 and anti-epitope-specific IgG antibodies were measured using an indirect ELISA protocol. Briefly, peptide epitopes diluted in PBS at an optimal concentration were used to coat microtiter 96-well plates overnight at 4°C. Following blocking with 3% BSA in PBS, the plates were incubated for 1.5h at RT with mouse sera derived from Balb/c mice immunized twice (day 0, 21) with different amounts of mRNA-LNP (0.5-10 μg), diluted 1:20 in assay buffer. Following incubation with a secondary anti-mouse IgG antibody conjugated with horseradish peroxidase (HRP), the plate was developed using TMB as substrate, and the optical density was measured at 450 nm with an ELISA plate reader.

Conclusions: Immunization of mice with inventive compositions comprising the mRNA transcripts encoding for each one of the preferred epitopes NPB, PR and PhtE of SEQ ID NO:4, SEQ ID NO:23 and SEQ ID NO:113, and thus encoding the very preferred first pneumococcal antigen, second pneumococcal antigen and third pneumococcal antigen, encapsulated in said lipid nanoparticles (LNP) led to the generation of detectable IgG responses for all three epitopes. The results demonstrate that a vaccine comprising the inventive specific combination of the three pneumococcal antigens and epitopes can be delivered using more than one vaccine platform to produce antigen specific immune responses to the three *S. pneumoniae* antigenic epitopes contained in the vaccine.

CLAIMS

1. A composition comprising

(a) a first pneumococcal antigen comprising a first pneumococcal peptide epitope or a nucleic acid encoding said first pneumococcal antigen, wherein said first pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

QQAEEDYARRSEEEYNRLX_{19}QQQ (SEQ ID NO:1),

wherein X_{19} represents an amino acid selected from T and P; or

a variant of said amino acid sequence, wherein up to 2 amino acids in other than in position denoted with X in SEQ ID NO:1 are replaced by any amino acid;

(b) a second pneumococcal antigen comprising a second pneumococcal peptide epitope or a nucleic acid encoding said second pneumococcal antigen, wherein said second pneumococcal peptide epitope comprises, preferably consists of, the amino acid sequence

$X_1X_2PX_4PEX_7X_8AX_{10}X_{11}PX_{13}PX_{15}X_{16}APX_{19}X_{20}APAPX_{25}PEX_{28}PA$ (SEQ ID NO:2),

wherein X_1 represents an amino acid selected from E, P or A;

wherein X_2 represents an amino acid selected from T, A or P;

wherein X_4 represents an amino acid selected from A, T or Q;

wherein X_7 represents an amino acid selected from A, P or Q;

wherein X_8 represents an amino acid selected from P, Q or E;

wherein X_{10} represents an amino acid selected from E or P;

wherein X_{11} represents an amino acid selected from Q or A;

wherein X_{13} represents an amino acid selected from K or A;

wherein X_{15} represents an amino acid selected from A or E;

wherein X_{16} represents an amino acid selected from P or Q;

wherein X_{19} represents an amino acid selected from Q, K or E;

wherein X_{20} represents an amino acid selected from P or Q;

wherein X_{25} represents an amino acid selected from K, A or Q;

wherein X_{28} represents an amino acid selected from K or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:2 are replaced by any amino acid; and

(c) a third pneumococcal antigen comprising a third pneumococcal peptide epitope or a nucleic acid encoding said third pneumococcal antigen, wherein said third pneumococcal peptide epitope comprises, preferably consists of, the amino sequence

$YX_2TX_4HGX_7HYHYX_{12}X_{13}GX_{15}VX_{17}YDA$ (SEQ ID NO:3),

wherein X_2 represents an amino acid selected from V, A or I;
wherein X_4 represents an amino acid selected from S or P;
wherein X_7 represents an amino acid selected from D or N;
wherein X_{12} represents an amino acid selected from Y, I or F;
wherein X_{13} represents an amino acid selected from N, P or F;
wherein X_{15} represents an amino acid selected from K, S or D;
wherein X_{17} represents an amino acid selected from P, L, H or Q; or

a variant of said amino acid sequence, wherein up to 3 amino acids in other than in positions denoted with X in SEQ ID NO:2 are replaced by any amino acid; and

wherein preferably said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen have a length of at most 50 amino acids, further preferably of at most 45 amino acids.

2. The composition of claim 1, wherein

said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4 to 21;

said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23 to 112; and

said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to 172.

3. The composition of claim 1, wherein

said first pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:4 to 6;

said second pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NOs:23 to 25; and

said third pneumococcal peptide epitope comprises, preferably consists of, an amino sequence selected from any one of the amino acid sequences of SEQ ID NO:113 to 115.

4. The composition of any one of the claims 1-3, wherein at least two of said first, said second and said third pneumococcal antigen are covalently linked by way of at least one connecting moiety forming a multi-epitope pneumococcal antigen, preferably a multi-epitope branched pneumococcal antigen.

5. The composition of any one of the preceding claims, wherein said composition further comprises an adjuvant.

6. The composition of any one of the preceding claims, wherein said composition further comprises at least one carrier, wherein preferably at least one of said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen or at least one of said nucleic acid encoding said first pneumococcal antigen, said second pneumococcal antigen and said third pneumococcal antigen is linked to or is complexed with said at least one carrier.

7. The composition of any one of the preceding claims, wherein said composition further comprises at least one carrier, wherein said first, said second and said third pneumococcal antigen are linked to said at least one carrier, either directly or by way of a linker.

8. The composition of any one of the preceding claims, wherein said composition comprises a first carrier, a second carrier and a third carrier, wherein

(a) said first carrier is a first synthetic virus-like particle (SVLP), wherein said first SVLP comprises at least one first conjugate, wherein said first conjugate comprises a first lipopeptide building block (LBB) and said first pneumococcal antigen, wherein said first lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said first pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker;

(b) said second carrier is a second synthetic virus-like particle (SVLP), wherein said second SVLP comprises at least one second conjugate, wherein said second conjugate comprises a second lipopeptide building block (LBB) and said second pneumococcal antigen, wherein said second lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said second pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker; and

(c) said third carrier is a third synthetic virus-like particle (SVLP), wherein said third SVLP comprises at least one third conjugate, wherein said third conjugate comprises a third lipopeptide building block (LBB) and said third pneumococcal antigen, wherein said third lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

9. The composition of any one of the claims 1-7, wherein said composition comprises a bi-epitope carrier and single epitope carrier, wherein

(a) said bi-epitope carrier is a bi-epitope synthetic virus-like particle (SVLP), wherein said bi-epitope SVLP comprises at least one bi-epitope conjugate, wherein said bi-epitope conjugate comprises a bi-epitope lipopeptide building block (LBB) and a bi-epitope pneumococcal antigen, preferably a bi-epitope branched pneumococcal antigen, wherein said bi-epitope lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said bi-epitope pneumococcal antigen, preferably said bi-epitope branched pneumococcal antigen, is covalently linked to said peptide moiety, either directly or via a linker;

(b) said single epitope carrier is a single epitope SVLP, wherein said single epitope SVLP, comprises at least one conjugate, wherein said conjugate comprises a lipopeptide building block (LBB) and said first, said second or said third pneumococcal antigen, wherein said lipopeptide building block comprises, preferably consists of,

(i) a peptide moiety comprising at least one coiled coil peptide chain segment, and

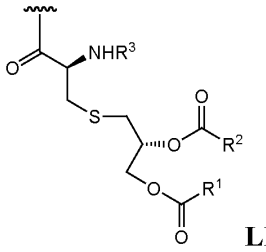
(ii) a lipid moiety comprising two or three, preferably two hydrocarbyl chains;

wherein said peptide moiety is covalently linked to said lipid moiety, either directly or via a coupling moiety, and

wherein said third pneumococcal antigen is covalently linked to said peptide moiety, either directly or via a linker.

10. The composition of claim 8 or claim 9, wherein

- said coiled coil peptide chain segment comprises 3 to 8 repeat units, and wherein said repeat unit consists of the sequence IEKKIE-X0 (SEQ ID NO:173), wherein X0 represents an amino acid, and wherein preferably said repeat unit consists of the sequence selected from IEKKIEG (SEQ ID NO:174), IEKKIEA (SEQ ID NO:175) or IEKKIES (SEQ ID NO:176), further preferably wherein said repeat unit consists of the sequence IEKKIES (SEQ ID NO:176); and
- said lipid moiety comprises formula **LM-II**



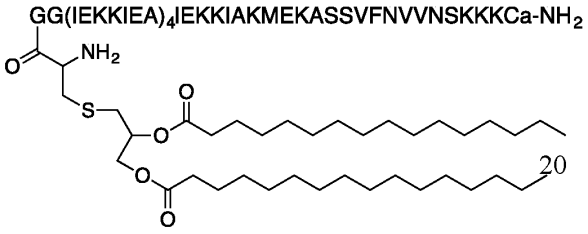
LM-II

wherein R^1 and R^2 are independently C_{11}-$_{15}$alkyl, preferably wherein R^1 and R^2 are independently -$C_{11}H_{23}$, -$C_{13}H_{27}$ or -$C_{15}H_{31}$, and wherein further preferably R^1 and R^2 are -$C_{15}H_{31}$; and

wherein R^3 is hydrogen or -C(O)C_{11}-$_{15}$alkyl, preferably wherein R^3 is H or -C(O)$C_{15}H_{31}$; and

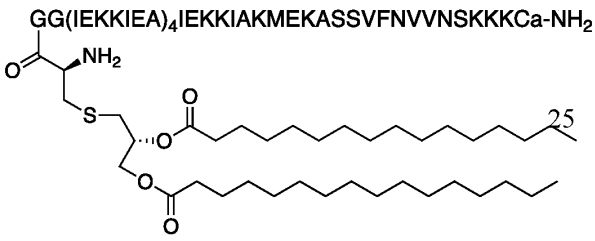
wherein the wavy line in formula **LM-II** indicates the linkage site to said peptide moiety, and wherein preferably said lipid moiety is linked to the N-terminus of said peptide moiety.

11. The composition of any one of the claims 8 to 10, wherein said lipopeptide building block is of the formula **LBB-1**, **LBB-2**, **LBB-3**, **LBB-4**, **LBB-5**, or **LBB-6** (SEQ ID NO:227-232), wherein preferably said lipopeptide building block is of the formula **LBB-4** or **LBB-5**, further preferably wherein said lipopeptide building block is of the formula **LBB-5**.



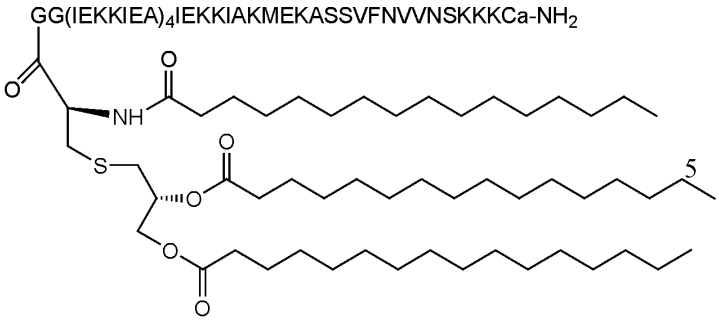
SEQ ID NO:227

LBB-1



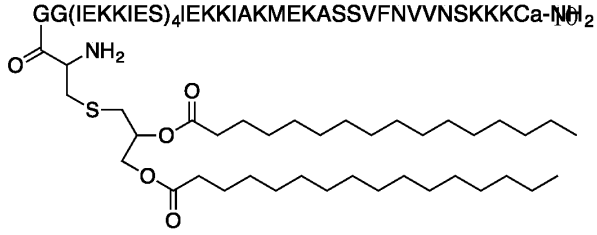
SEQ ID NO:228

LBB-2



SEQ ID NO:229

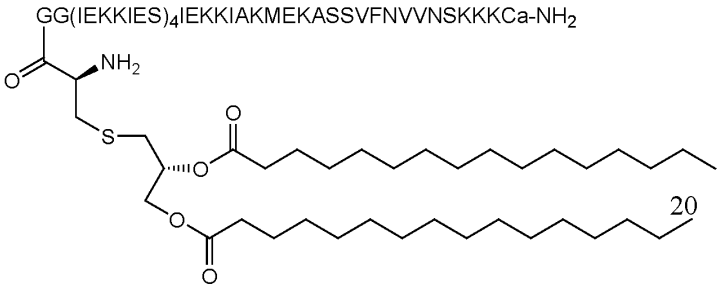
LBB-3



SEQ ID NO:230

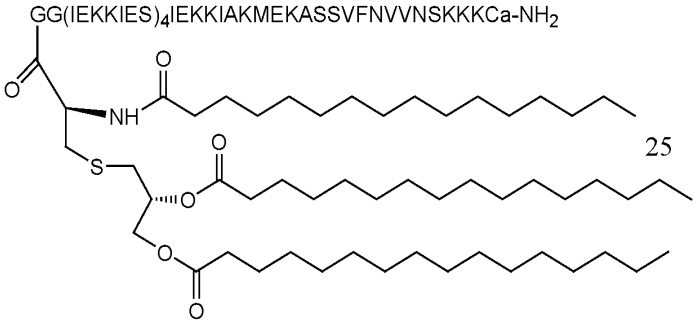
LBB-4

15



SEQ ID NO:231

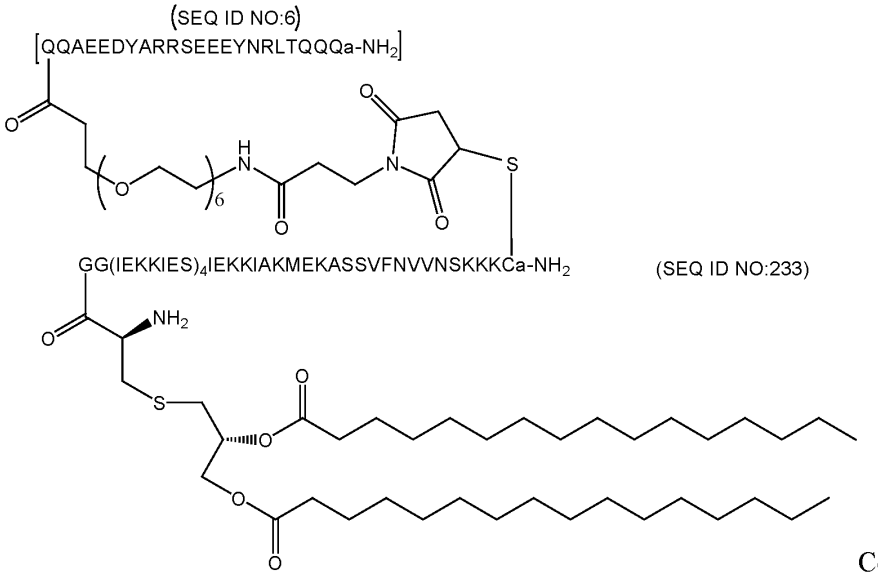
LBB-5



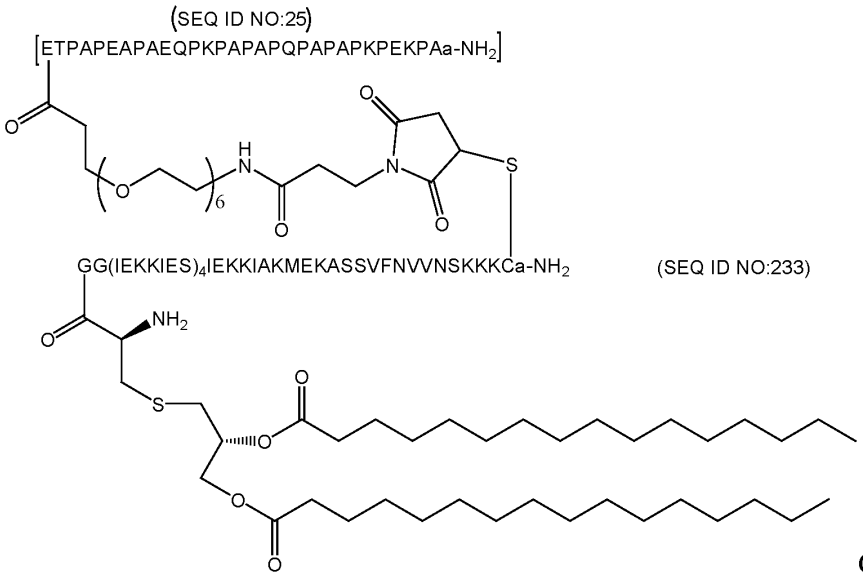
SEQ ID NO:232

LBB-6

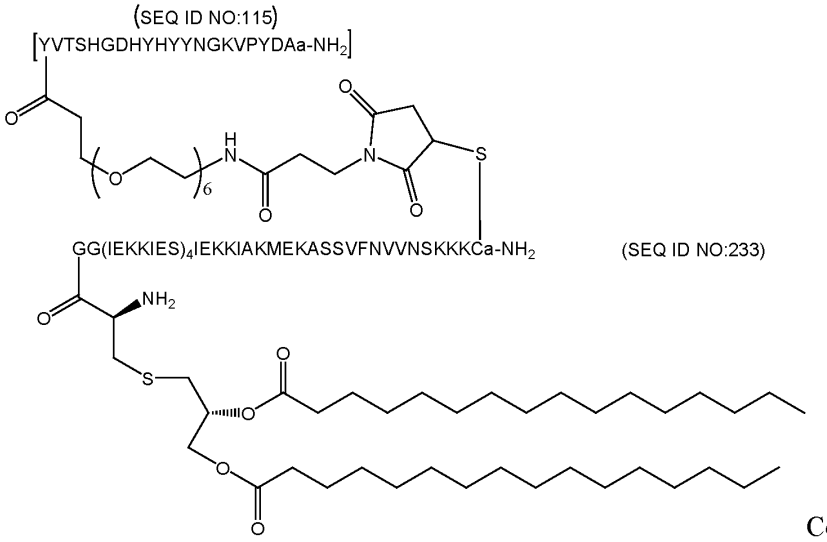
30 12. The composition of claim 8, wherein said first conjugate is Conjugate **1**, said second conjugate is Conjugate **2**, and said third conjugate is Conjugate **3**



Conjugate **1**

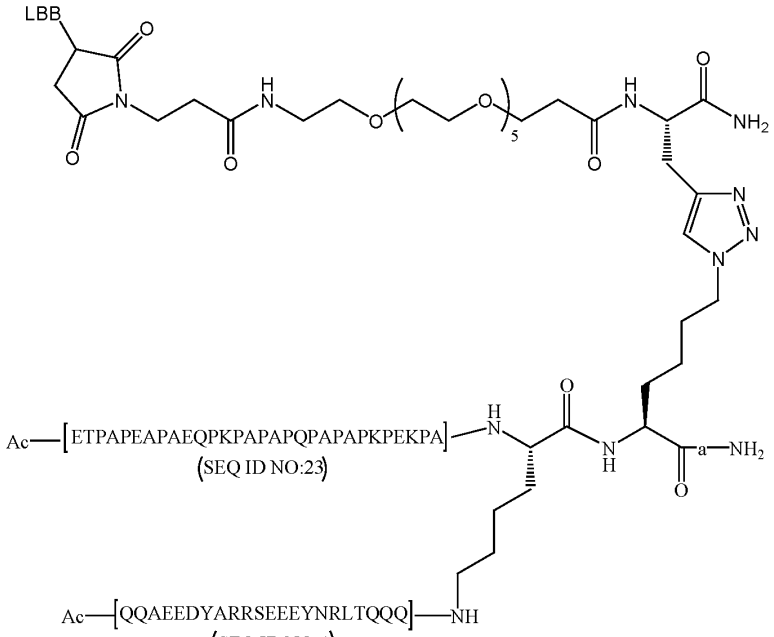


Conjugate **2**



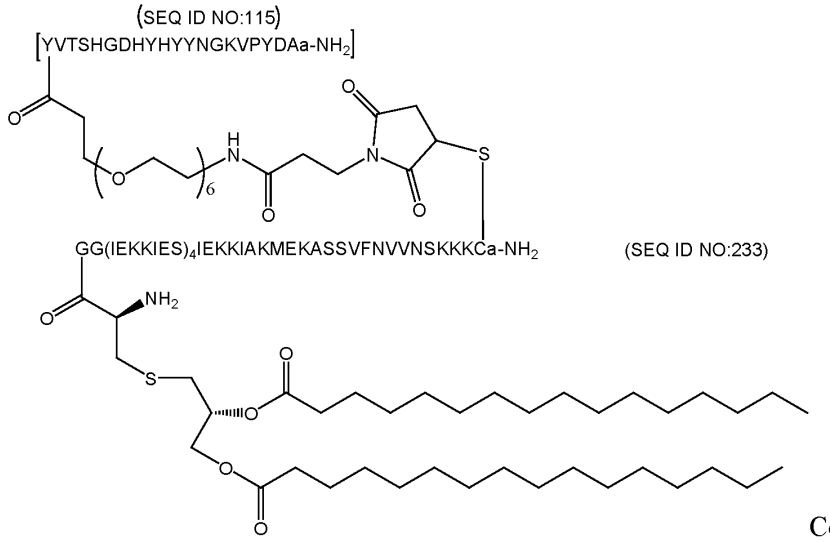
Conjugate **3.**

13. The composition of claim 9, wherein said bi-epitope conjugate is Conjugate **21**, and said conjugate is Conjugate **3**



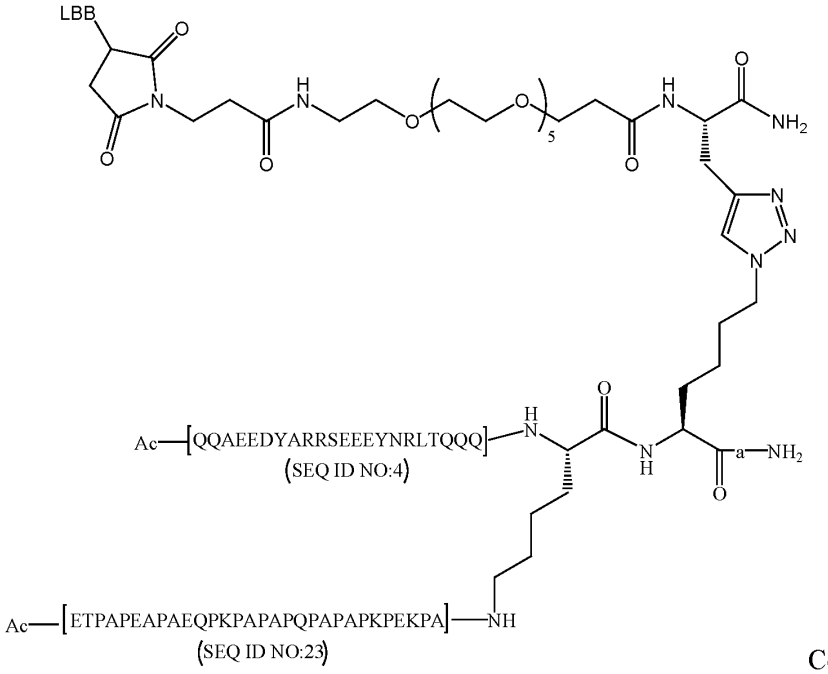
Conjugate **21**,

wherein said LBB is of the formula **LBB-5**,



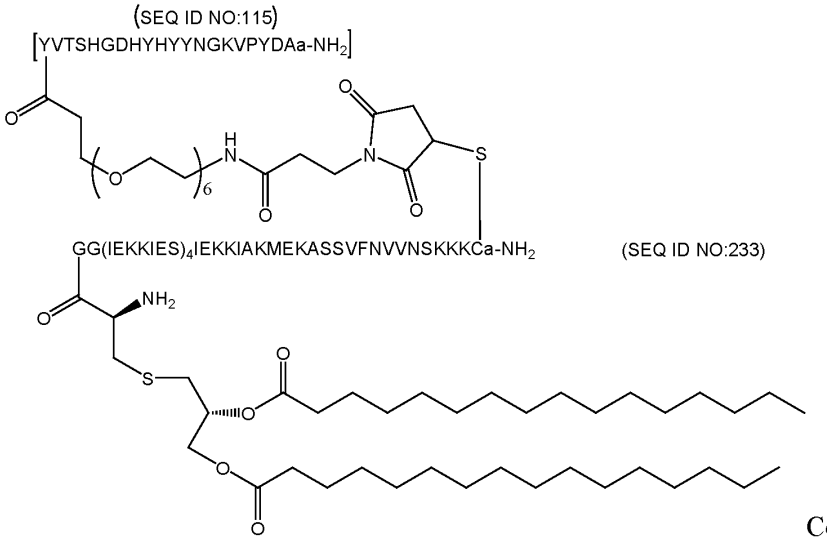
Conjugate **3**,

or wherein said bi-epitope conjugate is Conjugate **22**, and said conjugate is Conjugate **3**



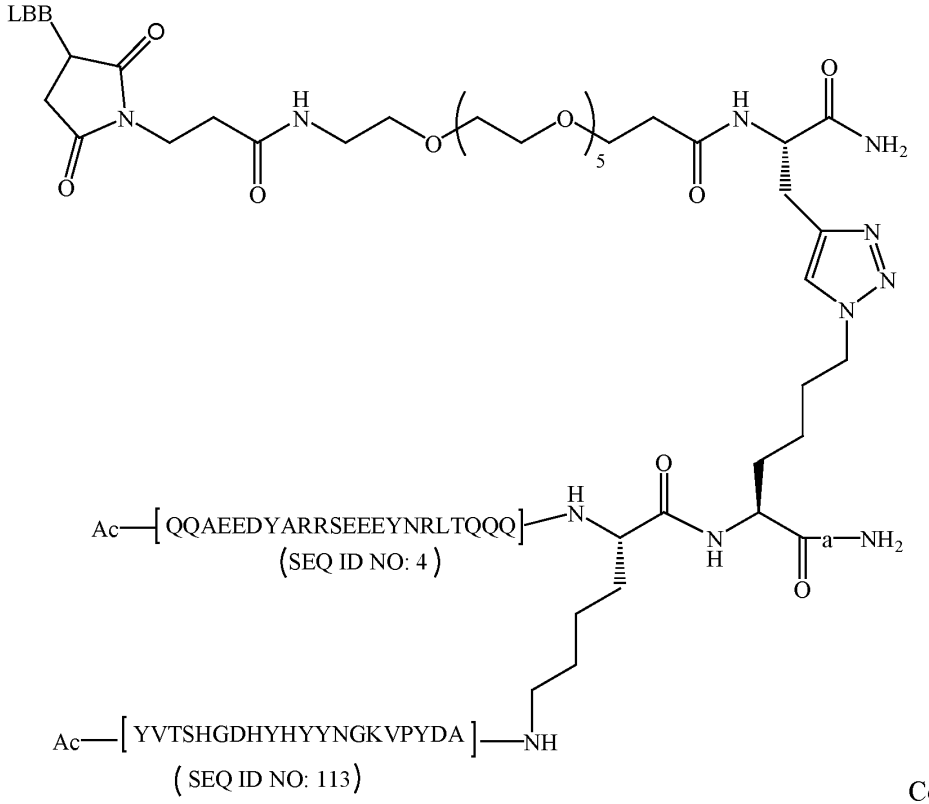
Conjugate **22,**

wherein said LBB is of the formula **LBB-5**,



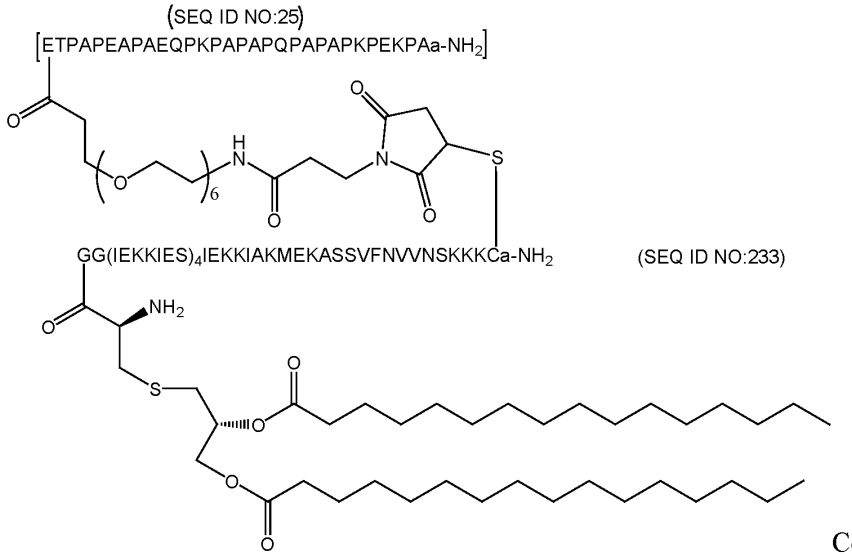
Conjugate **3**,

or wherein said bi-epitope conjugate is Conjugate **23**, and said conjugate is Conjugate **2**



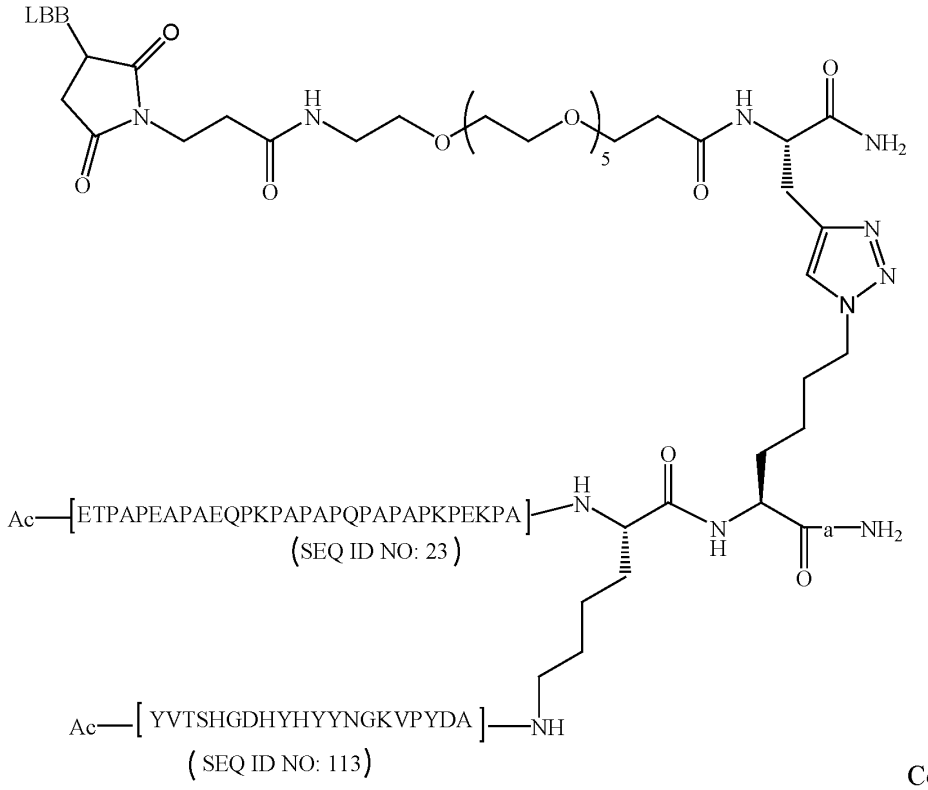
Conjugate **23**,

wherein said LBB is of the formula **LBB-5**,



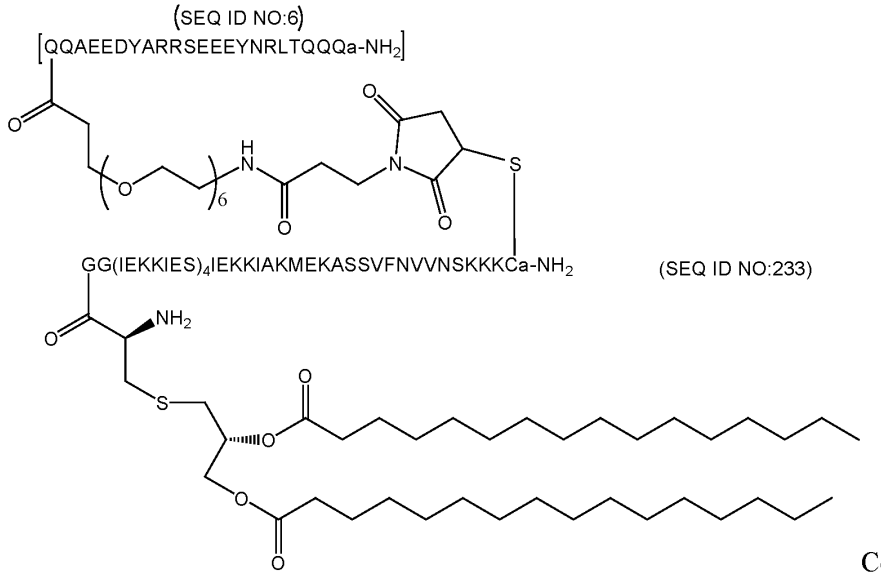
Conjugate **2,**

or wherein said bi-epitope conjugate is Conjugate **24**, and said conjugate is Conjugate **1**



Conjugate **24**,

wherein said LBB is of the formula **LBB-5**,



Conjugate **1**.

14. A pharmaceutical composition comprising a composition of any one of the claims 1 to 13 and pharmaceutically acceptable carrier.

15. A composition of any one of the claims 1 to 13, or an pharmaceutical composition of claim 14 for use in a method of preventing or treating a *Streptococcus pneumoniae* infection.