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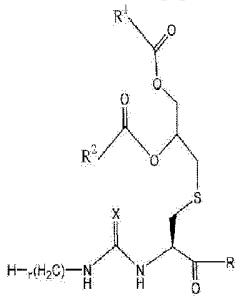
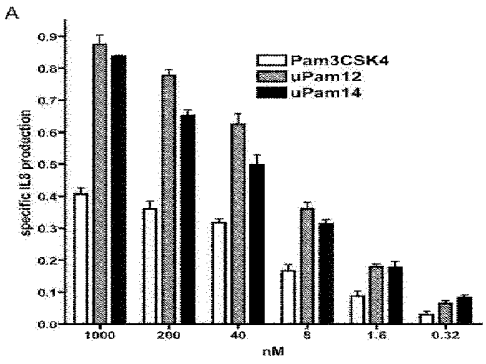
(54) **Adjuvant compound.**

(57) The invention is directed to a compound according to the above formula [1]



wherein R1 and R2 are branched or straight alkyl groups having 10 to 17 carbon atoms, n is 0 to and including 18, X is S or O and R is en organic group comprising one or more peptides. The invention is also directed to the use of said compound as en adjuvant. The invention is also directed to a composition comprising said compound and the use of said composition, for example as a vaccine composition.



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ADJUVANT COMPOUND

Field of the invention

The present invention relates to a novel compound suited as adjuvant in a vaccine composition, a process to prepare the compound and a composition comprising the compound.

Background of the invention

Vaccination strategies have been used for decades primarily to foster a protective immunity to protect patients from developing a disease after contact with an infectious agent. To this end live attenuated, dead or disrupted pathogens, pathogen preparations, or purified or recombinant components of the pathogens have been administered to patients to elicit a specific immune response to antigenic components of the respective pathogen. The components, which stimulate such an immune response can be, for example, pathogen specific proteins, polysaccharides or lipids. The specific immune response against antigens comprised within pathogens can be further stimulated by the co-administration of adjuvants. Adjuvants are known in the art to accelerate, prolong, or enhance the quality of the specific immune response to the antigen or antigens and are currently employed as part of vaccines. The proposed advantages of adjuvants include their ability to: 1) direct and optimize immune responses that are appropriate for the vaccine; 2) enable mucosal delivery of vaccines; 3) promote cell-mediated immune response; 4) enhance the immunogenicity of weaker immunogens such as highly purified or recombinant antigens; 5) reduce the amount of antigen or the frequency of immunization required to provide protective immunity; 6) improve efficacy of vaccines in individuals with reduced or weakened immune responses such as newborns, the aged, and immunocompromized patients.

Adjuvants have diverse mechanisms of action. One set of adjuvants act through toll-like receptors. Toll-like receptors (TLR) recognize specific patterns of microbial components, especially those from pathogens, and regulate the activation of both innate and adaptive immunity. Immature dendritic cells mature in response to these microbial components. As of yet, 13 members of the TLR-family have been identified. TLR are expressed by phagocytic cells such as monocytes, macrophages and dendritic cells. TLR activation through ligand binding leads to signal transduction

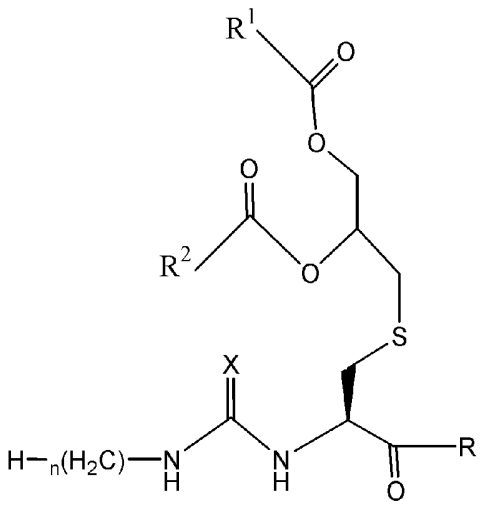
events either in a MyD88-dependent pathway (NF-[kappa]β) or MyD88-independent pathway (IFR-3). A known lipopeptide adjuvant which interacts with toll-like receptor 2 (TLR2) is the so-called Pam3Cys-lipopeptide. According to Renate Spohn et al. (Vaccine 22 (2004) 2494-2499), the Pam3Cys-SK4 variant was found to be the most effective additive for electing a cellular immune response in mice. Another advantage of Pam3Cys-SK4 is that it is chemically stable and can be produced in large quantities at high quality. A Pam3Cys-lipopeptide, Pam3Cys—Ser-(Lys)4(Aca-Aca-Biotin).2trifluoroacetate is commercially available from for example Enzo Life Sciences International Inc, Plymouth Meeting, PA, USA for use as an adjuvant.

WO-A-2009/072767 describes the use of a mixture of the Pam3Cys-SK4 and polyinosinic:polycytidylic acid as adjuvant for a vaccine.

The object of the present invention is to improve the immune response of a Pam3Cys-like lipopeptide.

Summary of the invention

The present invention is directed to an improved Pam3Cys-like lipopeptide, inducing a better immune response. The new compound is represented by



(1)

wherein R^1 and R^2 are branched or straight alkyl groups having 10 to 17 carbon atoms, n is 0 to and including 18, X is S or O and R is an organic group comprising one or more peptides.

Applicants found that the compound according to the invention is able to induce an improved immune response by functionally stimulating TLR2 as compared to the

known Pam3Cys-SK4. Without wishing to be bound to the following theory, applicants believe that the higher stimulation level is achieved by exchanging the bridging -CH_2-group in the N-terminal palmitoyl moiety of the known compound into a -NH-bridging group. The fatty chains of the compound fit in the defined pockets in the dimeric receptor and it is believed that by incorporating the bridging –NH- group, a hydrogen bridge is created. This results in a tighter binding of the ligand to the receptor, which in turn is beneficial to achieve the desired adjuvant activity.

Brief description of the Figures.

Figure 1 shows the Pam3Cys-SK4 compound according to the state of the art and two examples of compounds according to the present invention.

Figure 2 shows a synthesis scheme for the preparation of a compound according to the present invention.

Figure 3 shows the test results wherein TRL2-transfected HEK cells and dendritic cells were incubated with the state of the art compounds and compounds according to the invention.

Detailed description of the invention

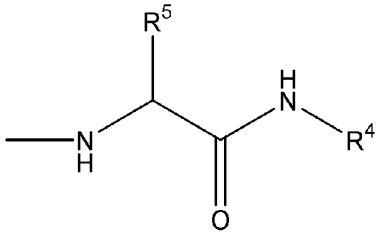
The compound (1) according to the present invention may have group X which is S or O. The naturally occurring O is preferred for synthetic ease. The S atom is a well known variant for the skilled person.

R^1 and R^2 are branched or straight alkyl groups having 10 to 17 carbon atoms and preferably straight alkyl groups having 10 to 17 carbon atoms. A preferred group R^1 and R^2 is a straight chain alkyl group with 15 carbon atoms. Groups with less carbon atoms, resulting in a less lipophilic version of the compound, may be advantageous because these may have better solubility properties and therefore exhibit more predictable behaviour in solution.

In formula (1) n is 0 to and including 18, preferably at least 4 and more preferably from 11 to and including 15. Applicants found positive results for compounds wherein n is 11 and wherein n is 13.

R is an organic group comprising one or more peptides. Examples of peptides are SSNASK4, SR8, RPDRY-NH_2 and QPDRY-NH_2. A preferred peptide is SK_m, wherein m is 0, 1, 2, 3, 4 or 5. Preferably m is 4. The SK4 is also known as

SerLysLysLysLys. The S part of the SK_n peptide and more preferably the S part of the SK4 peptide may suitably be modified as described below and may optionally be further coupled to an antigen. The preferred modified SK_m peptide is presented by:



(2)

in which R^4 represents a K_m peptide part, optionally further coupled to an antigen, and R^5 is a relatively small group comprising one to six atoms chosen from carbon, nitrogen and/or oxygen. m is preferably 4. Examples of possible groups R^5 are C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, C1-C6 hydroxyalkyl, C1-C6 mercaptoalkyl, C1-C6 aminoalkyl cycloalkyl, C1-C6-cyanoalkyl, C1-C6-azidooalkyl, C1-C6-haloalkyl, aromatic 5 or 6-membered rings containing carbon, nitrogen oxygen or sulphur, saturated 3-6 membered heterocyclic rings containing carbon, nitrogen oxygen or sulphur. R^5 is preferably a $–CH_2$-OH group, CH_2-CH_3 group CH_2-CN, group, CH_2CCH group, CH_2NH_2 group, CH_2Cl group, CH_2N_3 group. Configuration of the asymmetric carbon to which R^5 is attached can be L- or D and preferably L enantiomer.

Group R may comprise an antigen and more preferably group R^4 in formula (2) is further coupled to an antigen. The antigen may be directly coupled to the K_m peptide part or via a spacer molecule. This coupling may for example be a covalent bond or ionic bond.

The invention is also directed to a process to prepare the novel compounds starting from the corresponding peptide R-H and prepared by standard solid phase peptide synthesis protocol involving the coupling of a palmitoylated cysteine building block (Fmoc-Pam_2-Cys-OH) to the peptide R-H and a final treatment with alkylisocyanate according to H-$(CH_2)_n$-N=C=O. Preferably the following steps are performed:

(a) Solid-phase peptide synthesis obtaining immobilized and side-chain protected peptide R-H,

(b) coupling of Fmoc-Pam_2-Cys-OH with the immobilized and side-chain protected peptide R-H obtained in step (a)

(c) cleavage of the N-terminal Fmoc-group from the Pam2Cys-moiety

(d) reacting the product of step (c) with an isocyanate according to H-(CH2)n-N=C=O,

(e) performing an acid-mediated deprotection and cleavage from the solid phase of the product of step (b) and

(f) performing a RP HPLC purification.

The above process is illustrated by Figure 2 and below for the synthesis of U-Pam-14 and U-Pam-12. Figure 2 describes the solid-phase synthesis of target compounds U-Pam-14 and U-Pam-12, wherein the following terms are defined as:

TentaGelb S RAM is functionalized copolymer of polystyrene and polyethylene glycole provided with Rink-amide linker (a common solid phase for peptide synthesis)

NHBoc is tert-butyloxycarbonyl protected amino group

tBuO is tert-butyl ester

Fmoc-Pam2Cys-OH is Fluorenylmethyloxycarbonyl--S-[2,3-bis(palmitoyloxy)propyl]-L-cysteine.

PyBOP, is (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; a phosphonium activating reagent commonly used in peptide chemistry

DIPEA is diisopropylethylamine, an organic base commonly used in peptide chemistry

TFA is trifluoroacetic acid

NMP is N-methylpirrolidone, a solvent

DCM is dichloromethane, a solvent

The solid-phase peptide synthesis of step (a) is known and for example described (Dick, F. Peptide Synthesis Protocols. *In:* M.W. Pennington and B. M. Dunn (eds.) Methods in Molecular Biology, Vol. 35, pp. 63-72. Totowa: Humana Press Inc., 1994.) In step (a) immobilized and side chain protected peptide **1** is assembled starting from TentaGel S resin equipped with Rink amide linker (Tentagel S RAM in Scheme 1). The synthesis as illustrated is suitably performed in a fully automated fashion on ABI 433A peptide synthesizer applying Fmoc/OtBu chemistry with HCTU as the coupling reagent and DIPEA as the base. It should be understood that other peptide synthesizers known in the art may also be used. Upon the final

Fmoc-cleavage using for example a 20% piperidine in NMP the peptide resin (**1**) is suitably removed from the instrument, washed with DCM and dried. In step (b) the Fmoc-Pam_2Cys-OH is suitably coupled manually to the peptide resin **1** to give fully protected peptide resin **2**. Phosphonium coupling reagent PyBOP is used in this coupling step and the base (DIPEA) is suitably added in two portions to prevent base-catalyzed side reactions. After piperidine-mediated cleavage of Fmoc-group in step (c), the resin is suitably washed with NMP and the resulting immobilized N-lipohexapeptide with free N-terminal amino group was treated in step (d) with tetradecyl isocyanate in DCM/NMP mixture overnight to give immobilized and fully protected UPam-14 (**3**). The product was cleaved in step (e) from the resin with concomitant removal of the side chain protecting groups by acidolysis with suitably 95 % TFA in the presence of H_2O and TIS as cation scavengers. Subsequent in step (f) RP HPLC purification on C_4 phase and lyophylization furnished pure UPam-14 (**5**) as a white solid. The same protocol starting from resin **2** may be employed for the preparation of UPam-12 (**6**) except that dodecyl isocyanate is used instead of tetradecyl isocyanate on the stage of the introduction of urea connected N-terminal lipophilic chain to immobilized lipohexapeptide.

The compounds according to the invention as described above are suitably used as part of a medicament or vaccine. The invention is thus directed to a composition comprising the compound according to the invention for use as a medicament. More preferably the invention is directed to a composition comprising the compound according to the invention to enhance a TLR2 mediated innate immune reaction in a patient. The compound may be used in a so-called mono-therapy wherein in a stand-alone treatment the existing immune system is stimulated, for example for local administration in the lymphoid drainage area of a tumor.

Suitably the compound is used together with an antigen. The invention is therefore also directed to a composition comprising this compound for use as a preventive or therapeutic vaccine composition. In particular, the invention is directed to a vaccine composition comprising the compound according to the invention as an adjuvant and at least one antigen, wherein the antigen may be present as a separate compound or coupled to the compound according to the invention as described above. Preferably the antigen is part of the compound, wherein the antigen is coupled to the adjuvant compound described above. Such a linkage has the advantage that, in use, an enhanced immune response by simultaneous stimulation

of antigen presenting cells, in particular dendritic cells, that internalize, metabolize and display antigen is achieved.

The antigen may be any material that can induce an immune response by the immune system of animal or human. It can be a full length biomacromolecule or a fragment thereof. The antigen can for example be synthetic material, purified subunits of a protein, a whole microbe or a mixture thereof. Preferably the antigen is a synthetically produced peptide, oligonucleotide or oligosaccharide and more preferably it has been obtained after purification.

The antigen is preferably selected as a single or multiple component from the group consisting of a protein of a pathogen, a recombinant protein, a peptide, a hapten, a polysaccharide, a glycoprotein, a lipopolysaccharide, a DNA molecule, a cDNA molecule, an RNA molecule (all polynucleotides), a cancer cell and a micro-organism.

A preferred composition comprises a compound according to the invention as adjuvant and at least one viral antigen or bacterial antigen, for example TBC; tetanus and Helicobacter Pylori, or parasite antigen or tumor antigen suitable for treating or preventing viral or parasitic or bacterial infections or treating or preventing cancer or comprises a compound according to the invention, wherein group R comprises a viral antigen or bacterial antigen, for example TBC and tetanus, or parasite antigen or tumor antigen suitable for treating or preventing viral or parasitic or bacterial infection or treating or preventing cancer.

Suitable viral antigens are influenza virus antigen, such as for example HA: haemaglutinin or neuraminidase antigen; human papilloma virus (HPV) antigen, such as E6 , E7; human immunodeficiency virus (HIV) antigen, such as for example GP120, GP140, GP160; vesicular stomatitis virus antigen, for example vesicular stomatitis virus glycoprotein; cytomegalovirus (CMV) antigen; hepatitis virus antigens, such as for example hepatitis A(HAV), B(HBV), C(HCV), D(HDV) and G(HGV): L-HBsAg, S-HBsAg, M-HBsAg, pre S; respiratory syntytial virus (RSV) antigen; SV40 virus antigen, such as Large T, small T; EBV antigen, such as EBNA, Kaposi Sarcoma Virus (KSV) antigen, Human T-Lymphotropic Virus-1(HTLV-1) antigen, Merkel cell virus (MCV) antigen or herpes simplex virus antigen.

Suitable parasite antigens may be derived from protozoa, nematoda, trematoda or cestoda, such as Cryptosporidium hominis or parvum; Schistosoma haematobium, mansoni or japonicum; Plasmodium falciparum, malariae, vivax or ovale; Leishmania

major, tropica, aethiopica, Mexicana, donovani, infantum or braziliensis; Toxoplasma Gondii.

Suitable bacterial antigens may be antigens derived from Mycobacterium Tuberculosis, Streptococcus pneumoniae, Staphylococcus Aureus, Vibrio cholera, Neisseria meningitides.

Tumor antigens are antigens expressed on tumor cells. This group of antigens comprises antigens derived from proteins that are expressed solely on tumors and not or only in a limited amount on normal adult cells, antigens derived from proteins that are overexpressed on tumors as compared to normal adult cells and antigens derived from proteins that have been mutated in tumors.

Suitable antigens include antigens derived from infectious agents that cause diseases such as cancers and/or premalignant conditions. Examples of such infectious agents are HPV, which causesdiseases such as a cervical cancer, head and neck cancer, Penile cancer, Vulva cancer, Anal cancer, nasopharyngeal cancer, CIN, VIN, PIN, VAIN and AIN, HCV and HBV, which are involved in liver carcinoma, SV40, which is involved in mesothelioma, HTLV-1, which is involved with T cell leukemia/lymphoma, Merkel cell virus, which is involved with Merkel cell carcinoma and KSV, which is involved with Kaposi sarcoma.

The above vaccine compositions may be used as a preventive or therapeutic vaccine composition for both acute or persistent infections or disease caused thereby.

The vaccine composition is also preferably used as a preventive or therapeutic vaccine composition designed to elicit specific immune responses against non-viral cancers. A preferred vaccine composition comprises a compound according to the invention as adjuvant and at least one non viral cancer-associated tumor antigen or comprises a compound according to the invention, wherein group R comprises a non viral cancer-associated tumor antigen. The cancer to be treated or be prevented may be a brain cancer, renal cell carcinoma, a melanoma, a leukemia, a lung cancer, a stomach cancer, an esophageal cancer, a thyroid cancer, a pancreatic cancer, a breast cancer, a prostate cancer, an ovarian cancer, a uterine cancer, a testicular cancer, a cholangioma, a liver cancer, a colon cancer, a gastrointestinal cancer, a bladder cancer, or a rectal cancer. In addition pre-malignant lesions may be treated or prevented by use of the vaccine composition. Pre-malignant lesions are lesions that have undergone genetic changes that predispose cells to become cancer cells.

These pre-malignant lesions may evolve into cancers over time. Examples of suitable tumor antigens are gp100, MART-1, MAGE-1, BAGE, GAGE, HAGE, tyrosinase, CEA (cancer embryonic antigen), p53, PSA (prostate specific antigen), PSMA (prostate specific membrane antigen); PRAME, HER2/neu, MAGE-1, MAGE-2, MAGE-3, NY-ESO-1, MUC-1, SART-1 or SART-3, XAGE-1B, Tyrosinase, TERT (telomerase reverse transcriptase), WT1 , Survivin-2B, gp75, MDM2, telomerase, al[rho]h-1 fetoprotein, CA125, CA15-3, CA19-9, G250, HER2, BCR-ABL, Ras, PML-RARα, PR1, SSX-2, HSP70 or a peptide analogue derived from any of the above mentioned viral, non-viral, tumor, bacterial or parasite antigens.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from any of the abovementioned viral, non-viral, tumor, bacterial or parasite antigens, but preferably from the high risk human papilloma virus (HPV)-specific E6 and E7 oncoproteins as described in WO02/070006 and WO2008/147187, which publications are hereby incorporated by reference.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from the non-viral tumor antigen p53 as described in WO2008/147186, which publication is hereby incorporated by reference.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from the non-viral tumor antigen PRAME as described in WO2008/118017, which publication is hereby incorporated by reference.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from the non-viral tumor antigen NY-ESO-1 as described in WO98/14464, which publication is hereby incorporated by reference.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from the non-viral tumor antigen XAGE-1B as described in US6,630,574, US6,504,010, US7,425,607, US6,686,447, which publications are hereby incorporated by reference.

A preferred antigen comprises peptides comprising CD4 and/or CD8 T cell epitopes that are derived from the non-viral antigens derived from *Mycobacterium tuberculosis* as described in WO06/04389, which publication is hereby incorporated by reference.

Such a peptide may comprise additional amino acids than the ones originating from an antigen or may entirely be made of or consist of an amino acid sequence originating from such antigen. The length of the contiguous amino acid sequence

from one of the above-defined antigens comprised within the peptide, preferably is at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 amino acids and/or preferably no more than 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 60, 50, 45, 40, 35, 33 or 30 amino acids, more preferably the length of the contiguous amino acid sequence from one of the above-defined antigens comprised within the peptide is 19-45, even more preferably 22-40 amino acids, even more preferably 30-35 and most preferably 33-35 amino acids. In another preferred embodiment, the peptide of the invention consists of any of the contiguous amino acid sequence from the antigen as defined herein, whereby it is understood that no amino acids are appended to either end of the contiguous amino acid sequence from the antigen that are not contiguous with this amino acid sequence in the sequence of the native antigen. These peptides may be easily synthesized and are large enough to be taken up by professional antigen presenting cells, processed by the proteasome and have sufficient physical capacity and length to contain at least one HLA class I and/or at least one HLA class II epitope. Optionally a peptide may comprise N- or C-terminal extensions, which may be amino acids, modified amino acids or other functional groups that may for instance enhance bio-availability, cellular uptake, processing and/or solubility.

The vaccine composition of the present invention can additionally include, in addition to the adjuvant and an antigen, one or more effective ingredients having the same or similar effect with them. For example the vaccine composition according to the invention may comprise one or more adjuvants, in addition to the adjuvant according to the present invention. These other adjuvants may be admixed to the vaccine composition according to the invention or may be administered separately to the mammal or human to be treated. Examples of suitable other adjuvants to be used in combination with the adjuvant compound according to the invention are Montanide adjuvant, such as Montanide ISA-51 or Montanide ISA 720 (Seppic France), Freund's adjuvant or IFA,. Resiquimod; imiquimod; Poly IC:LC (Hiltonol); ISCOMS; CpG and GLA;MPL.

The vaccine composition may also comprise compounds like for example detoxified Lipid A, clinical grade CpG or other appropriate immunomodulatory agent or antibody such as CTLA-4 blocking or CD40 agonistic antibodies or agonistic antibodies against other members of the TNF receptor family such as OX40, CD27, 4-1-BB (CD137) or 4-1-BB and/or CD40 ligands, OX40 ligands or functional

fragments and derivates thereof, as well as synthetic compounds with similar agonistic activity. These compounds can be mixed or conjugated to either the compound according to the invention and/or to the specific antigen in the vaccine.

The vaccine composition can also include, in addition to the above-mentioned effective ingredients, one or more pharmaceutically acceptable carriers for the administration. The pharmaceutically acceptable carrier can be selected or be prepared by mixing more than one ingredients selected from a group consisting of saline, sterilized water, Ringer's solution, buffered saline, dextrose solution, maltodextrose solution, glycerol and ethanol. Other general additives such as anti-oxidative agent, buffer solution, bacteriostatic agent, etc., can be added. In order to prepare injectable solutions such as aqueous solution, suspension and emulsion, diluents, dispersing agents, surfactants, binders and lubricants can be additionally added.

The specific formulation of the vaccine composition of the present invention, ways of administration and the use of pharmaceutically acceptable excipients are known in the art and for instance described in Remington; The Science and Practice of Pharmacy, 21st Edition 2005, University of Sciences in Philadelphia. Vaccine compositions and medicaments of the invention are preferably formulated to be suitable for intravenous or subcutaneous, or intramuscular administration, although other administration routes can be envisaged, such as mucosal administration or intradermal and/or intracutaneous administration, e.g. by injection or via a patch. Intradermal administration is preferred herein.

In a preferred embodiment, the vaccine composition is formulated to be suitable for intradermal administration or application. Intradermal is known to the skilled person. In the context of the invention, intradermal is synonymous with intracutaneous and is distinct from subcutaneous. A most superficial application of a substance is epicutaenous (on the skin), then would come an intradermal application (in or into the skin), then a subcutaneous application (in the tissues just under the skin), then an intramuscular application (into the body of the muscle).

The intradermal administration of the vaccine composition is very attractive since the injection of the vaccine is realized at or as close by as possible to the site of the disease resulting in the local activation of the disease draining lymph node, resulting in a stronger local activation of the immune system. In a preferred embodiment, the intradermal administration is carried out directly at the site of the

lesion or disease. At the site of the lesion is herein understood to be within less than 5, 2, 1, 0.5, 0.2 or 0.1 cm from the site of the lesion.

In addition, a preferred embodiment comprises delivery of the antigen and adjuvant compound as part of the vaccine composition in a slow release vehicle such as mineral oil (e.g. Montanide ISA 51) ,PLGA based particles or scaffolds, dextran based particles or scaffolds, poly active based particles or scaffolds, liposomes, virosomes. Preferably for intradermal delivery the vaccine composition is administered in a composition comprising in addition one or more immunologically inert pharmaceutically acceptable carriers, e.g. buffered aqueous solutions at physiological ionic strength and/or osmolarity (such as e.g. PBS).

It is furthermore encompassed by the present invention that the administration of at least one vaccine composition of the invention may be carried out as a single administration. It may also be possible that the various active compounds of the vaccine are administered sequentially and/or using different ways or different sites of administration. Alternatively, the administration of at least one vaccine composition may be repeated if needed.

The invention shall be illustrated by the following non-limiting examples.

Example 1

To illustrate the advantages of the compounds according to the invention two variants of the established TLR2/TLR1 ligand Pam_3CysSK_4 containing the CH_2 to NH substitution were prepared. These two compounds named U-Pam-14 and U-Pam-12 differ in the length of the fatty chain attached to the N-terminus of the Cys residue, U-Pam-14 being an exact isostere of palmitoyl moiety of the natural ligand while UPam-12 contains a shortened chain. Figure 1 shows the state of the art Pam_3CysSK_4 ligand and the U-Pam-14 and U-Pam-12 ligand according to the invention. A circle shows where the $-CH_2-$ bridge has been replaced by the -NH- bridge.

All reagents and solvents used in the solid phase peptide synthesis were purchased from Bachem and Biosolve and used as received. Fmoc-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH was purchased from Bachem, Fmoc-amino acids, HCTU and PyBOP from Novabiochem. Tentagel based resins were ordered from Rapp Polymere. LC/MS was conducted on a JASCO system using a Vidac C4 analytical column (4.6 x 50 mm, 5 µm particle size, flow 1.0 mL/min.) or an Alltima CN

analytical column (4.6 x 50 mm, 3 µm particle size, flow 1.0 mL/min.). Absorbance was measured at 214 and 256 nm.

Solvent system:

A: 100% water,

B: 100% acetonitrile,

C: 1% TFA/H2O.

Gradients of B in 10% C were applied over 15 minutes unless stated otherwise. Purifications were conducted on the Gilson preparative HPLC system, supplied with a semi preparative Vidac C4 column (10 x 250 mm, 5 µm particle size, flow 5.0 mL/min.).

Solvent system:

A: 100% water,

B: 100% acetonitrile,

C: 1% TFA/H2O.

Gradients of B in 10% C were applied over 3 CV unless stated otherwise. The UV absorption was measured with 214 and 256 nm. The solid-phase peptide synthesis was performed on an ABI (Applied Biosystems) 433A automated instrument applying Fmoc based protocol starting from Tentagel-RAM resin according to established methods. The consecutive steps performed in each cycle applied for Fmoc-Lys(Boc)-OH were:

1) Deprotection of the Fmoc-group with 20% piperidine in NMP for 15 min;

2) NMP wash;

3) Coupling of the appropriate amino acid using a five-fold excess.

Briefly, the Fmoc amino acid (0.25 mmol) was dissolved in 0.25 M HCTU in NMP (1 mL), the resulting solution was transferred to the reaction vessel followed by 0.5 mL of 1.0 M DIPEA in NMP to the initiate the coupling. The reaction vessel was then shaken for 45 min;

4) NMP wash;

5) capping with 0.5 M acetic anhydride in NMP in presence of 0.5 mmol DIPEA;

6) NMP wash;

7) Final Fmoc removal with 20% piperidine in NMP for 15 min;

8) NMP wash;

9) DCM wash.

H-Ser(OtBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Rink-Tentagel (1)

Peptide synthesis was performed on a 1 mmol scale using an ABI 433A automated instrument applying Fmoc based protocol starting form Rink Amide S Tentagel (loading 0.26 mmol/g). The resin, after final Fmoc deprotection, was washed with NMP and DCM and dried. The resulting resin 1 was used in the next step.

General procedure coupling Fmoc-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH

The Tentagel S Ram resin 1 loaded with H-Ser(OtBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Rink-Tntagel was treated with a 0.5 mL stock solution of 0.18 M Fmoc-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH in 0.22 M PyBop in DCM:NMP (2:1). The resulting mixture was activated with 2 x 44 µmol Dipea over 15 min. and reacted by shaking for 18h followed by NMP and DCM wash. The resin was swelled in DCM:NMP again and divided in portions of 10 µmol.

General procedure for isocyanate addition

The 10 µmol resin loaded with Fmoc-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser(tBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) 2 was swelled in DCM:NMP (1:1) and treated with 3 x 3 min 20% piperidine in NMP for Fmoc-deprotection. After a thorough NMP wash the resin was suspended in 1 mL DCM:NMP (1:1) and treated with tetradecyl isocyanate or dodecyl isocyanate (25 µL) . The mixture was shaken for 18 h, washed with NMP and DCM and air dried. The resin was treated for 104 minutes with a cleavage cocktail TFA/TIS/H2O (95/2.5/2.5). The solution was filtered and precipitated with Et2O (50 mL) and stored at -200C for 18h. The Et2O was centrifuged, removed and the precipitated was dissolved by sonification in 1 ml MeCN : H2O : tBuOH (1:1:1). Of each 50µL product was diluted with 50µL MeCN : H2O : tBuOH (1:1:1) for LCMS analysis (Vidac C4 column). Obtained sequences were diluted with another 0.5 mL MeCN : H2O : tBuOH (1:1:1) and were purified on a semipreparative Vidac C4 column (10 x 250 mm, 5 µm particle size, flow 5.0 mL/min, 60-100% B.).

The thus obtained compounds according to the invention had the following properties:

Upam-14:
1-tetradecyl-urea-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys-NH_2 was obtained:

0.89 mg (0.59 µmol, 6%),
LCMS: 50-90% B,
rt 8.23 min.
Bruto formula $C_{80}H_{156}N_{12}O_{12}S$ calculated 1509.17, found ESI-MS: [M+H]+: 1510.6 (calculated 1510.2), [M+H]2+: 756.0 (calculated 755.8). HRMS [M+H+] calcd for $C_{80}H_{156}N_{12}O_{12}S$ 1510.17592, found 1510.17670.

Upam-12
1-dodecadecyl-urea-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys-NH_2
0.89 mg (0.59 µmol, 6%),
LCMS: 50-90% B
rt 8.06 min.
Bruto formula $C_{79}H_{154}N_{12}O_{12}S$ calculated 1495.15, found ESI-MS: [M+H]+: 1496.3 (calculated 1496.16).

Example 2

The two compounds U-Pam-12 and U-Pam-14 as obtained in Example 1 were tested in comparison to unmodified Pam3CysSK4 for their functional capacity to activate a human TLR2 expressing reporter cell line HEK-TLR2 (Figure 3A) and a murine dendritic cell line (Figure 3B).

In the test live TLR2-transfected HEK cells and dendritic cells ($5x10^4$ cells/well) were incubated with titrating concentrations of the respective Pam compounds in culture medium and incubated at 37 ^oC. After 24 hours, supernatants were harvested and the presence of IL-8 or IL-12 cytokines respectively, was measured by specific sandwich ELISA assays.

Both cell types were significantly more stimulated by the U-Pam compounds than the unmodified Pam3CysSK4. Both compounds increased the maximal stimulation level at least twofold and were calculated to be at least 100-fold more effective as based on the concentration of the compound needed to reach similar stimulation levels.

It was found that the compound according to the invention can functionally stimulate TLR2 from both human and mouse origin in low (pM to nM) concentrations. The active concentrations are lower than those of the unmodified TLR2 ligand. Additionally, the physiologically important dendritic cells can be activated to produce the immunologically relevant cytokine IL-12. This cytokine is crucially important to facilitate efficient priming of specific T lymphocytes to viruses and/or tumor-antigens. Therefore, a composition comprising said compound as adjuvant can effectively be used to increase the immunogenicity of antigen and thereby improving the efficacy of a vaccine.

It is believed that the use of a compound according to the invention will result in an improved immune response, meaning a more robust innate immune system activation as well as a more robust adaptive immune system activation, expressed in a higher T cell response and/or a higher antibody response, in comparison to immune stimulation with the known Pam3Cys-SK4.

Example 3

This example illustrates the synthesis of a UPam-14 derivative in which R5 group (CH2-OH) is replaced by CH2-CH3. This compound named here Upam-14-Abu.

H-Abu-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Rink-Tentagel (2)

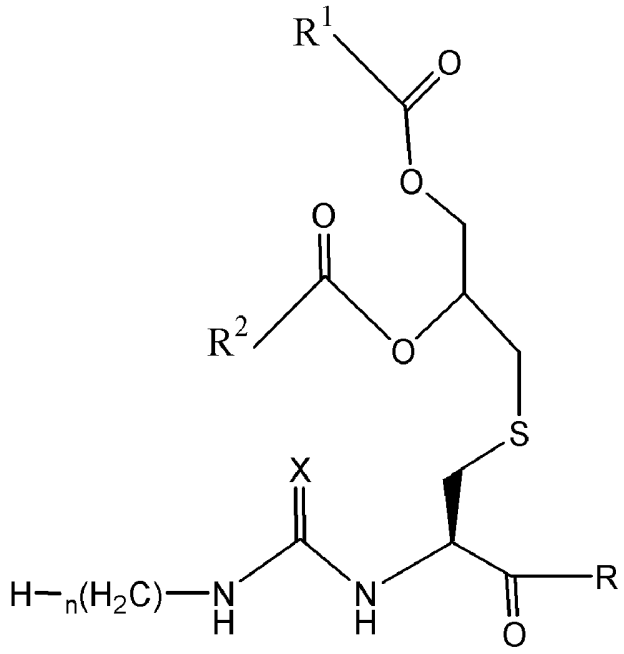
Peptide synthesis was performed as described in Example 1 for H-Ser(OtBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Rink-Tentagel (1) with the only difference that Fmoc-Abu-OH was applied instead of Fmoc-Ser(OtBu)-OH to introduce 2-aminobutyric acid residue instead of serine residue of Upam-14. Subsequent synthetic and purification steps were identical to those described in Example 1.

The thus obtained compound according to the invention had the following properties:

Upam-14-Abu
1-tetradecyl-urea-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Abu-Lys-Lys-Lys-Lys-NH2
3.22 mg (2.13 µmol, 21%),
LCMS: 50-90% B
rt 8.31 min.
Bruto formula C81H158N12O11S calculated 1507.19, found ESI-MS: [M+H]+: 1508.5 (calculate 1508.2), [M+H]2+: 755.1 (calculated 754.6). HRMS [M+H+] calcd for C81H158N12O11S 1508.19665, found 1508.19725.

CONCLUSIES

1. Verbinding, weergegeven door



de bovenstaande formule [1], waarin R^1 en R^2 vertakte of rechtketenige alkylgroepen zijn met 10 tot 17 koolstofatomen, n gelijk is aan 0 tot en met 18, X gelijk is aan S of O, en R een organische groep is die één of meerdere peptiden omvat.

2. Verbinding volgens conclusie 1, waarbij X gelijk is aan O.

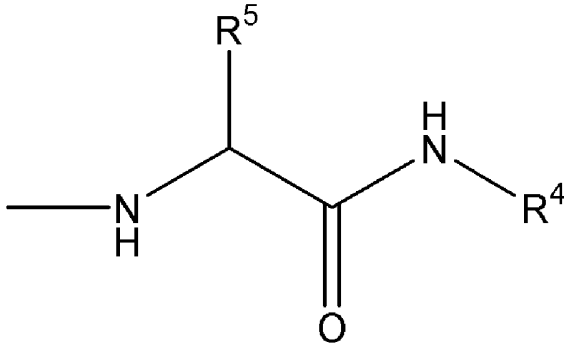
3. Verbinding volgens conclusie 1 of conclusie 2, waarbij R^1 en R^2 rechtketenige alkylgroepen zijn met 10 tot 17 koolstofatomen.

4. Verbinding volgens één der conclusies 1-3, waarbij R^1 en R^2 rechtketenige alkylgroepen zijn met 15 koolstofatomen.

5. Verbinding volgens één der conclusies 1-4, waarbij n gelijk is aan 11, 12, of 13.

6. Verbinding volgens één der conclusies 1-5, waarbij R gelijk is aan SK_m, waarbij m gelijk is aan 0, 1, 2, 3, 4, of 5, eventueel verder verbonden met een antigen.

7. Verbinding volgens één der conclusies 1-5, waarbij de groep R wordt weergegeven door



waarbij R^4 een K_m peptidedeel is, eventueel verder verbonden met een antigen, en waarbij R^5 een relatief kleine groep is die één tot zes atomen omvat die geselecteerd worden uit koolstof, stikstof, en/of zuurstof.

8. Verbinding volgens conclusie 7, waarbij R^5 een $-CH_2$-OH groep is, dan wel een CH_2-CH_3 groep, een CH_2-CN groep, een CH_2CCH groep, een CH_2NH_2 groep, een CH_2Cl groep, of een CH_2N_3 groep.

9. Verbinding volgens conclusie 8, waarbij het asymmetrische koolstof waarop de R^5 gebonden is, L-enantiomeer is.

10. Verbinding volgens één der conclusies 7-9, waarbij een antigeen rechtstreeks verbonden is met het K_m peptidedeel, dan wel door middel van een spacermolecule R4 een antigen omvat.

11. Verbinding volgens één der conclusies 6-10, waarbij m gelijk is aan 4.

12. Werkwijze voor het bereiden van een verbinding volgens één der conclusies 1-11, vertrekkende van de overeenstemmende peptide R-H, en bereid door gebruik te maken

van een standaard vaste-fase peptidesyntheseprotocol waarbij een ge-palmitoyleerde cysteïne bouwsteen (Fmoc-Pam_2-Cys-OH) gebonden wordt op het peptide R-H, en waarbij een uiteindelijke behandeling wordt doorgevoerd met alkylisocyanaat volgens H-($CH_2)_n$-N=C=O.

13. Samenstelling die een verbinding bevat volgens één der conclusies 1-10, met het oog op een gebruik als geneesmiddel.

14. Samenstelling volgens conclusie 13, met het oog op een gebruik als preventieve of therapeutische vaccinsamenstelling.

15. Samenstelling volgens conclusie 13 of 14, waarbij de verbinding volgens één der conclusies 1-10 is verbonden met een antigen, of waarbij een antigen aanwezig is in de vorm van een afzonderlijke verbinding.

16. Samenstelling volgens conclusie 15, waarbij het antigen wordt geselecteerd als een enkel- of meervoudige component uit de groep die bestaat uit een proteïne van een pathogeen, een recombinante proteïne, een peptide, een hapteen, een polysaccharide, een glycoproteïne, een lipopolysaccharide, een DNA-molecule, een cDNA-molecule, een RNA-molecule, een kankercel, en een micro-organisme.

17. Samenstelling volgens conclusie 15, waarbij het antigen een tumor-antigen, een viraal antigen, een bacterieel antigen, of een parasitair antigen is.

18. Samenstelling volgens conclusie 15, waarbij het antigen een antigen is dat is afgeleid van infectieuze middelen die cancereuze en/of premaligne toestanden veroorzaken.

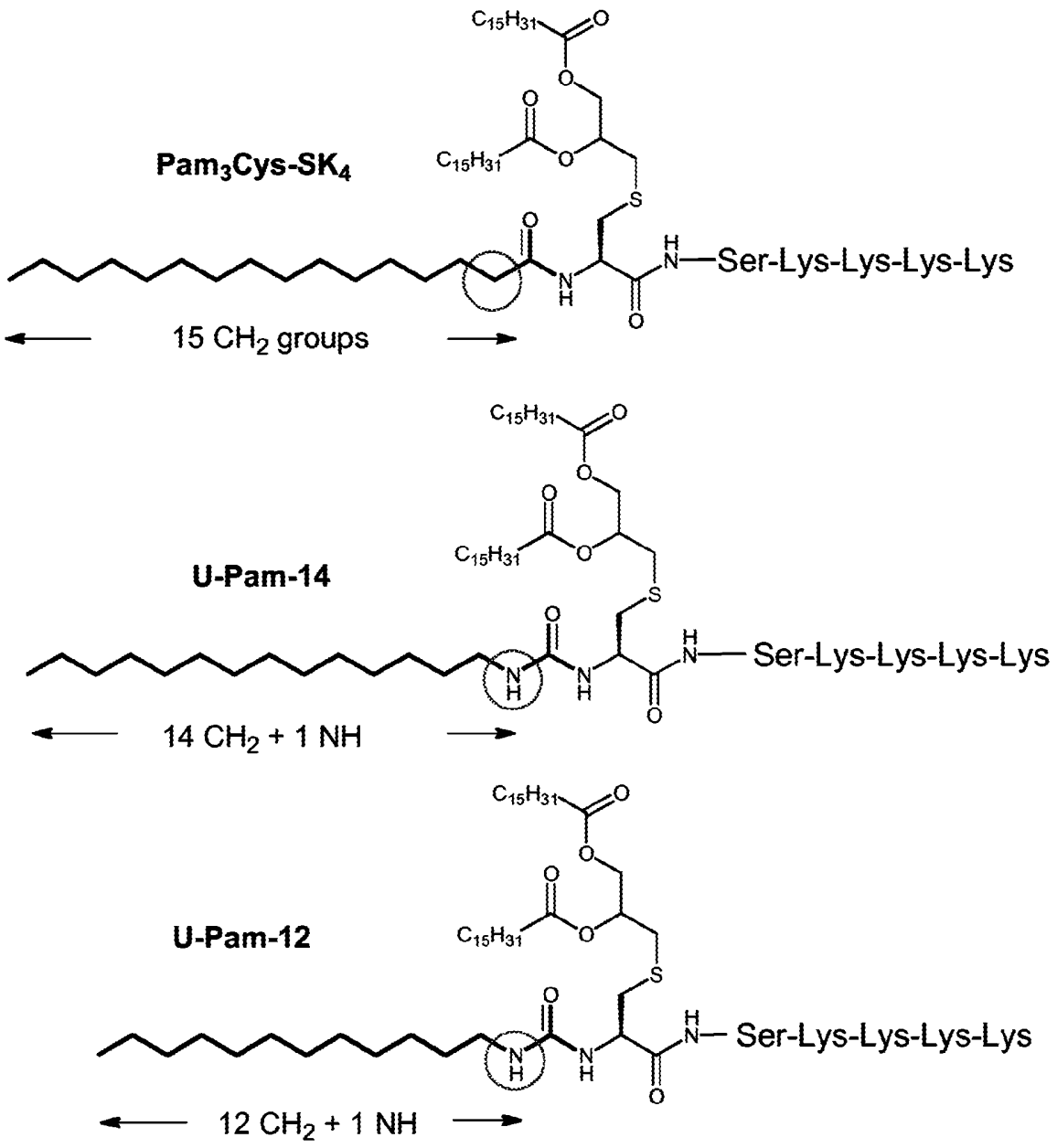

Pam3Cys-SK4
C15H31
Ser-Lys-Lys-Lys-Lys
15 CH2 groups
U-Pam-14
14 CH2 + 1 NH
U-Pam-12
12 CH2 + 1 NH

Fig. 1

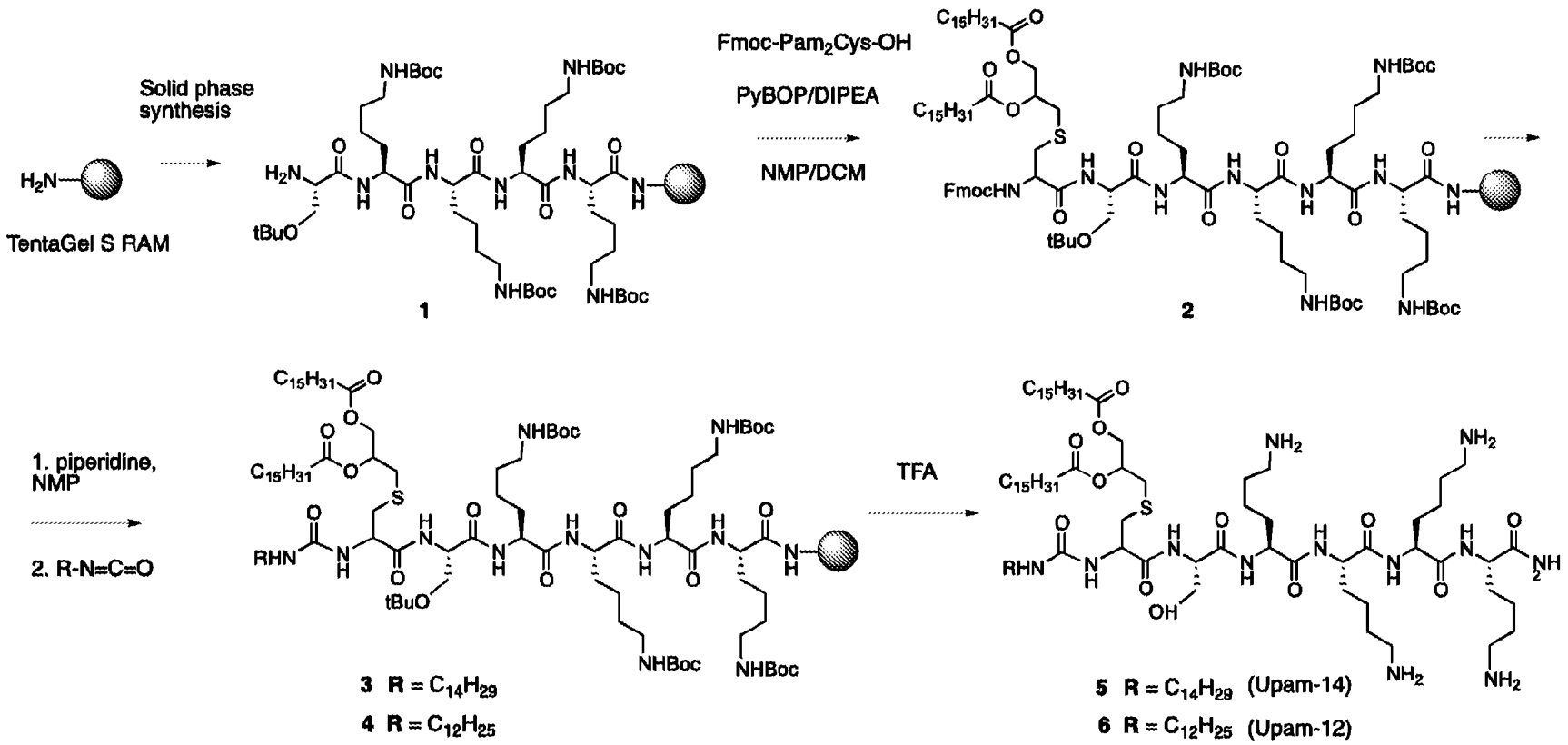

Solid phase synthesis
TentaGel S RAM
1
Fmoc-Pam2Cys-OH
PyBOP/DIPEA
NMP/DCM
2
1. piperidine, NMP
2. R-N=C=O
3 R = C14H29
4 R = C12H25
TFA
5 R = C14H29 (Upam-14)
6 R = C12H25 (Upam-12)

Fig.2 1

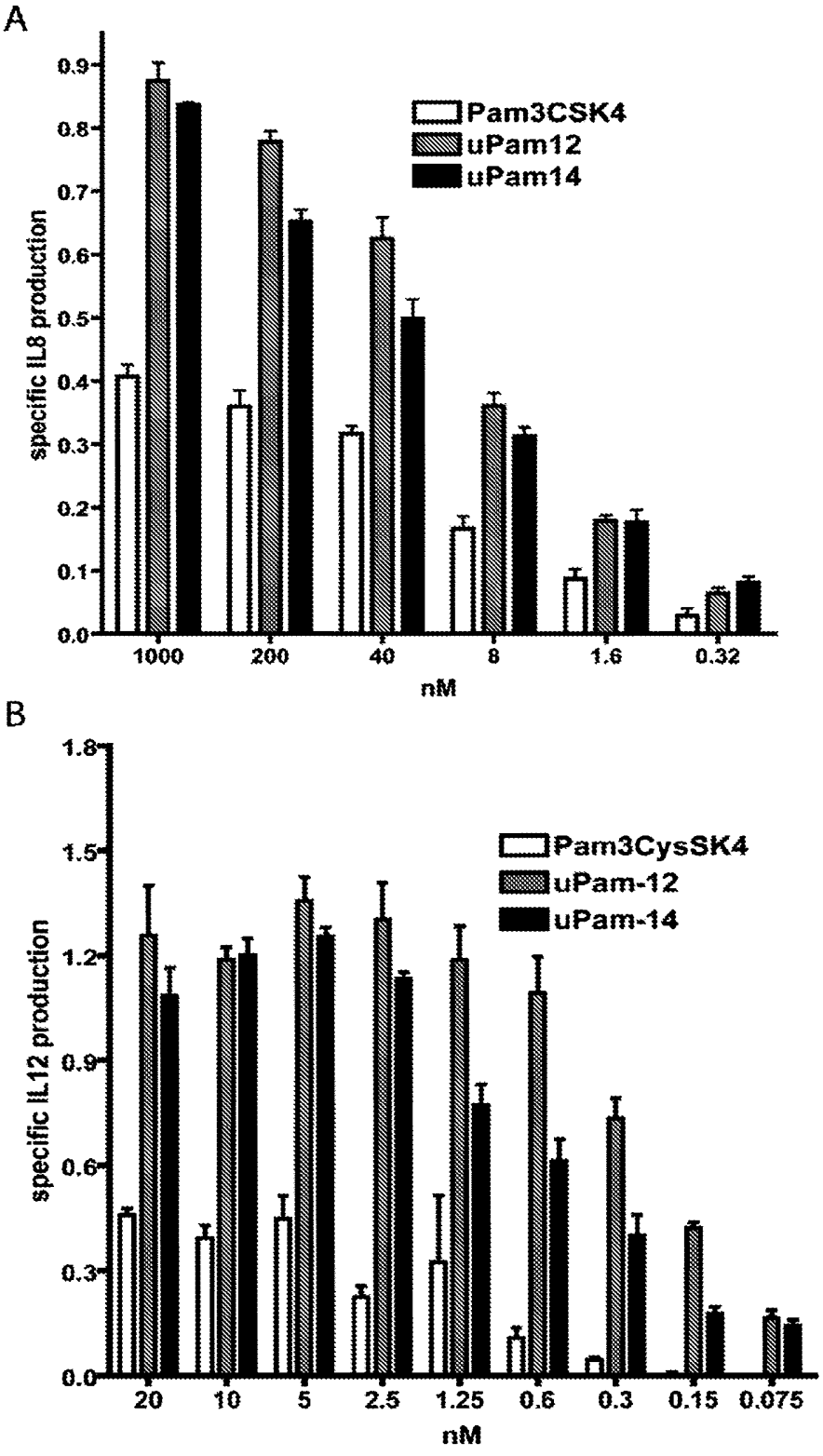

A
specific IL8 production
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Pam3CSK4
uPam12
uPam14
1000
200
40
8
1.6
0.32
nM
B
specific IL12 production
0.0
0.3
0.6
0.9
1.2
1.5
1.8
Pam3CysSK4
uPam-12
uPam-14
20
10
5
2.5
1.25
0.6
0.3
0.15
0.075
nM

Fig. 3

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	P117118NL00
Nederlands aanvraag nr. **2007536**	Indieningsdatum **05-10-2011**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Academisch ziekenhuis Leiden h.o.d.n. LUMC**	
Datum van het verzoek voor een onderzoek van internationaal type **12-11-2011**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN 57185**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

A61K39/39

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**A61K**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐ **GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐ **GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2007536

A. CLASSIFICATIE VAN HET ONDERWERP
INV. A61K39/39
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
A61K

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	TSUNEAKI HIDA ET AL: "Synthesis and Biological Activities of TAN-1511 Analogues.", THE JOURNAL OF ANTIBIOTICS, deel 48, nr. 7, 1 januari 1995 (1995-01-01), bladzijden 589-603, XP55023889, ISSN: 0021-8820, DOI: 10.7164/antibiotics.48.589 * tabel 5; verbinding 30 *	1-18
A	EP 0 431 327 A1 (HOECHST AG [DE] AVENTIS PHARMA GMBH [DE]) 12 juni 1991 (1991-06-12) * conclusies *	1-18

-/--

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
5 april 2012	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Winger, Rudolf

1

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2007536

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	MOYLE PETER M ET AL: "Self-adjuvanting lipopeptide vaccines", CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS BV, BE, deel 15, nr. 5, 1 januari 2008 (2008-01-01), bladzijden 506-516, XP009104575, ISSN: 0929-8673, DOI: 10.2174/092986708783503249 * bladzijde 508; figuur 1 * -----	1-18

Formulier PCT/ISA/201 (vervolg tweede blad) (Januari 2004)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2007536

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)		Datum van publicatie
EP 0431327	A1 12-06-1991	AT	137118 T	15-05-1996
		DE	3937412 A1	16-05-1991
		DE	59010300 D1	30-05-1996
		DK	0431327 T3	12-08-1996
		EP	0431327 A1	12-06-1991
		ES	2087111 T3	16-07-1996
		GR	3019859 T3	31-08-1996
		IE	904051 A1	22-05-1991
		JP	3057748 B2	04-07-2000
		JP	4054131 A	21-02-1992
		PT	95824 A	13-09-1991

Agentschap NL
Ministerie van Economische Zaken,
Landbouw en Innovatie

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN57185	05.10.2011		NL2007536

International Patent Classification (IPC)
INV. A61K39/39

Applicant
Academisch Ziekenhuis Leiden LUMC, et al

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

Examiner
Winger, Rudolf

Application number

WRITTEN OPINION

NL2007536

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	1-18
	No:	Claims	
Inventive step	Yes:	Claims	1-18
	No:	Claims	
Industrial applicability	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents of the search report and the passages identified therein:

D1 TSUNEAKI HIDA ET AL: "Synthesis and Biological Activities of TAN-1511 Analogues.",
THE JOURNAL OF ANTIBIOTICS,
deel 48, nr. 7, 1 januari 1995 (1995-01-01), bladzijden 589-603,
XP55023889,
ISSN: 0021-8820, DOI: 10.7164/antibiotics.48.589

D2 EP 0 431 327 A1 (HOECHST AG [DE] AVENTIS PHARMA GMBH [DE]) 12 juni 1991 (1991-06-12)

D3 MOYLE PETER M ET AL: "Self-adjuvanting lipopeptide vaccines",
CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS BV, BE,
deel 15, nr. 5, 1 januari 2008 (2008-01-01), bladzijden 506-516,
XP009104575,
ISSN: 0929-8673, DOI: 10.2174/092986708783503249

1.1 Document D1 discloses a N-$ClCH_2CONHCO$-Pam_2Cys-peptide and its effect on proliferation of bone marrow cells.

1.2 Document D2 discloses a vaccine consisting of a conjugate of at least one triacyl-S-glycerylcystein bound to a protein, which contains at least one killer T-cell epitope, of a virus, of a bacterium, of a parasite or of a tumour antigen.

1.3 Document D3 reviews lipid based peptide vaccines including different triacyl-S-glycerylcystein based ones as well as analysis of the structure-activity relationship which at R_1 only suggests Pam or H.

2 Claim 1 relates to a compound represented by Formula (1), claim 12 to a process for its preparation, claims 13 and 14 to its medical use and claim 15 to the coupling to an antigen.

Document D1 differs in that the N-substituent is not only alkyl (and that it is used for different medical purposes).

Documents D2 or D3 which can be regarded as being the prior art closest to the subject-matter of claim 1 differ in the N-substituent.

In the application it has been shown that this substitution led to a significantly increased stimulation of two cell types (example 2 / fig. 3).

The problem to be solved by the present invention may be regarded as to provide improved lipopeptide adjuvants.

The solution to this problem is considered as involving an inventive step because non of the prior art documents disclose or suggest the substitution as made in formula (1), even less so with the expectation of an improved immune response.

Thus, the subject-matter of independent claims 1, 12 and 13-15 as well as of the dependent claims meet the requirements of novelty and inventive step.