Description

Field of the Invention

The present invention provides novel lipophilic disaccharide-tripeptide compounds having improved immunological efficacy, and a liposome encapsulated composition comprising said compounds.

Background of the Invention

Intact microbial agents are known to have immunological effects. These effects include both immuno-adjuvant efficacy and antitumor effects, in both experimentally induced and human malignancies. The active components, consisting of the peptidoglycan cell wall skeleton and trehalose dimycolate, have been isolated from mycobacteria. These active components, especially when attached to mineral oil or squalene, are known to be as active as the intact microbial agents. See, for example, E. Ribi et al., Ann. NY Acad. Science, U.S.A., 277, 228-236 (1976).

The cell wall skeleton of Nocardia rubra (N-CWS) is also known to activate macrophages. Given intravenously, oil-attached N-CWS can cure some rats with experimental pulmonary metastases. See, for example, S. Sone et al., Cancer Immunology Immunotherapy, 12, 203-209 (1982). Smaller, water soluble monomeric units of the cell wall peptidoglycans have been demonstrated to be adjuvant active. Adjuvants are compounds causing stimulation of the immune system of a human or other mammal which result in an increased production of antibodies and in an enhancement of the protective reaction of the organism, e.g., against infection. Such monomeric units have also shown antitumor activity when given intravenously, for example, in mice bearing the Lewis lung carcinoma or the MCA mammary carcinoma. See, for example, G. Sava et al., Cancer Immunology Immunotherapy, 15, 84-86 (1983).

The active components of these organisms have been isolated, purified, and synthesized. These components are glycopeptides constituting a broad class of organic compounds which include a sugar part and a peptide part. Glycopeptides found in the cell are known to retain not only adjuvant activity, as evidenced by their ability to increase the antibody response, but also possess antitumor activity, as evidenced by their ability to activate macrophages to become cytotoxic and destroy tumor cells. For example, muramyl dipeptide (MDP) (e.g., N-acetylmuramyl-L-alanyl-D-isoglutamine) and a large number of MDP derivatives are known to have antitumor macrophage activation properties. An example of a lipophilic disaccharide tripeptide derivative of the base compound muramyl dipeptide (MDP) is described in WO90/00396. These disclosed compounds are glycerol-ended peptides.

Both the in vitro and in vivo antitumor activity of mono- and disaccharide peptides is increased by their incorporation into liposomes. Lipophilic derivatives of immunomodulators and/or antitumor agents are known. Such compounds are useful for efficiently incorporating these agents into liposomes for targeting macrophages and activating macrophages to the cytotoxic state.

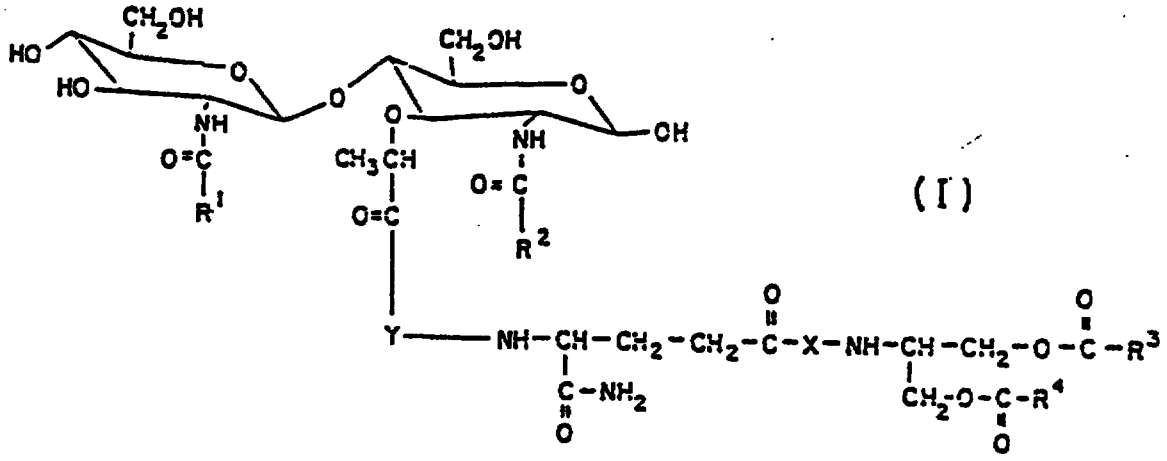
Both the intact microbial agents and many MDP analogues have shown an undesirable level of toxicity. Intact microbial agents, used alone or in an oil-water emulsion, such as Freund's adjuvant, can cause an increased sensitivity to histamine, granuloma formation, and hyperplasia of the liver and spleen. In particular, the administration of N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanyl-phosphatidylethanolamine, when given in repeated doses, has caused the undesirable toxic reaction of generalized vasculitis (See, D.G. Brown et al., in Immunotoxicology, A. Belin et al., Ed; Martin Hijhof, Pub.; 1987, pp. 219-233).

Therefore, there is a need for novel glycopeptide compounds which have improved adjuvant and/or antitumor activity, which are readily incorporated into liposomes, and which have acceptable toxicity in dosages exceeding anticipated effective human dosages.

Summary of the Invention

The present invention provides novel lipophilic disaccharide-tripeptide compounds. The compound of the invention is preferably encapsulated into multilamellar liposomes, which can be formed from, for example, phosphatidyl choline and phosphatidyl glycerol. The compounds are effective in activating human monocytes. These compounds have acceptable levels of toxicity in dosages exceeding anticipated human dosages.

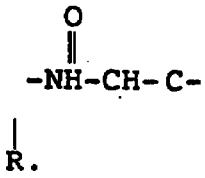
The compounds of the invention have the formula (I):



(I)

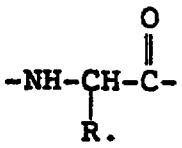
wherein R^1 is a (C_1-C_9)alkyl group, R^2 is a (C_1-C_5)alkyl group, R^3 and R^4 are individually (C_5-C_{30})alkyl groups having about 0-4 double bonds.

X is a spacer group that does not substantially adversely affect the activity or toxicity of the compound. X is particularly a single bond, or any peptidyl residue comprising one or more amino acids. X is preferably any amino acid residue of the general formula:



More preferably X is any naturally occurring amino acid residue or an enantiomorph of any naturally occurring amino acid residue. Particularly useful compounds of the present invention include an X group selected from the group consisting of neutral naturally occurring amino acid residues, and more particularly neutral aliphatic naturally occurring amino acids, and the enantiomorphs of these amino acid residues. L, D, or DL mixtures of valine and alanine, are particularly preferred.

Y is any amino acid residue of the general formula:



Preferably Y is any naturally occurring amino acid residue or an enantiomorph of any naturally occurring amino acid residue. Particularly useful compounds of the present invention include a Y group selected from the group consisting of neutral naturally occurring amino acid residues, and more particularly neutral aliphatic naturally occurring amino acids, and the enantiomorphs of these amino acid residues. L, D, or DL mixtures of threonine, alanine, valine, and serine are particularly preferred. L-threonine and L-alanine are most preferred.

The pharmaceutically acceptable salts of the compounds, and a liposome comprising a compound of the above formula I, are also within the scope of the invention. In addition to the L-enantiomorphs and the D-enantiomorphs, DL-mixtures of amino acids can also be used in the present compositions.

Detailed Description of the Invention

Chemical Structure of Compounds I

The compounds of the formula (I) according to the present invention are novel lipophilic disaccharide-tripeptides. The compounds of formula I include a glucosamine (Glc) derivative having an acyl group with about 2 to 10 carbons attached to the nitrogen. Preferably, the acyl group has 2 carbons (acetyl) forming N-acetylglucosamine (GlcNAc).

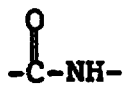
The N-acylglucosamine moiety is attached to an N-acylmuramyl moiety. The acyl functionality attached to the nitrogen of the muramyl group has about 2 to 6 carbons, preferably 2 carbons, forming an N-acetylmuramyl group (MurNAc). The alternating disaccharide GlcNAc-MurNAc is a naturally occurring disaccharide, found in bacterial cell walls as part of a polymeric glycopeptide. See, U.S. Patent No. 4,395,399.

The disaccharide moiety of the compounds of formula I. N-acylglucosamine-N-acylmuramate, is bonded to the N-terminus of a peptidyl moiety, Y, through the lactyl ether linkage at the number 3 position on the muramyl group. The amino acid residue, Y, is bonded to D-isoglutamine (isoGln), which is bonded through the C-terminus to the group, X.

Neutral naturally occurring amino acid residues for use in the X and Y positions are of particular interest. Neutral naturally occurring amino acids include neutral aliphatic, neutral thioaliphatic, neutral aromatic, and neutral heterocyclic amino acids. Neutral aliphatic naturally occurring amino acids include: glycine (Gly), alanine (Ala), serine (Ser), threonine (Thr), valine (Val), leucine (Leu), and isoleucine (Ile). Neutral thioaliphatic amino acids include: cysteine (Cys), cystine (CyS-Cys), and methionine (Met). Neutral aromatic amino acids include phenylalanine (Phe) and tyrosine (Tyr). Neutral heterocyclic amino acids include: proline (Pro), hydroxyproline (Hyp), and tryptophan (Trp). Other naturally occurring amino acids, include: the acidic amino acids aspartic acid (Asp) and glutamic acid (Glu); and the basic amino acids, histidine (His), lysine (Lys) and arginine (Arg).

Preferred peptidyl moieties include L-alanyl-D- . isoglutaminyl-L-alanine (L-Ala-D-isoGln-L-Ala); D-alanyl-D-isoglutaminyl-L-alanine (D-Ala-D-isoGln-L-Ala); and D-alanyl-D-isoglutaminyl-D-alanine (D-Ala-D-isoGln-D-Ala). The disaccharide-tripeptide portion of the novel compound may be referred to as N-acylglucosaminyl-N-acylmuramyl-tripeptide.

The lipophilic end of the compounds of the invention comprise a derivative of glycerol substituted with two acyl groups, the acyl groups individually having between 7 and 31 carbons, preferably between 13 and 24 carbons (i.e., R^3 and R^4 are individually (C_{12} - C_{23})alkyl groups), and about 0 to 4 double bonds, preferably about 0 to 1 double bond. Preferably both acyl groups have 16 carbons [C_{16}] (i.e., R^3 and R^4 are individually (C_{15})alkyl groups) to form a dipalmitoyl-glycerol (DPG) derivative. In the compounds of formula I the glycerol derivative is attached to the C-terminus of the terminal amino acid of X, through an amide linkage:



attached to the number two carbon of the glycerol backbone.

Novel compounds of the present invention can generally be described as N-acylglucosaminyl-N-acylmuramyl-tripeptide-diacyl-serinol compounds. Preferred compounds are GlcNAcMurNAc-L-Ala-D-isoGln-L-Ala-NHDPG; GlcNAcMurNAc-L-Ala-D-isoGln-D-Ala-NHDPG; and GlcNAcMurNAc-D-Ala-D-isoGln-D-Ala-NHDPG.

The compounds of formula I may also be used as the pharmaceutically acceptable salts of the formulas above. Such salts include the amine salts which are derived from the epsilon amino groups of lysine and the organic acids such as citrate, lactic, malic, methane sulfonic, p-toluene sulfonic, and the like; as well as inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid, and the like. Salts such as (lower) alkyl sulfate and halides can also be used. If X or Y have a free carboxyl side chain, such as with aspartic or glutamic acid, such salts could include those derived from inorganic bases such as KOH, NaOH, and the like, from NH,OH, or from organic amines. For isolation or purification of the compound, pharmaceutically unacceptable salts may also be used. However, only the pharmaceutically acceptable, nontoxic salts can be used therapeutically, and are therefore preferred.

Liposomes

The liposomes are generally produced from phospholipids or other lipid substances and are formed of mono- or multilamellar hydrated liquid crystals. They are customarily used in dispersions in an aqueous carrier medium. The use of liposomes incorporating the compounds of formula I results in an increase in the adjuvant and anti-tumor activity. Also, an increase in humoral and/or cellular mediation immune responses is often observed. Thus, the compounds of formula I are preferably included in liposomes.

There are a number of conventional procedures to form liposomes. Any nontoxic, physiologically acceptable and metabolizable lipid, capable of forming liposomes, can be used. The most usual lipids are the phospholipids, and notably the phosphatidyl cholines (lecithins), both natural and synthetic. Phospholipids may also be used, for example, the phosphatidyl serines, the phosphatidyl inositides, or the sphingomyelines. Other lipids can also be used, which have been described, for example, by W. R. Hargreaves and D. W. Deamer (Conference on Liposomes and Their Uses in Biology and Medicine, Sept. 14-16, 1977, New York Acad. Sci.) and in Biochem., 18, 3759 (1978).

Traditional techniques and apparatus can be employed to form the liposomes according to the invention. These techniques are described in, for example, Chapter IV of the work entitled "Methods in Cell Biology", edited by David M. Prescott, Volume XIV, 1976, Academic Press, New York, page 33, et seq.

Another method of encapsulating the active compounds of formula I into a liposome involves casting a film of phospholipid (with or without a charged lipid) by evaporation from a solution in an organic solvent, and then dispersing the film in a suitable aqueous medium. In the case of lipid-soluble, biologically active compounds, that is, those which associate with the lipid layers rather than with the aqueous phase of the liposomes, the compound is usually cast as a film together with a phospholipid, using a common organic solvent. In the case of water-soluble, biologically active compounds, the compound is typically encapsulated in liposomes by dispersing a cast phospholipid film with an aqueous solution of the compound. The encapsulated compound is then separated from the free compound by centrifugation, chromatography or some other suitable procedure.

The lipophilic end of the compounds of formula I enhance the incorporation of the compounds into liposomes. The compounds of formula I are preferably incorporated into a liposome having a bilayer membrane consisting essentially of 1-palmitoyl-2-oleoyl-phosphatidyl choline (PC) and dioleoyl phosphatidyl glycerol (PG) in a weight ratio of about 5:1 to 1:1, preferably about 7:3. These compounds are commercially available from Avanti Polar Lipids, of Pelham, Alabama.

Preferred methods which can be used to encapsulate the compounds of formula I into a liposome are described in U.S. Patent No. 4,370,349 which is incorporated herein by reference. The methods comprise either: (1) dissolving the necessary substances in a suitable solvent and then freeze-drying the solution, storing the resulting freeze-dried mixture, and, when desired, reconstituting it into an aqueous liposome preparation, or (2) preparing an aqueous liposome preparation by any known method and freeze-drying the preparation. When desired, the freeze-dried product can be made up into an aqueous liposome preparation. The freeze-dried mixtures disperse easily when shaken with an aqueous medium, and use of the freeze dried liposomes results in liposome preparations having a narrower size distribution than a corresponding preparation obtained by dispersing a cast film. This is advantageous to the reproducibility of the therapeutic effect of liposome preparations. Generally, the compositions in the form of liposomes can contain, in addition to the compounds of formula I, any constituents, stabilizers, preservatives, excipients, or other active substances capable of being used in the injectable solutions or emulsions presented previously for administration of muramyl peptide compounds.

Delivery

The compounds of formula I, preferably incorporated into liposomes, may be used for their adjuvant and/or antitumor activity, and may be administered orally or parenterally, preferably by injection.

The invention relates in particular to medicinal adjuvant and antitumor compositions, containing the compounds of formula I, in association with a pharmaceutically acceptable carrier vehicle. Compositions of this type which are particularly preferred are constituted by the injectable solutions containing an effective immunomodulating amount of the product of the invention. Sterile solutions in an aqueous, preferably isotonic liquid, such as saline isotonic solutions or isotonic solutions of glucose, are advantageously used for this purpose. A simple solution in distilled water can also be used. It is also possible to use injection media containing an oily phase, especially water-in-oil emulsions. Such emulsions are obtained in particular with metabolizable vegetable oils, such as are described in the French Patent Application No. 75-04003 (FR-A-2 313 078). That French patent application corresponds to the U.S. co-pending patent application

Ser. No. 656,738 of Audibert et al., filed on February 9, 1976 (US-A-4 125 603), based on said French priority patent application Ser. No. 75-04003. The preferred carrier vehicle is the freeze-dried liposomes described above.

The adjuvant and antitumor compositions of the invention may also be administered in various forms, by using for this purpose vehicles suitable for the selected method of administration. For example, unit dosage forms will be used in the form of cachets, compressed tablets or hard or soft gelatine-capsules, for oral administration, and aerosols or gels for the application to mucous membranes.

The compositions may also be in lyophilized form so as to permit the extemporaneous preparation of the adjuvant and antitumor compositions. A pharmaceutically advantageous form comprises unit doses of about 200 micrograms to 10 milligrams of Compound I or II per meter^2 of body surface area.

Manufacture

Compounds of the invention, for example, 4-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl]-2-acetamido-2-deoxy-3-O-[D-2-propanoyl-L-alanyl-D-isoglutaminyl-L-alanyl-2,3-dipalmitoyl-sn-serinol]-D-glucopyranose (GlcNAcMurNAc-L-Ala-D-isoGln-L-Ala-NHDPG (Compound IA), may be prepared from commercially available materials, in about nine major steps. The steps do not necessarily have to be performed in the order described as will become apparent from the description below.

The first step involves the preparation of a blocked amino acid-diacyl serinol. This is the lipophilic portion of Compound I attached to the C-terminus of the spacer group X, e.g., an amino acid residue.

The blocked amino acids or peptides employed as starting materials in the synthesis are either commercially available in the blocked form or are obtained by known methods of peptide chemistry. Blocking groups or protecting groups that can readily be split off are those known from peptide and sugar chemistry. For hydroxy groups the following are suitable examples: acyl radicals, for example lower alkanoyl radicals, such as acetyl; aroyl radicals, such as benzoyl; and especially radicals derived from carbonic acid derivatives, such as benzyloxycarbonyl or lower alkoxycarbonyl, or alkyl, especially tert-butyl, benzyl, optionally substituted by nitro, (lower) alkoxy or by halogen, triphenylmethyl or tetrahydropyranyl, each optionally substituted by halogen or by lower alkoxy such as methoxy, or optionally substituted alkylidene radicals that bond the oxygen atoms in the 4-and 6-position. Such alkylidene radicals are preferably a lower alkylidene radical, e.g., the methylidene, isopropylidene, or propylidene radicals, or alternatively, an optionally-substituted benzylidene radical.

For blocking C-terminal carboxy groups, suitable moieties include tert-butyl, benzyl, or benzhydryl. For protection of free amino groups, tert-butyloxycarbonyl or benzyloxycarbonyl groups can be used.

These blocking groups can be cleaved in a manner known in the art, such as acid hydrolysis. Benzyl or benzylidene radicals also can be removed by hydrogenolysis, for example using hydrogen in the presence of a noble metal catalyst, such as a palladium or platinum catalyst.

The second step in the preparation of a compound of the invention involves removal of the blocking group to form X-diacyl-serinol, where X, for example, is an amino acid residue as described above, such as L-alanine.

The third step involves isolation of the disaccharide moiety GlcNAcMurNac. Several methods are known in the art for obtaining this disaccharide moiety. One method involves the isolation from a suitable bacteria, for example Micrococcus lysodeikticus (dried cells are commercially available from Sigma Chemical Co., St. Louis, MO). The disaccharide that is obtained is N-acetylglucosaminyl-N-acetylmuramate. The isolation of this disaccharide from a biomass of Micrococcus lysodeikticus is known and described in the literature. It involves enzymatic hydrolysis of the cell walls obtained from the biomass of Micrococcus lysodeikticus by means of trypsin and lysozyme and a further purification in a column packed with Dowex® 1 X 8 (acetate form) 200-400 mesh (O. Hoshino, U. Zenavi, P. Sinay, R. W. Jeanloz, J. Biol. Chem. 247, No. 2, 381 (1972); and N. Sharon, T. Osawa, H. M. Flowers, R. W. Jeanloz, J. Biol. Chemistry, 241, 223 (1966). Also, see, U. S. Patent No. 4,427,659, which is incorporated herein by reference. A second method by which the disaccharide moiety GlcNAcMurNAc and disaccharide peptides may be obtained is by means of total chemical synthesis. See for example S. Kusomoto et al., Bull. Chem. Soc. Jpn., 59, 1414 (1986); M. Kiso et al., Carbohydrate Res., 104, 253 (1982); and A. Hasegawa et al., Carbohydrate Res., 100, 234 (1982); S. Kusumoto et al., Tetahedron Letters, 45, 4407 (1978); and R. Fhyuri et al., Agric. Biol. Chem., 50 2561 (1986). A third method by which the disaccharide moiety, preferably linked to appropriate amino acid residues, may be produced is through the use of recombinant DNA technology.

Enzymatic methods have also been described, by which disaccharide peptides may be obtained. Such methods generally yield compounds containing the natural L-meso-2,5-diaminopimelic acid at the third amino acid position herein designated by "X". See, for example, S. Kawata et al., Agric. Biol. Chem., 48,

1783 (1984); D. Keglevic et al., Biochimica et Biophysica Acta, 585, 273 (1979). Through the use of an enzymatic hydrolysis using gamma-D-glutamyl-meso-diaminopimelate endopeptidase I isolated from Bacillus sphaericus 9602 or other appropriate bacterium, it is also possible to enzymatically isolate the disaccharide dipeptide GlcNAc-MurNAc-L-Ala-D-isoGln. [See M. Guinand et al., Eur. J. Biochem., 143, 359 (1984)]. Reference is also made to U.S. Patent Nos. 4,395,399; 4,515,891; and 4,545,932.

In the disaccharide isolated above, R^1 and R^2 are both $-CH_3$, forming acetyl groups on both the muramyl and glucosamyl functionalities. The analogous compounds, where R^1 is a (C_1-C_3)alkyl group and R^2 is a (C_1-C_5)alkyl group, can be prepared by methods known in the art. For example, the acetyl group can be hydrolyzed by a strong base, for example, as described in P. H. Gross and R. W. Jeanloz, J. Org. Chem., 32, 2761 (1967). Then an acylating agent, corresponding to the R^1 or R^2 which is desired to be introduced, such as an acid anhydride or chloride, may be used to attach the desired R^1 or R^2 group to the muramyl or glucosaminyl functionality.

The next step involved the preparation of the dipeptide-Y-D-isoglutamine, which is blocked on both ends. For example, in Compound IA where Y is L-alanine, BOC-L-alanyl-D-isoglutamine, commercially available from United States Biochemical Co. of Cleveland, Ohio (USBC), must be treated in a manner known in the art to terminate the C-terminus isoglutamine residue with a suitable blocking agent, such as a benzyl ester (-OBn). BOC refers to N-tert-butoxycarbonyl, a blocking group. Thus, BOC-L-Ala-D-isoGln-OBn is preferably formed.

The next step involves the removal of the blocking group from the alanine by a known method to form, for example, L-Ala-D-isoGln-OBn. The next step involves coupling the N-acylglucosamine-N-acylmuramyl functionality with the alanine-isoglutamine moiety. The condensation reaction is conducted in an inert solvent medium, in the presence of a condensation agent, preferably Woodward's Reagent K (N-ethyl-5-phenylisoxazolium-3'-sulphonate),at a temperature of about 0°C to 25°C in one stage. See, U. S. Patent No. 4,395,399.

The next step involves removal of the blocking group by conventional means to form the unblocked disaccharide-dipeptide, for example, 4-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl]-2-acetamido-2-deoxy-3-O-[D-2-propanoyl-L-alanyl-D-isoglutamine]-D-glucopyranose (GlcNAcMurNAc-L-Ala-D-isoGln).

A compound according to Formula I, where the lipophilic group is attached via an amide linkage (designated NHDPG), for example, 4-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl]-2-acetamido-2-deoxy-3-O-[D-2-propanoyl-L-alanyl-D-isoglutaminyl-L-alanyl-2-(1,3-dipalmitoyloxy) propylamide]-D-glucopyranose, (GlcNAcMurNAc-L-Ala-D-isoGln-L-Ala-NHDPG) (Compound IA), is prepared by the coupling of the disaccharide dipeptide, GlcNAcMurNAc-L-Ala-D-isoGln, to the lipophilic alanine amide, L-Ala-NHDPG. The disaccharide dipeptide is prepared as described above. The required amide is prepared by the following three-step synthetic sequence:

(A) The blocked amino acid, BOC-L-Ala, is coupled with serinol (SerOH) by conventional means to give BOC-L-Ala-SerOH.

(B) BOC-L-Ala-SerOH is reacted with two moles of palmitic acid to yield the dipalmitate, BOC-L-Ala-NHDPG.

(C) The BOC-protecting group is removed by conventional means to form the unblocked lipophilic alanine amide, L-Ala-NHDPG.

The final synthetic step involves the coupling of the GlcNAcMurNAc-L-Ala-D-isoGln with the lipophilic amide by conventional techniques to form the Compound IA.

The compounds of formula I are preferably encapsulated into liposomes as described herein above. Preferably the compound of the invention is combined with phosphatidyl choline and phosphatidyl glycerol. Typically the phospholipids are dissolved in tert-butanol at a concentration of about 100 mg per ml. Appropriate amounts of the PC and PG in tert-butanol are mixed to give a weight ratio of about 7:3. Compound I is weighed out and added to a given volume of the lipids to give a final concentration of, for example, about 1 mg per 5 ml. The material is then passed through a millipore filter and the composition is dispensed into vials. The vials are frozen, typically at -20°C and lyophilized typically at about 20°C for 18 hours. The vials are then sealed under an inert gas, such as argon.

The present invention is further described by way of the following non-limiting examples:

EXAMPLE 1

GlcNAcMurNAc

Dried cells (15.0 grams) of Micrococcus lysodeikticus (commercially available from Sigma Chemical Co., St. Louis, MO), was suspended in 200 ml of distilled water and disrupted by stirring at high speed with

250 g of 0.1 mm glass beads for 90 minutes at 4°C. The cell wall skeletons (CWS) were removed from the glass beads by decantation and then centrifuged at 1200 x g for 30 minutes. The supernatant was removed from the pellet (intact cells), then centrifuged at 10,000 x g for 50 minutes. The supernatant was removed and the resulting pellet (crude CWS) was washed three times by suspension in 100 ml of distilled H_2O and centrifugation at 10,000 x g for 70 minutes. The resulting pellet was suspended in 150 ml of distilled water and then placed in a boiling water bath for 30 minutes. After cooling to ambient temperature, the resulting slurry was centrifuged at 10,000 x g. The supernatant was removed and the pellet slurried in 60 ml of 0.5 M ammonium acetate (NH_4OAc) buffer (pH 7.60). The resulting slurry was treated with 10.0 mg of porcine pancreas trypsin (Sigma, 14,600 BAC units/mg), and incubated at 37°C for 20 hours. After several washes with distilled H_2O, the CWS pellet was slurried in 60 ml of 0.05 M NH_4OAc buffer (pH 6.30), treated with egg-white lysozyme (Sigma, 56,000 units/mg, 10.0 mg), and incubated at 37°C for 19 hours.

The crude preparation was dialyzed to remove the enzymes and undigested cell walls. Final purification was achieved by ion exchange chromatography on Dowex®-1 resin (acetate form) by elution with an acetic acid (HOAc) gradient. The column fractions were pooled based on UV absorbance and thin layer chromatography (TLC) (silica gel, 50:39:8:3 $CHCl_3$/MeOH/H_2O/NH_4OH, 5% H_2SO_4/EtOH and heating). Positive identification of the disaccharide product was obtained from colorimetric analysis of muramic acid and total hexosamines, and fast atom bombardment mass spectrometry. Approximate yields were 120 mg GlcNAcMurNAc from 15 g of dried cells.

EXAMPLE 2

BOC-L-Ala-D-isoGln-OBn

Benzyl alcohol (77.0 mg, 0.71 mMol), DMAP (33.0 mg, 0.27 mMol), and BOC-L-alanyl-D-isoglutamine (USBC, 159.0 mg, 0.50 mMol) were dissolved in 5 ml of CH_2Cl_2 and 2 ml of N,N-dimethylformamide (DMF). This solution was cooled in an ice-water bath to 4°C, treated with EDCI (118.0 mg, 0.61 mMol) and stirred at 4°C for 30 minutes, then at room temperature for 15 hours. After removing the solvents in the rotary evaporator, the residue was partitioned between 20 ml of EtOAc and 10 ml of H_2O. The layers were separated and the aqueous layer extracted with another 20 ml of EtOAc. The organic fractions were combined, then successively extracted with saturated $NaHCO_3$ (2 x 20 ml) and H_2O (2 x 20 ml). After drying over Na_2SO_4, the solvent was removed on the rotary evaporator leaving a waxy solid, which was recrystallized from EtOAc-petroleum ether to yield 141 mg (69%) of BOC-L-Ala-D-isoGln-OBn as white fluffy solid.

EXAMPLE 3

L-Ala-D-isoGln-OBn

BOC-L-Ala-D-isoGln-OBn (120 mg, 0.294 mMol) was treated with 10 ml of 1N HCl/HOAc and the resulting solution stirred at RT for 2 hours. The solvent was then removed on the rotary evaporator to yield a colorless oil, which was taken up in 3 ml of MeOH, then precipitated by the dropwise addition of 20 ml of diethyl ether. After stirring for one hour at RT, the product was collected on a filter, washed with ether, then dried under high vacuum to yield 88 mg of the hydrochloride salt of L-Ala-D-isoGln-OBn as a white solid.

EXAMPLE 4

Coupling of GlcNAcNurNAc with L-Ala-D-isoGln-OBn

A total of 200 mg of GlcNAcMurNAc (MW 496.47, 0.405 mMol) was dissolved in 15 ml of DMF and then treated with 0.95 ml of a solution containing 42.94 mg/ml of triethylamine (TEA) in DMF (0.403 mMol). The solution was cooled with magnetic stirring in an ice bath and then treated with 139.63 mg (95% pure, 0.524 mMol) Woodward's Reagent K. The slurry was then stirred in an ice-water bath for one hour, then at room temperature for 10 minutes. Then a solution containing 152.3 mg (0.443 mMol) of the HCl salt of the L-Ala-D-isoGln-OBn in 8.0 ml of DMF to which was added 1.05 ml (0.443 mMol) of the TEA/DMF solution was added via a pressure equalizing funnel over a period of 10 minutes. The solution was stirred at RT for 18 hours and then allowed to stand for an additional 96 hours. The reaction was followed during this time by TLC and allowed to go as far as possible to completion.

The DMF was removed in a rotary evaporator under high vacuum (approximately 50 microns) at 25 • C to yield a reddish oil that was further dried under high vacuum.

The oil was taken up in 5 ml of H_2O and applied to a 1.7 x 7 cm column of Dowex® 1 X 8 resin (200-400 mesh, acetate form). The column was washed with 50 ml of H_2O and the entire colorless eluate applied to a 1.7 x 7 cm column of Amberlite® IR-120 P resin (16-20 mesh, H^+ form). The column was washed with 50 ml of H_2O and the eluate and washings were combined. This material was taken to dryness in a rotary evaporator under aspirator vacuum at 25 • C to yield a colorless oil. This was dried under high vacuum (50-75 microns) overnight during which time it solidified to a glassy solid. This was taken up in 20 ml of H_2O and lyophilized to yield 181 mg of GlcNAcMurNAc-L-Ala-D-isoGln-OBn as a snow white fluffy solid.

EXAMPLE 5

GlcNAcMurNAc-L-Ala-D-isoGln

The protected material prepared (170 mg) in Example 4 was dissolved in a solution of H_2O (30 ml) and acetic acid (1.0 ml). The solution was added to 100 mg of 5% Pd/C (by weight of the palladium, C is powdered charcoal, from Matheson, Coleman, and Bell of Norwood, Ohio) in a 500 ml Parr hydrogenation bottle and hydrogenated at 20 PSIG for 24 hours. The catalyst was removed and washed with water (3 x 10 ml), and the filtrate and washings were combined and lyophilized to yield 150 mg (100%) of GlcNAcMurNAc-L-Ala-D-isoGln as a white solid. The product was further dried under high vacuum for 48 hours then tightly capped and stored at 4 • C.

LARGE SCALE PREPARATION OF GlcNAcMurNAc-L-Ala-D-isoGln

EXAMPLE 6

BOC-L-Ala-D-isoGln-OBn (V)

BOC-L-Ala-D-isoGln (USBC, 1.587 g, 5.0 mMol), benzyl alcohol (540.7 mg, 5.0 mMol), and DMAP (305 mg, 2.5 mMol) were dissolved in 40 ml of CH_2Cl_2 and 10 ml of DMF, and the resulting solution was cooled in an ice-water bath to 4 • C with magnetic stirring. EDCI (1.150 g, 6.00 mMol) was added, and the reaction stirred in the ice bath for one hour, then at room temperature for 17 hours. After removal of the solvents on the rotary evaporator, the oily residue was partitioned between 50 ml of H_2O and 150 ml of EtOAc, the layers separated, and the organic layer further extracted with saturated aqueous $NaHCO_3$ (3 x 50 ml) and H_2O (3 x 50 ml). After drying over Na_2SO_4, the solvent was removed on the rotary evaporator to yield a colorless oil that was further dried under high vacuum, during which it solidified to a waxy solid. Recrystallization from EtOAc-hexane yielded 1.318 g (65%) of Compound V as a snow-white solid.

A TLC (silica; EtOAc/pyridine/HOAc/H_2O, 30:2:0.6:1; HCl spray, then ninhydrin) of the product revealed a single spot of R_f 0.90.

L-Ala-D-isoGln-OBn (VI)

The protected dipeptide ester V (2.08 g, 5.10 mMol) was treated with 100 ml of 1N HCl/HOAc, and the resulting solution was allowed to stand at room temperature for two hours. After removal of the solvents on the rotary evaporator and further drying under high vacuum, the product was crystallized from MeOH-ether to yield 1.68 g (95.8%) of Compound VI as its hydrochloride salt.

Preferred Method of Preparation of GlcNAcMurNAc (VII)

Cell Disruption

Micrococcus lysodeikticus (Sigma, 200 g), in the form of dried cells, was suspended in distilled water for 24 hours, then disrupted with a Microfluidics Corporation laboratory Microfluidizer® (Model M-110Y). This was driven by a Powerex® GI-25 air compressor at a normal operating air pressure of 82 PSIG, which resulted in a hydraulic pressure of 19,000 PSIG. Approximately ten passes through the interaction chamber at these operating conditions were required to effect nearly total (95-98%) disruption of the cells. The resulting slurry was then centrifuged for 30 minutes at 1000 x g, the supernatant (containing CWS) removed from the small pellet (intact cells), then centrifuged for two hours at 10,000 x g. The resulting supernatant

was carefully removed by decantation and discarded. The white, somewhat greasy, pellet was suspended in 1,500 ml of distilled water, then carefully warmed with magnetic stirring to 90°C and held at that temperature for 30 minutes. After cooling to RT, the slurry was centrifuged at 10,000 x g for 2.5 hours, and the supernatant carefully removed and discarded. The resulting pellet (containing CWS) was then washed once by suspension in a total of 2000 ml of water, followed by centrifugation at 10,000 x g for 2.5 hours.

Trypsin Treatment

The pellet from above was suspended in 2000 ml of 0.05 M sodium phosphate buffer (pH 7.60) and the pH of the slurry corrected by 7.60 by the addition of 10 N NaOH. Trypsin (porcine pancreas, type IX, 300 mg, 17,000 BAEE units/mg protein) and toluene (10 drops) were added and the resulting slurry incubated at 37°C for 20 hours. The pH was then corrected to 7.60 with 10 N NaOH, an additional 100 mg of trypsin was added, and the mixture again incubated at 37°C for 24 hours. The slurry was then centrifuged at 10,000 x g for 2.5 hours, the supernatant carefully removed and discarded, and the white pellet (containing deproteinized CWS) washed several times with water by suspension-centrifugation until a ninhydrin test of the supernatant was negative.

Lysozyme Digestion

The pellet (i.e., deproteinized CWS) from above was suspended in 1500 ml of 0.1 M NH_4OAc (pH 6.30), and the pH of the slurry was corrected to 6.30 with dilute HOAc. Lysozyme (egg-white, 400 mg, 49,000 U/mg) and toluene (5 drops) were added, and the slurry was incubated at 37°C for 24 hours, during which time the slurry cleared up considerably. Then, an additional 200 mg of lysozyme was added, and the mixture was incubated for another 24 hours at 37°C. The reaction mixture was then centrifuged at 10,000 x g for 2.5 hours. The clear, yellowish supernatant was removed from the small pellet, then dialyzed through an Amicon PM-10 membrane. The colorless outer dialysate was concentrated to dryness on the rotary evaporator, and the resulting residue repeatedly dissolved in water, then concentrated to dryness in order to remove the last traces of the NH_4OAc buffer. The yellow syrupy residue was finally dissolved in 400 ml of water, then lyophilized to yield 20.8 g of a yellow, gummy solid.

Purification of Disaccharide

The entire 20.8 g of the lysozyme digest from above was dissolved in 100 ml of water, then applied to a 4.3 x 63 cm column of Dowex® 1 (acetate form, 200-400 mesh) resin. The column was then eluted with a gradient comprised of 4000 ml of H_2O (starting) and 4000 ml of 0.8 N HOAc (final) at a flow rate of 3.5 ml/minute. Fractions of 21 ml were collected and analyzed by TLC as described in Example 3A. The desired fractions were then pooled, concentrated to dryness on the rotary evaporator, then redissolved in water and lyophilized to yield 2.60 g of crude product as a slightly off-white solid.

For final purification, the 2.60 g of crude disaccharide was dissolved in 25 ml of water, then applied to a 3 x 55 cm column of Dowex® 1 (acetate form, 200-400 mesh) resin. The column was then eluted with a gradient comprised of 2000 ml of H_2O (starting) and 2000 ml of 0.8 N HOAc (final) at a flow rate of 3.0 ml/minute. Fractions of ca. 21 ml were collected and were analyzed by TLC as above. The desired fractions were pooled, concentrated to dryness on the rotary evaporator, then redissolved in 50 ml of water and lyophilized to yield 2.243 g or pure product as a snow-white solid.

GlcNAcMurNAc-L-Ala-D-IsoGln-OBn (VIII)

Prior to use, the DMF was dried over 4A molecular sieves, then distilled from ninhydrin. The TEA was distilled from sodium hydroxide (NaOH) pellets. Woodward's Reagent K was purified by dissolving 3.0 g of the commercial material (Aldrich) in 15 ml of 1N HCl, filtering through Whatman #2 paper, then precipitating by the addition of 120 ml of acetone. After filtering and washing with 100 ml of acetone, the reagent was dried under high vacuum for several hours.

The disaccharide Compound VII (2.00 g, 4.028 mMol) was dissolved in 100 ml of DMF, treated with TEA (0.62 ml, 447.5 mg, 4.431 mMol), cooled in an ice-water bath to near 4°C, then treated with Woodward's Reagent K (95%, 1.397 g, 5.24 mMol). The resulting slurry was stirred in the ice-water bath for one hour, then at room temperature for 10 minutes. Then, a solution containing the dipeptide benzyl ester (VI) (1.523 g, 4.43 mMol) and TEA (447.42 mg, 0.616 ml) in 50 ml of DMF was added via a pressure equalizing addition funnel over a period of 30 minutes. After the addition was completed, the reaction

mixture was stirred at room temperature for a total of 120 hours, during which the progress of reaction was monitored by TLC (silica; $CHCl_3/MeOH/H_2O/NH_4OH$, 50:25:4:2; 5% H_2SO_4/EtOH, heat). The solvent was then removed on the rotary evaporator and the oily residue further dried under high vacuum. This was then taken up in 50 ml of H_2O, then applied to a 2.5 x 17 cm column of Dowex 1 x 8 resin (200-400 mesh, acetate form) and eluted with 500 ml of H_2O. The entire eluate was concentrated to ca. 50 ml, then applied to a 2.5 x 17 cm column of Dowex 50 x 8 resin (100 mesh, H^+ form) and eluted with 500 ml of H_2O. The eluate was concentrated to ca. 50 ml, then lyophilized to yield 2.25 g (71%) of Compound VIII as a snow-white solid.

GlcNAcMurNAc-L-Ala-D-isoGln (IX)

The disaccharide dipeptide benzyl ester (VIII) (2.20 g, 2.80 mMol) was dissolved in 150 ml of H_2O and 3.0 ml of glacial acetic acid. To this was added 300 mg of 5% Pd/C, and the resulting slurry was hydrogenated at room temperature and 40 PSIG for 40 hours. The catalyst was then removed by filtration through a Celite pad, washed with H_2O (3 x 10 ml), and the filtrate and washings combined, concentrated to ca. 50 ml, then passed through a 1 ml column of Detoxi-Gel® (Pierce) at a flow rate of 8 ml/hr. The column was washed with 10 ml of H_2O, and the eluate and washings were combined, then lyophilized to yield 1.86 g (95.5%) of Compound IX as a white powder.

EXAMPLE 7

Preparation of Liposomes

The compound (IIA) was encapsulated into liposomes using the following procedure. One mg of IA as prepared in Example 11 was combined with 175 mg of 1-palmitoyl-2-oleoyl phosphatidyl choline (PC) and 75 mg of 1,2-dioleoyl phosphatidyl glycerol (PG), both commercially available from Avanti Polar Lipids, Pelham, Alabama. The PC and PG were previously dissolved in tert-butanol at a concentration of 100 mg lipid per ml, thus giving a 7:3 weight ratio of PC:PG in tert-butanol. Tert-butanol was then added to the 1 mg IA; 175 mg PC; 75 mg PG to give a final volume of 5.0 ml. The IA and lipid mixture was passed through a sterile millipore 0.22μ filter to remove any contaminants present. The filtrate was collected in a clean, sterile, round bottom flask which was capped with aluminum foil after filling. Five ml of the filtered mixture containing 1 mg of IA was dispensed into 10 ml vials. After the vials were filled, they were covered with sterile rubber serum stoppers. Each of the stoppers includes a slit in one side so that air can enter and leave the vial during lyophilization and stoppering. Sterile aluminum foil was placed over the vials and the vials were transferred to the tray drying chamber of the lyophilizer. The vials were then cooled to -20°C until the tert-butanol lipid mixture was frozen (approximately 30 to 60 minutes). The refrigeration was then turned off and the tray heater set for 10°C. The vials were then lyophilized for 18 hours. The lyophilizer containing the vials was purged with filtered sterile argon and evacuated three times. The lyophilizer containing the vials was then purged again with argon and the vials stoppered under argon at atmospheric pressure.

PREPARATION OF GlcNAcMurNAc-L-Ala-D-isoGln-L-Ala-NHDPG (IA)

EXAMPLE 8

BOC-L-Ala-SerOH

BOC-L-Ala (567 mg, 2 mMol) and HOBT•H_2O (505 mg, 3.3 mMol) were dissolved in 5 ml CH_2Cl_2 and 5 ml DMF, then treated with 1,3-dicyclohexylcarbodiimide (DCC, 680 mg, 3.3 mMol). The resulting solution was stirred at RT for one hour, then treated dropwise with a solution that contained 421 mg (3.3 mMol) of serinol hydrochloride (Aldrich) and 333 mg (3.3 mMol) of N-methylmorpholine in 5 ml of DMF. The reaction mixture was stirred at RT for 20 hours, then treated with 0.5 ml of glacial acetic acid and stirred for another hour. The solid reaction by-products were removed by filtration and washed with 20 ml CH_2Cl_2. The combined filtrate and washings was concentrated to dryness on the rotary evaporator then further dried under high vacuum. The oily residue was taken up in 50 ml of H_2O, cooled at 4°C for several hours, then filtered. The filtrate was concentrated to dryness on the rotary evaporator, then further dried under high vacuum to yield the crude product as a yellow oil.

For purification, the entire crude reaction product was dissolved in 25 ml of $CHCl_3$, then applied to a 2.5 x 20 cm column of BioSil A (100-200 mesh) that had been packed in $CHCl_3$. The column was then successively eluted with (A) 200 ml of $CHCl_3$, (B) 200 ml of 1% (v/v) MeOH in $CHCl_3$, (C) 200 ml of 2% MeOH in $CHCl_3$, (D) 200 ml of 4% MeOH in $CHCl_3$, (E) 200 ml of 6% MeOH in $CHCl_3$, (F) 200 ml of 10% MeOH in $CHCl_3$, and (6) 200 ml of 15% MeOH in $CHCl_3$. Fractions of 50-100 ml were collected and assayed by TLC (Eastman silica; ethyl acetate:pyridine:acetic acid:water, 30:2:0.6:1; HCl spray, then ninhydrin). The appropriate fractions (R_f 0.9) were combined, then concentrated to dryness to yield 739 mg (94%) of product as a white glassy solid.

EXAMPLE 9

BOC-L-Ala-NHDPG

The entire reaction product from Example 8 (739 mg, 2.82 mMol) was dissolved in 50 ml of CH_2Cl_2, then treated with palmitic acid (1.59 g, 6.2 mMol), DMAP (344 mg, 2.82 mMol) and EDCI (1.19 g, 6.2 mMol). The mixture was stirred at RT for 20 hours, and the reaction was followed by TLC (Eastman silica; hexane:2-propanol, 9:1; HCl spray, then ninhydrin). After removal of the solvent on the rotary evaporator, the resulting oily residue was partitioned between 100 ml of EtOAc and 100 ml of H_2O, the layers separated, and the organic layer further washed with H_2O (3 x 50 ml), then concentrated to dryness in the rotary evaporator to yield a colorless oil. This was dissolved in ca. 25 ml of $CHCl_3$:MeOH:H_2O (300:200:30), then applied to a 2.5 x 8 cm column of Whatman DE 52 resin (OH cycle) that had been packed in the same solvent. The column was washed with 500 ml of solvent, the entire eluate collected, then concentrated to dryness on the rotary evaporator to yield an oil that solidified while drying under high vacuum. There was obtained 2.02 g (97%) of crude product as a white amorphous solid.

For further purification, 1.0 g of this product was dissolved in 10 ml of hexane, then applied to a 1.5 x 28 cm column of BioSil A that had been packed in hexane. The column was then eluted with (A) 50 ml of hexane, (B) 100 ml of 2% (v/v) i-PrOH in hexane, (C) 100 ml of 4% i-PrOH in hexane, and (D) 100 ml of 6% i-PrOH in hexane. Fractions of ca. 20 ml were collected and assayed by TLC as above. The appropriate fractions were combined, concentrated to dryness, then further dried under high vacuum to yield 856 mg of pure product as a white amorphous solid.

EXAMPLE 10

L-Ala-NHDPG

850 mg (1.15 mMol) of the product from Example 9 was dissolved in 15 ml of CH_2Cl_2, then treated with 10 ml of TFA. After standing at RT for one hour, the solvents were removed on the rotary evaporator to leave a colorless oil that was repeatedly taken up in 10 ml-portions of hexane then concentrated to dryness on the rotary evaporator. After extensive drying under high vacuum, 856 mg (100%) of solid product was obtained as its trifluoroacetate salt.

EXAMPLE 11

GlcNAcMurNAc-L-Ala-D-IsoGln-L-Ala-NHDPG (IA)

The disaccharide dipeptide, GlcNAcMurNAc-L-Ala-D-isoGln, (see Example 5, 208.7 mg, 0.3 mMol) and HOBT•H_2O (53.3 mg, 0.348 mMol) were dissolved in 10 ml of DMF, then diluted with 7 ml of CH_2Cl_2. To this was added 66.4 mg (0.346 mMol) of EDCI, and the resulting solution was stirred at RT for 30 minutes.

A TEA solution was prepared by diluting 303 mg (0.42 ml) of TEA to 10 ml with CH_2Cl_2.

L-Ala-NHDPG (see Example 10, 226.2 mg, 0.3 mMol) was treated with 1.5 ml of CH_2Cl_2, then with 1.0 ml of the TEA solution. The resulting solution was added to the activated disaccharide dipeptide solution, and the reaction mixture was stirred at RT and the course of the reaction was monitored by TLC. The reaction was stopped after 20 hours, and the reaction mixture was filtered from the small amount of insoluble material, then concentrated to dryness on the rotary evaporator and further dried under high vacuum to yield a yellow semi-solid residue. This material was then purified by the procedure described in Example 20 (i.e., EtOAc extraction and dialysis against sterile H_2O) to yield 155 mg of a white electrostatic powder. For final purification, a 140 mg-sample was subjected to the same treatment as described in Example 20 (i.e., Sephadex LH-20 and Cellex D). Following the column treatments and lyophilization, the

product was obtained as a white electrostatic powder. Molecular weight (HRMS): (Molecular Ion + H)$^+$ Calc: 1316.8432; Found: 1316.8389.

PREPARATION OF GlcNAcMurNAc-L-Ala-D-IsoGln-D-Ala-NHDPG (IB)

EXAMPLE 12

BOC-D-Ala-SerOH

BOC-D-Ala-SerOH was prepared on a 1.0 mMol-scale by the DCC-HOBT mediated condensation of BOC-D-Ala and serinol following the procedure described in Example 8. The crude reaction product was purified by chromatography on a 1.7 x 24 cm column of BioSil A by successive elution with (A) 100 ml $CHCl_3$, (B) 100 ml of 1% (v/v) MeOH/$CHCl_3$, (C) 100 ml of 2% MeOH/$CHCl_3$, (D) 100 ml of 4% MeOH/$CHCl_3$, (E) 100 ml of 8% MeOH/$CHCl_3$, and (F) 100 ml of 12% MeOH/$CHCl_3$. The appropriate column fractions were combined and concentrated to dryness on the rotary evaporator. The oily residue was then taken up in 10 ml of CH_2Cl_2, filtered from the small amount of insoluble material, and again concentrated to dryness. The residue was further dried under high vacuum to yield 168 mg (68%) of product as a colorless viscous oil.

EXAMPLE 13

BOC-D-Ala-NHDPG

BOC-D-Ala-NHDPG was prepared on a 0.64 mMol-scale by the EDCI/DMAP mediated condensation of BOC-D-Ala-SerOH and palmitic acid following the procedure described in Example 9. After the coupling reaction and the ensuing workup, the crude product was purified by ion exchange (Cellex D, OH-cycle) and silica gel (BioSil A) chromatography as described in Example 9. The column fractions containing pure product were combined, concentrated to dryness on the rotary evaporator, then further dried under high vacuum to yield 310 mg (65.5%) of product as a white amorphous solid.

EXAMPLE 14

D-Ala-NHDPG

310 mg (0.42 mMol) of the product from Example 13 was dissolved in 10 ml of CH_2Cl_2, then treated with 5 ml of TFA. After standing at RT for one hour, the reaction was worked up exactly as described in Example 10 for the L-isomer. After extensive drying under high vacuum, there was obtained 315 mg (99.7%) of the trifluoroacetate salt of the product as a white amorphous solid.

EXAMPLE 15

GlcNAcMurNAc-L-Ala-D-IsoGln-D-Ala-NHDPG (IB)

The EDCI/HOBT mediated condensation of GlcNAcMurNAc-L-Ala-D-isoGln (see Example 5) with D-Ala-NHDPG (see Example 14) was carried out on a 0.24 mMol-scale following the procedure described in Example 11. Following the coupling reaction and the ensuing workup, the product was purified by the procedure described in Example 20 (i.e., EtOAc extraction and dialysis against distilled water) to yield, after lyophilization, 141 mg of a white powder.

For final purification, a 125 mg-sample of the above product was subjected to the two column procedure (i.e., Sephadex LH-20 and Cellex D) After lyophilization, the pure product was obtained as a white, electrostatic powder. Molecular weight (HRMS): (Molecular Ion + H)$^+$ Calc: 1316.8432; Found: 1316.8375.

PREPARATION OF GlcNAcMurNAc-D-Ala-D-isoGln-D-Ala-NHDPG (IC)

EXAMPLE 16

GlcNAcMurNAc-D-Ala-D-isoGln-OBn

GlcNAcMurNAc-D-Ala-D-isoGln-OBn was prepared on a 0.75 mMol-scale by the Woodward's Reagent K-mediated coupling of GlcNAcMurNAc (VII) with D-Ala-D-isoGln-OBn (prepared as described in Example 2 and 3) using the procedure in Example 4. Following the 72 hour-coupling reaction and the ensuing workup and lyophilization, 332 mg of product was obtained as a white powder.

EXAMPLE 17

GlcNAcMurNAc-D-Ala-D-isoGln

The entire reaction product from Example 16 (332 mg, 0.423 mMol) was deprotected by hydrogenation over a Pd/C catalyst using the procedure described in Example 5. Following workup and lyophilization, 313.1 mg of product was obtained as a glassy solid.

EXAMPLE 18

GlcNAcMurNAc-D-Ala-D-isoGln-D-Ala-NHDPG (IC)

The EDCI/HOBT mediated condensation of GlcNAcMurNAc-D-Ala-D-isoGln (see Example 17) with D-Ala-NHDPG (see Example 14) was carried out on a 0.25 mMol-scale following the procedure detailed in Example 11. Following the coupling reaction and the ensuing workup, the product was purified by EtOAc extraction and dialysis against distilled water to yield, after lyophilization, 102 mg of a white powder.

For final purification, a 90 mg-sample of the above product was subjected to the two column procedure (i.e., Sephadex LH-20 and Cellex D). After lyophilization, the pure product was obtained as a white, electrostatic powder. Molecular weight (HRMS): (Molecular Ion + H)$^+$ Calc: 1316.8432; Found: 1316.8444.

EXAMPLE 19

Activation of Monocytes In-Vitro by Drug Compounds as Measured by the Production of Tumor Necrosis Factor

Human peripheral blood monocytes were isolated by density gradient centrifugation on 46% Percoll. The cell number was adjusted to 5 x 10^5 cells per ml in RPM 1640 media containing 5% human AB negative serum. One ml containing 5 x 10^5 cells were placed in individual wells of a 24-well Plastek tissue culture plate. Various drugs in a volume of 0.5 ml were added at time zero. The cells were incubated at 37°C in a 5% CO_2 atmosphere. Aliquots (0.1 ml) of the supernatant were removed at 24, 48, and 72 hours and frozen at -80°C. These samples were subsequently assayed for tumor necrosis factor (TNF) activity using a commercially available ELISA Assay from T-Cell Sciences (Cambridge, MA). The results of a series of four similar experiments, set forth in Table 4, demonstrate significant levels of TNF production by all compounds as compared to a control.

Table 1

TNF Production by Monocytes Incubated with Compounds Suspended in Saline.		
Drug Compound	Concentration of Free Drug (μg/ml)	TNF Level (pg/ml)
1. Control	--	130
2. Compound IA	0.1	280
	1.0	1000
	10.0	1680
3. Compound IB	0.1	180
	1.0	350
	10.0	1980
4. Compound IC	0.1	280
	1.0	300
	10.0	250

EXAMPLE 20

Activation of Monocytes In-Vitro by Drug Compound Incorporated in Liposomes

Human peripheral blood monocytes were isolated by density gradient centrifugation on 46% Percoll. The cells were plated in 24-well microliter plates as described in Example 33. The compounds were prepared in liposomes as described in Example 7, and assayed for TNF production, as described in Example 19. The results of four separate experiments are set forth in Table 2, and show significant levels of TNF compared to controls.

Table 2

TNF Production by Monocytes Incubated with Compounds Incorporated in Liposomes		
Drug Compound	Concentration of Drug (μg/ml)	TNF Level (pg/ml)
Control	--	70
Compound IA	0.1	430
	1.0	800
	10.0	750

EXAMPLE 21

Activation of Monocytes In-Vitro as Measured by In-Vitro Tumor Cell Cytotoxicity

Human peripheral blood mononuclear cells were isolated by density gradient centrifugation on 46% Percoll. 1×10^5 of the mononuclear cells were plated onto Falcon microliter plates and allowed to adhere for two hours. The non-adherent cells were removed and fresh media added. The cells were allowed to incubate for 24 hours after which various concentrations of the drug either alone or incorporated in liposomes was added. After 24 hours, the plates were washed and 1×10^4 I^{125} labelled target cells were added. Following an additional 72 hours of co-incubation, the plates were washed and the residual radioactivity determined. The specific cytotoxicity was calculated from the formula:

$$\% \text{ cytotoxicity} = \frac{A-B}{A} \times 100$$

Where:

A = initial radioactivity

B = residual radioactivity

The results of cytotoxicity assay of various compounds alone or incorporated in liposomes at various concentrations is given in Table 3. All of the tested compounds, either alone or incorporated in liposomes, demonstrated ability to activate monocytes to be cytotoxic.

Table 3

In-Vitro Cytotoxicity of Various Compounds and Formulations			
Compound	Formulation	Concentration (μg/ml)	Cytotoxicity (%)
Control			1.4
Compound IA	PC/PG[1]	0.1	7.0
		1.0	7.0
		10.0	6.0
Compound IC	PC/PG[1]	0.1	10.0
		1.0	4.0
		10.0	12.0
Compound IA	PC/PS[2]	0.1	8.0
		1.0	5.0
		10.0	19.0
Compound IC		0.1	5.0
		1.0	16.0
		10.0	15.0
Compound IA		0.1	1.0
		1.0	4.0
		10.0	3.0
MDP		0.1	11.0
		1.0	9.0
		10.0	7.0

[1]PC/PG: phosphatidyl choline/phosphatidyl glycerol liposomes (7:3 molar ratio)
[2]PC/PS: phosphatidyl choline/phosphatidyl serine liposomes (7:3 molar ratio)

EXAMPLE 22

BOC-D-isoGln-OBn

Benzyl alcohol (476 mg, 4.4 mMol) BOC-D-isoGln (Sigma, 985.2 mg, 4.0 mMol) and DMAP (244 mg, 2.0 mMol) were dissolved in 30 ml CH_2Cl_2 and 20 ml DMF, then treated with EDCI (920 mg, 4.8 mMol), and the resulting solution stirred at RT for 24 hours. The reaction mixture was then concentrated to dryness on a rotary evaporator to yield an oily residue that was partitioned between 75 ml EtOAc and 25 ml H_2O. The layers were separated and the organic layer extracted with saturated $NaHCO_3$ (3 x 25 ml) and H_2O (3 x 25 ml). After drying over Na_2SO_4, the solvent was removed on the rotary evaporator to yield 1.206 g (90%) of a white solid, which was further purified by crystallization from diethyl ether/hexane to yield pure product as a snow-white solid.

EXAMPLE 23

D-isoGln-OBn

A sample (1.0g, 2.98 mMol) of the product from Example 22 was dissolved in 50 ml of 1N HCl/HOAc and the resulting solution allowed to stand at RT for two hours. The solvent was then removed on the rotary evaporator to yield a white solid that was further dried under high vacuum, then crystallized from MeOH/ether to yield 720 mg (90%) of the pure product as its hydrochloride salt.

EXAMPLE 24

BOC-L-Thr(OBn)-D-isoGln-OBn

BOC-L-Thr(OBn) (Sigma, 618.8 mg, 2.0 mMol), and N-hydroxysuccinimide (253.2 mg, 2.2 mMol) were dissolved in 5.0 ml DMF and 7.0 ml CH_2Cl_2. To this was added EDCI (421.7 mg, 2.2 mMol), and the resulting solution stirred at RT for one hour. Then, a solution that contained 440.4 mg (1.62 mMol) of the product from Example 23 and 164 mg (1.62 mMol) of N-methylmorpholine in 5.0 ml DMF was added and the reaction mixture was stirred at RT for 48 hours. The solvents were then removed on the rotary evaporator to yield an oily residue that was partitioned between 100 ml EtOAc and 50 ml H_2O. The layers were separated, and the organic layer further extracted with saturated $NaHCO_3$ (3 x 50 ml), then H_2O (3 x 50 ml). The organic layer was concentrated to dryness on the rotary evaporator, then further dried under high vacuum to yield crude product as a yellow glassy solid.

For further purification, the entire product was dissolved in 10 ml $CHCl_3$, then applied to a 2.5 x 18 cm column of BioSil A that had been packed in $CHCl_3$. The column was then successively eluted with (A) 200 ml $CHCl_3$, (B) 200 ml of 1% MeOH/$CHCl_3$, (C) 200 ml of 2% MeOH/$CHCl_3$, and (D) 200 ml of 3% MeOH/$CHCl_3$. Fractions of ca. 20 ml were collected, and aliquots (2ul) of each were assayed by TLC as described in Example 21. The appropriate fractions were combined, concentrated to dryness, then further dried under high vacuum to yield product as a white glassy solid.

For final purification, the entire product was crystallized from EtOAc/hexane to yield, after drying, 550 mg (65% overall) of pure product as a white glassy solid.

EXAMPLE 25

L-Thr(OBn)-D-isoGln-OBn

The product from Example 24 (540 mg, 1.044 mMol) was dissolved in 50 ml of 1N HCl/HOAc and the resulting solution stirred at RT for 1.5 hours. After removal of solvent on the rotary evaporator, the oily residue was further dried under high vacuum, then crystallized from MeOH/ether to yield, after extensive drying, 450mg of the hydrochloride salt of L-Thr(OBn)-D-isoGln-OBn as a white, amorphous solid.

EXAMPLE 26

GlcNAcMurNAc-L-Thr(OBn)-D-isoGln-OBn

GlcNAcMurNAc-L-Thr(OBn)-D-isoGln-OBn was prepared on a 0.60 mMol-scale by the Woodward's Reagent K-mediated coupling of GlcNAcMurNAc (VII) with the product from Example 25 using the procedure described in Example 4. Following the 96 hour-coupling reaction and the ensuing workup and lyophilization, 388 mg of product was obtained as a white powder.

EXAMPLE 27

GlcNAcMurNAc-L-Thr-D-isoGln

263 mg of the product from Example 26 was dissolved in 75 ml of 10% HOAc, then deprotected by hydrogenation over a Pd/C catalyst using the procedure described in Example 7. Following workup and lyophilization, 201 mg of product was obtained as a glassy tan solid.

PREPARATION OF GlcNAcMurNAc-L-Thr-D-isoGln-L-Ala-NHDPG

(ID) EXAMPLE 28

GlcNAcMurNAc-L-Thr-D-isoGln-L-Ala-NHDPG (ID)

The EDCI/HOBT mediated condensation of GlcNAcMurNAc-L-Thr-D-isoGln (see Example 26) with L-Ala-NHDPG (see Example 10) was carried out on a 0.1 mMol-scale following the procedure detailed in Example 11. After the 48 hour-coupling reaction, the reaction mixture was worked up, then purified as described in Example 11. The final column eluate was lyophilized to yield pure product as a white

electrostatic powder.

PREPARATION OF GlcNAcMurNAc-L-Ser-D-isoGln-L-Ala

EXAMPLE 29

BOC-L-Ser(OBn)-D-isoGln-OBn

This compound was prepared on a 1.0 mMol-scale using the same procedure described in Example 24 for the synthesis of the threonine analog. After workup and purification by both silica gel (BioSil A) chromatography and crystallization from EtOAc/hexane, pure product was obtained in a 79% yield as a white, amorphous solid.

EXAMPLE 30

L-Ser(OBn)-D-isoGln-OBn

The product from Example 29 (300 mg, 0.6 mMoL) was treated with 30 ml of 1N HCl/HOAc, and the resulting solution was allowed to stand at RT for 2 hours. After removal of the solvent on the rotary evaporator, the oily residue was further dried under high vacuum, then crystallized form MeOH/ether (see Example 3) to yield, after extensive drying, 234 mg of the hydrochloride salt of L-Ser(OBn)-D-isoGln-OBn as a white, amorphous solid.

EXAMPLE 31

GlcNAcMurNAc-L-Ser(OBn)-D-isoGln-OBn

GlcNAcMurNAc-L-Ser(OBn)-D-isoGln-OBn was prepared on a 0.20 mMol-scale by the Woodward's Reagent K-mediated coupling of GlcNAcMurNAc (VII) with the product from Example 30 using the procedure described in Example 4. Following the 120 hour-coupling reaction and the ensuing workup and lyophilization, 110 mg of product was obtained as a white powder.

EXAMPLE 32

GlcNAcMurNAc-L-Ser-D-isoGln

108 mg of the product from Example 46 was dissolved in 50 ml of 10% HOAc, then deprotected by hydrogenation over a Pd/C catalyst using the procedure described in Example 5. Following workup and lyophilization, 95 mg of the crude product was obtained as a white glassy solid.

For further purification, the entire 95 mg of product was slurried in 2.0 ml of $CHCl_3$, then treated with sufficient MeOH to effect solution. This was then applied to a 0.7 x 28 cm column of BioSil A that had been packed in $CHCl_3$. The column was then successively eluted with (A) 50 ml of $CHCl_3$, (B) 50 ml of 20% MeOH/$CHCl_3$, (C) 100 ml of 40% MeOH/$CHCl_3$, (D) 100 ml of 60% MeOH/$CHCl_3$, and (E) 100 ml of 80% MeOH/$CHCl_3$. Fractions of ca. 5.0 ml were collected and aliquots (2λ) of each were assayed by TLC (Merck silica; $CHCl_3$:MeOH:H_2O:con NH_4OH, 50:25:4:2; 5% H_2SO_4/EtOH; heat). The tubes containing material with R_f 0.0 were combined, concentrated to dryness, then redissolved in 10 ml of H_2O and lyophilized to yield pure product as a white, electrostatic solid.

PREPARATION OF GlcNAcMurNAc-L-Val-D-isoGln-L-Ala

EXAMPLE 33

BOC-L-Val-D-isoGln-OBn

BOC-L-Val-D-isoGln-OBn was prepared on a 1.0 mMol-scale using the same procedure described in Example 24 for the synthesis of the threonine analog. After workup and purification by both silica gel (BioSil A) chromatography and crystallization from the EtOAc/hexane, pure product was obtained in a 60% yield as a white, amorphous solid.

EXAMPLE 34

L-Val-D-isoGln-OBn

The product from Example 33 (175 mg, 0.40 mMol) was treated with 20 ml of 1N HCl/HOAc, and the resulting solution was allowed to stand at RT for 2 hours. After removal of the solvent on a rotary evaporator, the oily residue was further dried under high vacuum, then crystallized from MeOH/ether (see Example 3) to yield, after thorough drying, 134 mg of the hydrochloride salt of L-Val-D-isoGln-OBn as a white, amorphous solid.

EXAMPLE 35

GlcNAcMurNAc-L-Val-D-isoGln-OBn

GlcNAcMurNAc-L-Val-D-isoGln-OBn was prepared on a 0.20 mMol-scale by the Woodward's Reagent K-mediated coupling of GlcNAcMurNAc (VII) with the product from Example 50 using the procedure detailed in Example 4. Following the 96 hour-coupling reaction and the ensuing workup and lyophilization, 95 mg of product was obtained as a white powder.

EXAMPLE 36

GlcNAcMurNAc-L-Val-D-isoGln

95 mg of the product from Example 35 was dissolved in 50 ml of the 10% HOAc, then deprotected by hydrogenation over a Pd/C catalyst using the procedure described in Example 5. Following workup and lyophilization, 87 mg of the crude product was obtained as a white glassy solid. This material was then purified by chromatography on a silica gel column exactly as described in Example 47. Fractions containing material with R_f 0.05 were combined, concentrated to dryness, then redissolved in 10 ml of H_2O and lyophilized to yield pure product as a white powder.

EXAMPLE 37

Activation of Monocytes In-Vitro by Drug Compounds and Formulations as Measured by the Production of Interleukin-1-beta and/or TNF

Human peripheral blood monocytes were isolated by density gradient centrifugation on 46% Percoll.

The cell number was adjusted to 5 x 10^5 cells per ml in RPM 1640 media containing 5% human AB negative serum. One ml containing 5 x 10^5 cells were placed in individual wells of 24-well Plastek tissue culture plate. Various drugs in a volume of 0.5 ml were added at time zero. The compounds were also formulated in liposomes as described in Example 7, and added in a volume of 0.5 ml to individual wells of a 24-well Plastek tissue culture plate. The cells were incubated at 37°C in a 5% CO_2 atmosphere. Aliquots (0.1 ml) of the supernatant were removed at 24 hours and frozen at -20°C. These samples were subsequently assayed for TNF as described in Example 33 and Interleukin-1-beta (IL-1β) using a commercially available ELISA kit from Cistron (Pine Brook, NJ).

The results of a series of two separate experiments set forth in Tables 4 and 5 demonstrate significant levels of IL-1β and/or TNF production by all compounds and formulations of the invention as compared to controls.

TABLE 4 (Experiment 1)

TNF Level (pg/ml) at 24 Hours

Following Incubation of Monocytes with Various Compounds and Formulations

Compound	TNF (pg/ml) at given Concentration of Compound (µg/ml)			
	0.01	0.1	1.0	10.0
1D GlcNAcMurNAc-L-Thr-D-isoGln-L-Ala-NHDPG		0	250	1060
1D in Liposomes	10	280	180	220
MDP	400	430	380	360
GlcNAcMurNAc-L-Thr-D-isoGln	200	440	1050	1050
Empty Liposomes	70	60	80	70

TABLE 5 (Experiment 1)

Interleukin-1-beta Level (pg/ml) at 24 Hours

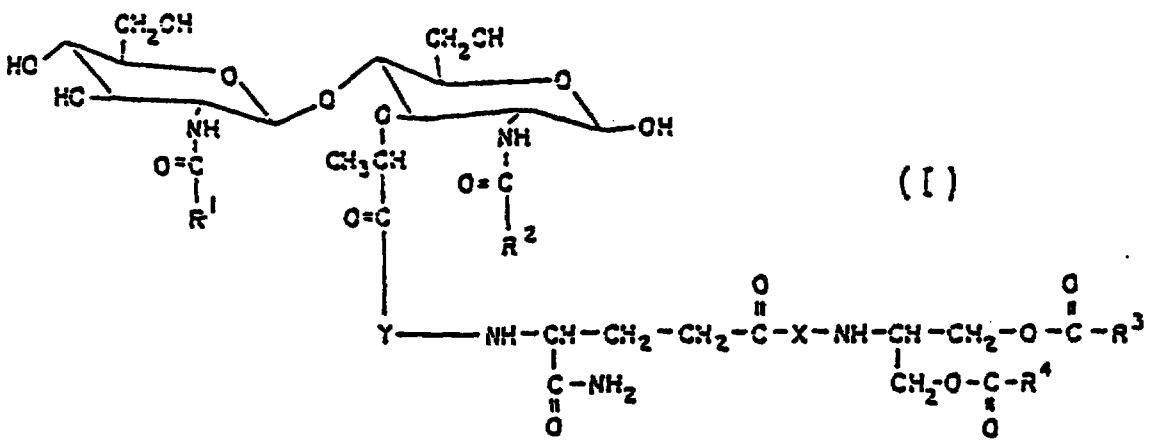
Following Incubation of Monocytes with Various Compounds and Formulations

Compound	IL-1-beta (pg/ml) at given Concentration of Compound (µg/ml) 0.01	0.1	1.0	10.0
ID GlcNAcMurNAc-L-Thr-D-isoGln-L-Ala-NHDPG	10	16	8	90
ID in Liposomes	40	14	40	40
MDP	180	260	226	240
GlcNAcMurNac-L-Thr-D-isoGln	50	70	230	630
Empty Liposomes	14	0	4	0

EP 0 527 803 B1

Claims

1. A compound of the formula:



(I)

wherein R^1 is $(C_1\text{-}C_9)$alkyl, R^2 is $(C_1\text{-}C_5)$alkyl, R^3 and R^4 are individually $(C_6\text{-}C_{30})$alkyl groups having about 0-4 double bonds, X is a single bond or any peptidyl residue comprising one or more amino acid residues; and Y is any amino acid residue; and the pharmaceutically acceptable salts thereof.

2. The compound of claim 1 wherein R^1 is $-CH_3$.

3. The compound of claim 2 wherein R^2 is $-CH_3$.

4. The compound of claim 1 wherein X is any amino acid residue.

5. The compound of claim 4 wherein X is any naturally occurring amino acid residue or an enantiomorph of any naturally occurring amino acid residue.

6. The compound of claim 5 wherein X is selected from L-valine, D-valine, L-alanine, and D-alanine.

7. The compound of claim 1 wherein Y is any naturally occurring amino acid residue or an enantiomorph of any naturally occurring amino acid residue.

8. The compound of claim 7 wherein Y is selected from threonine, alanine, valine, and serine.

9. The compound of claim 8 wherein Y is selected from L-alanine and L-threonine.

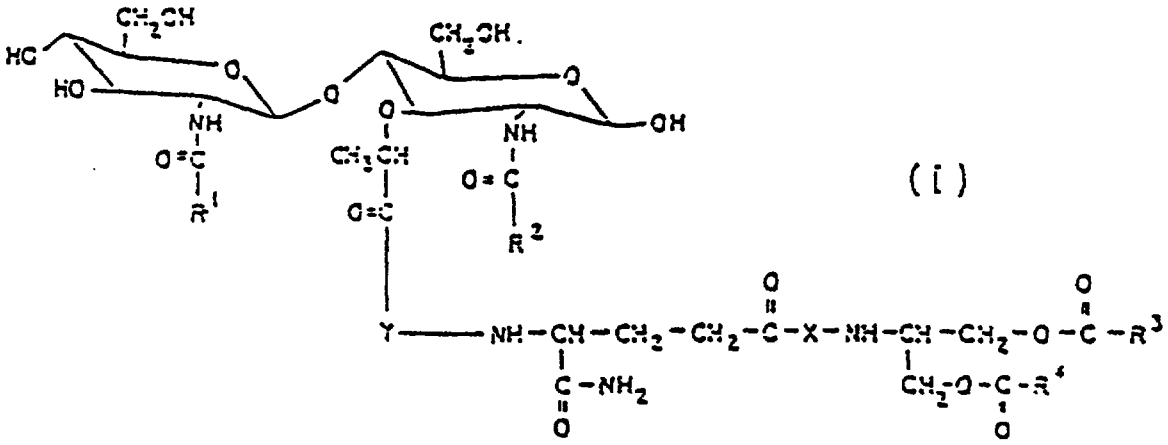
10. The compound of claim 1 wherein R^3 and R^4 are individually $(C_{12}\text{-}C_{23})$alkyl groups having about 0-1 double bonds.

11. The compound of claim 10 wherein R^3 is a (C_{15})alkyl group.

12. The compound of claim 11 wherein R^4 is a (C_{15})alkyl group.

13. The compound of claim 10 wherein R^1 and R^2 are $-CH_3$.

14. A liposome comprising a compound of the formula:



(I)

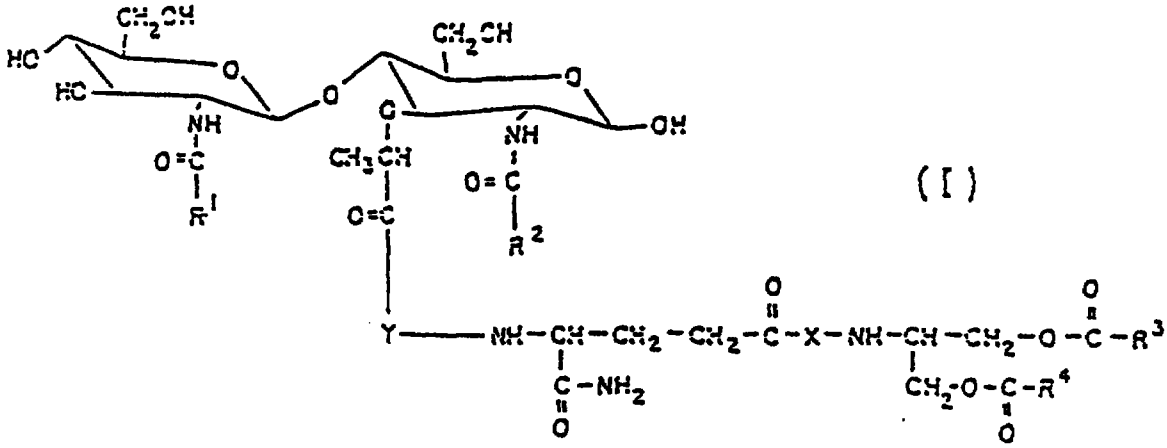
wherein R^1 is $(C_1\text{-}C_9)$alkyl, R^2 is $(C_1\text{-}C_5)$alkyl, R^3 and R^4 are individually $(C_6\text{-}C_{10})$alkyl groups having about 0-4 double bonds, X is a single bond or any peptidyl residue comprising one or more amino acid residues, and Y is any amino acid residue; and the pharmaceutically acceptable salts thereof.

15. Use of the compound or composition of Claim 1 or 14 for the manufacture of a medicament for therapeutic application in stimulating the immune response of a mammal.

16. A pharmaceutical composition comprising an effective amount of the compound of Claim 1 or 14 in a pharmaceutically acceptable vehicle.

Patentansprüche

1. Eine Verbindung der Formel



(I)

in der R^1 $(C_1\text{-}C_9)$-Alkyl ist, R^2 $(C_1\text{-}C_5)$-Alkyl ist, R^3 und R^4 individuell $(C_6\text{-}C_{30})$-Alkylgruppen mit ungefähr 0-4 Doppelbindungen sind, X eine Einfachbindung oder irgendein Peptidylrest umfassend eine oder mehrere Aminosäurereste ist, und Y irgendein Aminosäurerest ist; und die pharmazeutisch akzeptablen Salze davon.

2. Die Verbindung nach Anspruch 1, in der R^1 $-CH_3$ ist.

3. Die Verbindung nach Anspruch 2, in der R^2 $-CH_3$ ist.

4. Die Verbindung nach Anspruch 1, in der X irgendein Aminosaurerest ist.

* C L M S *

5. Die Verbindung nach Anspruch 4, in der X irgendein natürlich vorkommender Aminosäurerest oder ein Enantiomorph von irgendeinem natürlich vorkommenden Aminosäurerest ist.

6. Die Verbindung nach Anspruch 5, in der X ausgewählt ist aus L-Valin, D-Valin, L-Alanin und D-Alanin.

7. Die Verbindung nach Anspruch 1, in der Y irgendein natürlich vorkommender Aminosäurerest oder ein ein Enantiomorph irgendeines natürlich vorkommenden Aminosäurerestes ist.

8. Die Verbindung nach Anspruch 7, in der Y ausgewählt ist aus Threonin, Alanin, Valin und Serin.

9. Die Verbindung nach Anspruch 8, in der Y ausgewählt ist aus L-Alanin und L-Threonin.

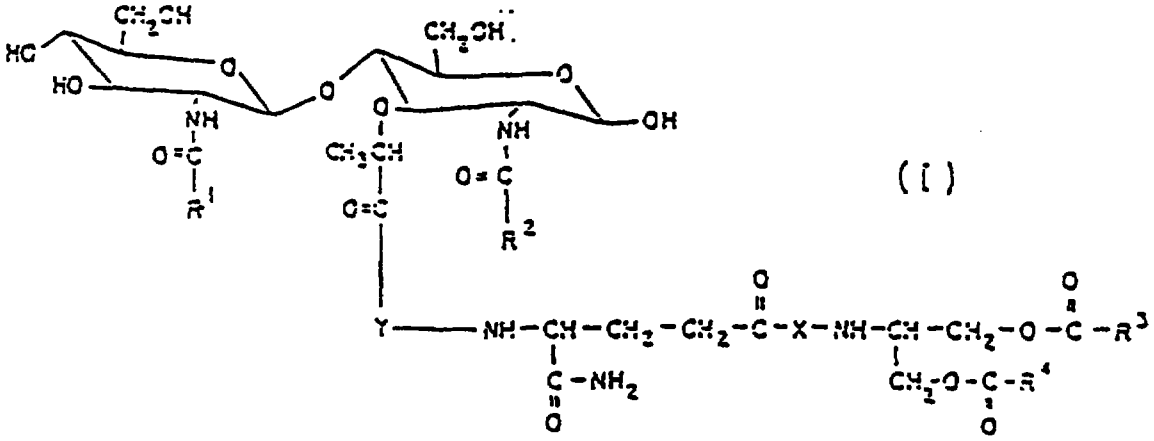
10. Die Verbindung nach Anspruch 1, in der R^3 und R^4 individuell (C_{12}-C_{23})-Alkylgruppen mit ungefähr 0-1 Doppelbindungen sind.

11. Die Verbindung nach Anspruch 10, in der R^3 eine (C_{15})-Alkylgruppe ist.

12. Die Verbindung nach Anspruch 11, in der R^4 eine (C_{15})-Alkylgruppe ist.

13. Die Verbindung nach Anspruch 10, in der R^1 und R^2 $-CH_3$ sind.

14. Ein Liposom enthaltend eine Verbindung der Formel



(I)

in der R^1 (C_1-C_9)-Alkyl ist, R^2 (C_1-C_5)-Alkyl ist, R^3 und R^4 individuell (C_6-C_{10})-Alkylgruppen mit ungefähr 0-4 Doppelbindungen sind, X eine Einfachbindung oder irgendein Peptidylrest ist umfassed eine oder mehrere Aminosäurereste und Y irgendein Aminosäurerest ist, und die pharmazeutisch akzeptablen Salze davon.

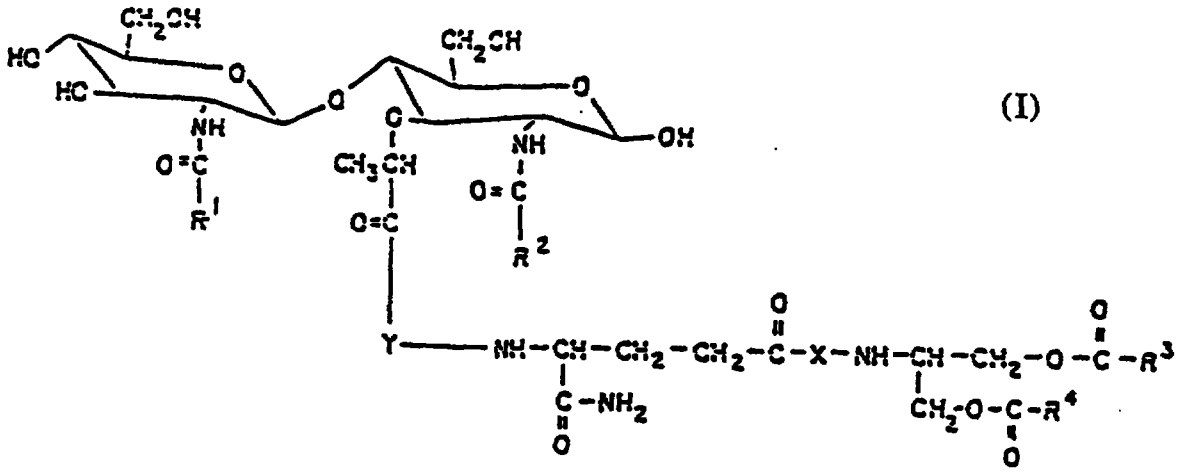
15. Verwendung der Verbindung oder Zusammensetzung nach Anspruch 1 oder 14 für die Herstellung eines Medikaments zur therapeutischen Anwendung bei der Stimulierung der Immunantwort eines Säugers.

16. Eine pharmazeutische Zusammensetzung enthaltend eine wirksame Menge der Verbindung nach Anspruch 1 oder 14 in einem pharmazeutisch akzeptablen Träger.

* C L M S *

Revendications

1. Composé de formule :



(I)

dans laquelle R^1 est un groupe alkyle en C_{1-9}, R^2 est un groupe alkyle en C_{1-5}, R^3 et R^4 sont individuellement des groupes alkyles en C_{6-30} ayant environ 0 à 4 doubles liaisons, X est une simple liaison ou un quelconque résidu peptidyle comprenant un ou plusieurs résidus d'acide aminé; et Y est un quelconque résidu d'acide aminé; et sels de celui-ci acceptables du point de vue pharmaceutique.

2. Composé suivant la revendication 1, dans lequel R^1 est le groupe $-CH_3$.

3. Composé suivant la revendication 2, dans lequel R^2 est le groupe $-CH_3$.

4. Composé suivant la revendication 1, dans lequel X est un quelconque résidu d'acide amine.

5. Composé suivant la revendication 4, dans lequel X est un quelconque résidu d'acide aminé d'origine naturelle ou un énantiomorphe d'un quelconque résidu d'acide aminé d'origine naturelle.

6. Composé suivant la revendication 5, dans lequel X est choisi parmi la L-valine, la D-valine, la L-analine et la D-alanine.

7. Composé suivant la revendication 1, dans lequel Y est un quelconque résidu d'acide aminé d'origine naturelle ou un énantiomorphe d'un quelconque résidu d'acide aminé d'origine naturelle.

8. Composé suivant la revendication 7, dans lequel Y est choisi parmi la thréonine, l'alanine, la valine et la sérine.

9. Composé suivant la revendication 8, dans lequel Y est choisi parmi la L-alanine et la L-thréonine.

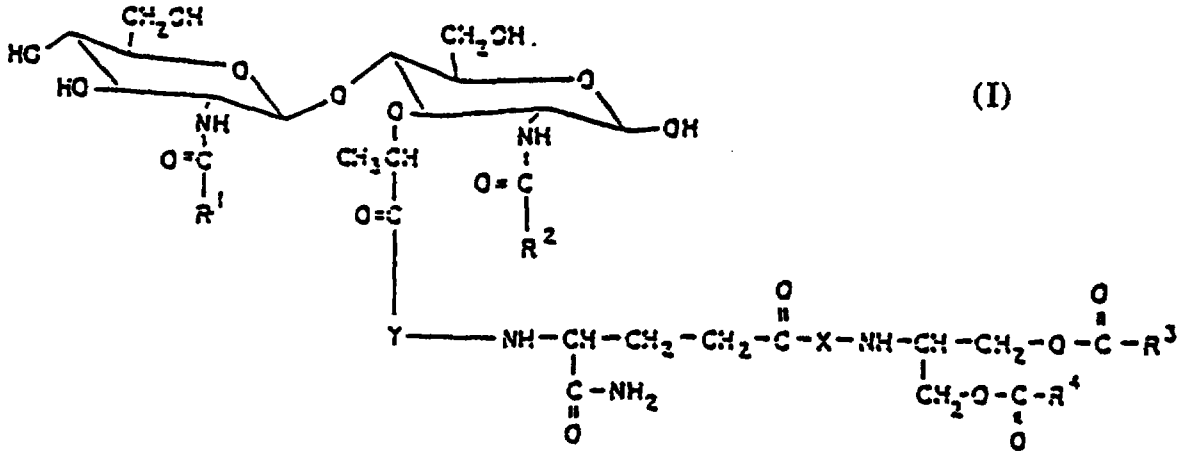
10. Composé suivant la revendication 1, dans lequel R^3 et R^4 sont individuellement des groupes alkyles en C_{12-23} ayant environ 0 à 1 double liaison.

11. Composé suivant la revendication 10, dans lequel R^3 est un groupe alkyle en C_{15}.

12. Composé suivant la revendication 11, dans lequel R^4 est un groupe alkyle en C_{15}.

13. Composé suivant la revendication 10, dans lequel R^1 et R^2 sont chacun le groupe $-CH_3$.

14. Liposome comprenant un composé de formule :



(I)

dans laquelle R^1 est un groupe alkyle en C_{1-9}, R^2 est un groupe alkyle en C_{1-5} R^3 et R^4 sont individuellement des groupes alkyles en C_{6-10} ayant environ 0 à 4 doubles liaisons, X est une simple liaison ou un quelconque résidu peptidyle comprenant un ou plusieurs résidus d'acide aminé, et Y est un quelconque résidu d'acide aminé; et ses sels acceptables du point de vue pharmaceutique.

15. Utilisation du composé ou de la composition suivant les revendications 1 ou 14 pour la fabrication d'un médicament pour l'application thérapeutique dans la stimulation de la réponse immune d'un mammifère.

16. Cmposition pharmaceutique comprenant une quantité efficace d'un composé suivant les revendications 1 ou 14 dans un véhicule acceptable du point de vue pharmaceutique.