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CA 2290724 C 2010/03/23

(11)(21) 2 290 724

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1998/05/05
(87) Date publication PCT/PCT Publication Date: 1998/11/26
(45) Date de délivrance/Issue Date: 2010/03/23
(85) Entrée phase nationale/National Entry: 1999/11/17
(86) N° demande PCT/PCT Application No.: US 1998/009115
(87) N° publication PCT/PCT Publication No.: 1998/052596
(30) Priorité/Priority: 1997/05/20 (US60/047,165)

(51) Cl.Int./Int.Cl. *A61K 38/12* (2006.01), *C07K 16/00* (2006.01), *C07K 17/00* (2006.01), *C07K 5/00* (2006.01), *C07K 7/00* (2006.01), *C07K 9/00* (2006.01)

(72) Inventeurs/Inventors:
THOMPSON, RICHARD CRAIG, US;
WILKIE, STEPHEN CHARLES, US

(73) Propriétaire/Owner:
ELI LILLY AND COMPANY, US

(74) Agent: FASKEN MARTINEAU DUMOULIN LLP

(54) Titre : HEXAPEPTIDES DE GLYCOPEPTIDES
(54) Title: GLYCOPEPTIDE HEXAPEPTIDES

(57) Abrégé/Abstract:
The present invention is directed to glycopeptides and more particularly to derivatives of the glycopeptide A82846B. In these derivatives, the leucyl has been removed to create "hexapeptides" of A82846B and its N^{DISACC} variations. These hexapeptides are useful as antibacterials and also as starting materials from which further antibacterial compounds are prepared.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/12, C07K 5/00, 7/00, 16/00, 17/00

A1

(11) International Publication Number: WO 98/52596

(43) International Publication Date: 26 November 1998 (26.11.98)

(21) International Application Number: PCT/US98/09115

(22) International Filing Date: 5 May 1998 (05.05.98)

(30) Priority Data:
60/047,165 20 May 1997 (20.05.97) US

(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: THOMPSON, Richard, Craig [US/US]; 763 North County Road, 900 West, Frankfort, IN 46041 (US). WILKIE, Stephen, Charles [US/US]; 8229 Quetico Drive, Indianapolis, IN 46268 (US).

(74) Agents: PAGE, Kathleen, R., S. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: GLYCOPEPTIDE HEXAPEPTIDES

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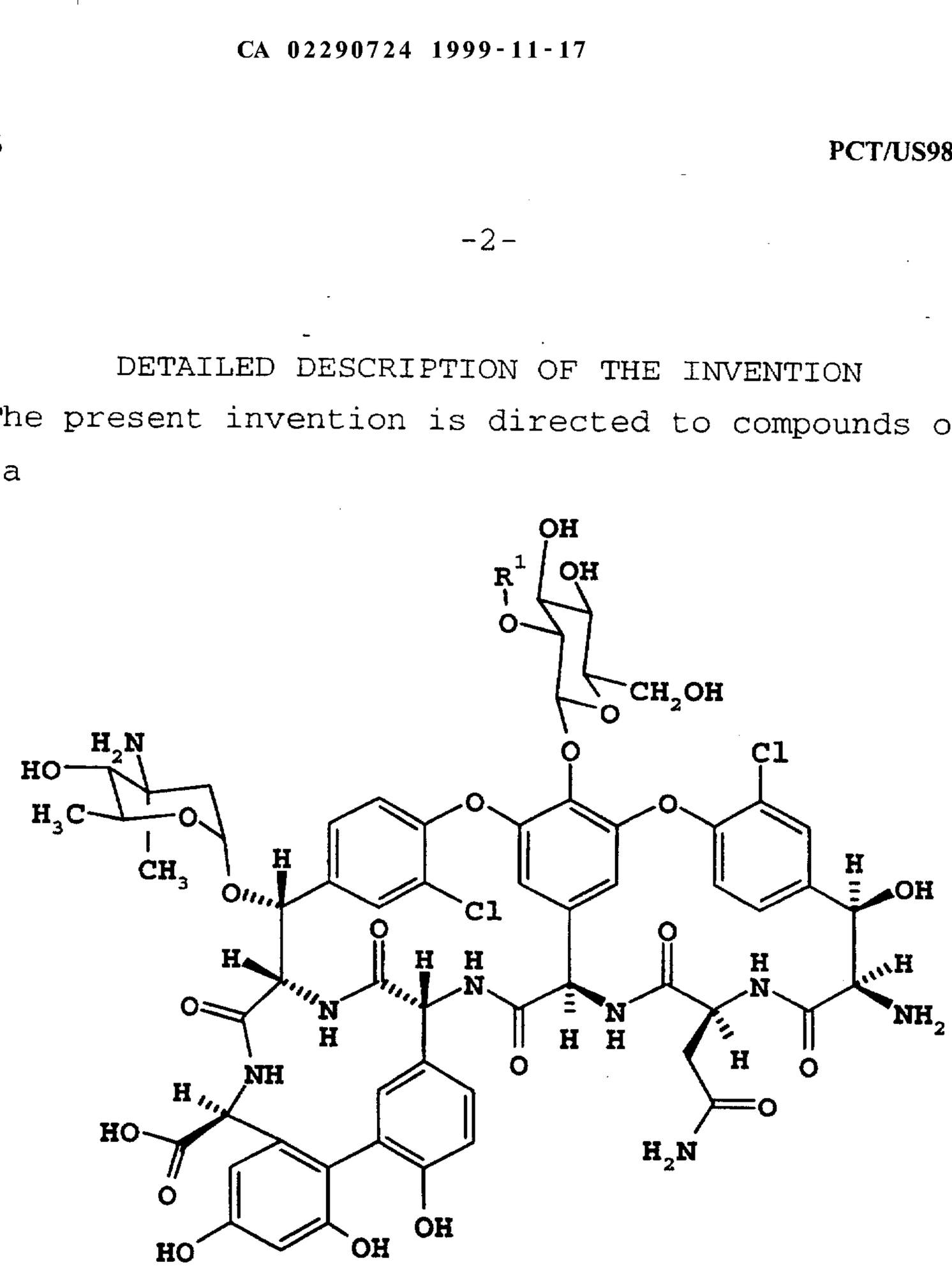
GLYCOPEPTIDE HEXAPEPTIDES

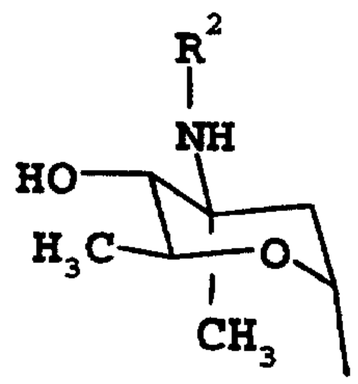
The present invention is directed to glycopeptides and more particularly to derivatives of the glycopeptide A82846B. In these derivatives, the leucyl has been removed to create "hexapeptides" of A82846B and its N^{DISACC} variations. These hexapeptides are useful as antibacterials and also as starting materials from which further antibacterial compounds are prepared.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compounds of the formula



wherein R^1 represents hydrogen or an epivancosaminyl radical of the formula



wherein R^2 represents hydrogen or $-CH_2-R^3$; and R^3 represents

alkyl of C_1-C_{11},

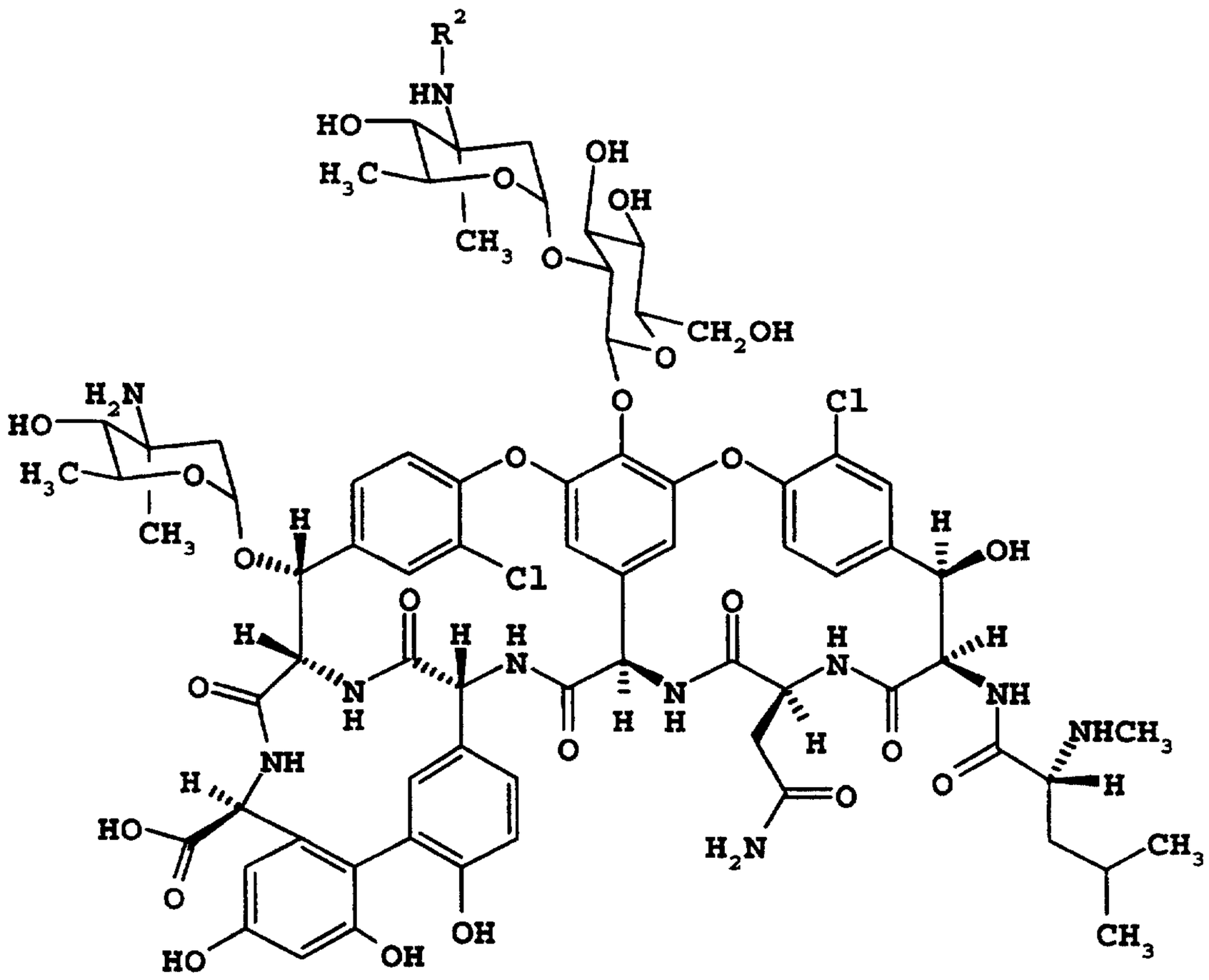
alkyl of C_1-C_{11}-R^4, or

R^4-$(O_{(0\ or\ 1)}$-$R^4)_{0\ or\ 1}$,

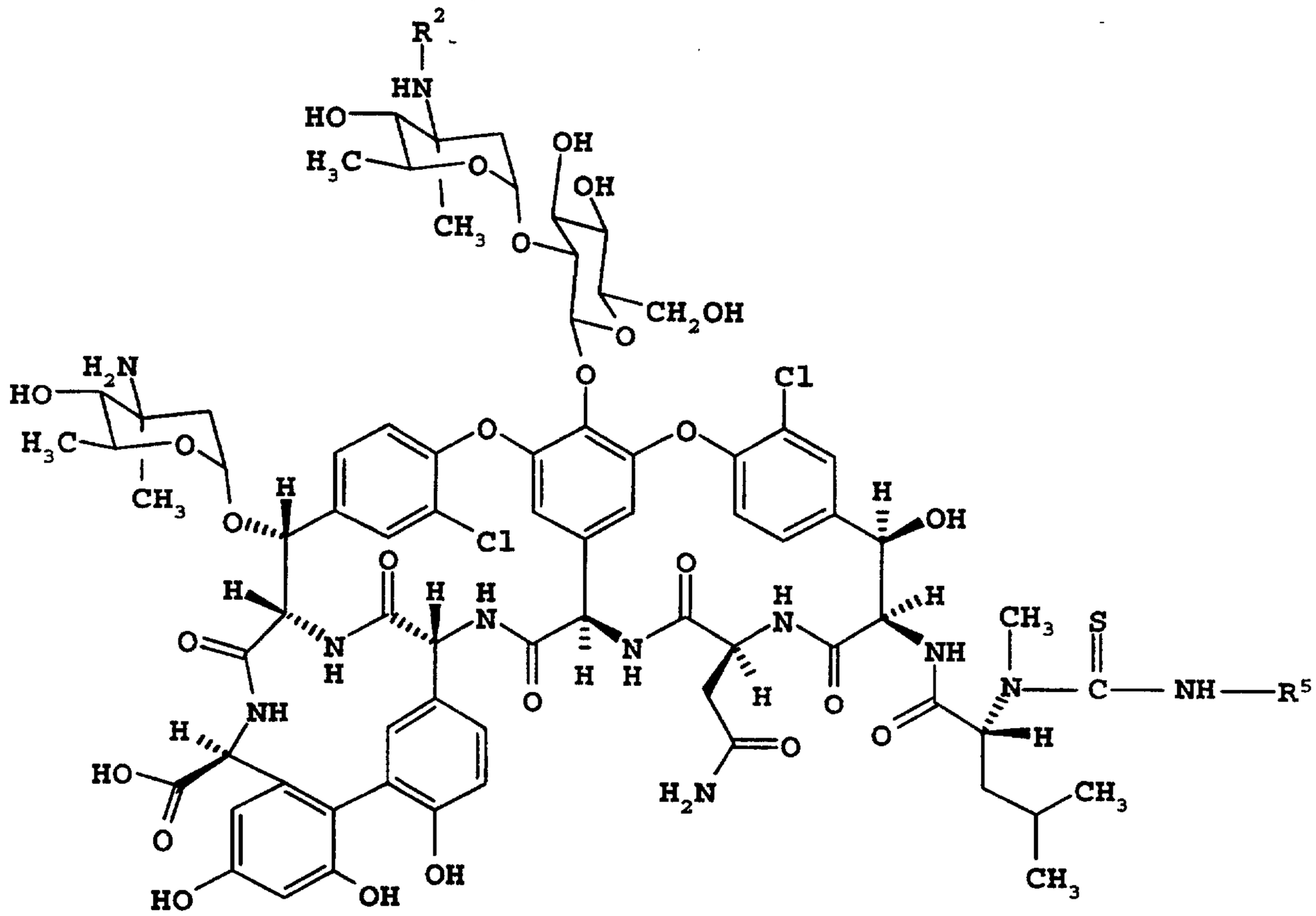
wherein each R^4 is independently phenyl or phenyl substituted by one or two substituents, each of which is

independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, or loweralkylthio of C_1-C_4, and pharmaceutically acceptable salts thereof.

The compounds of the present invention are prepared by an Edman degradation of a parent glycopeptide of the formula



wherein R^2 is as defined above. The Edman degradation is a two-step process for the cleavage of the N-terminal residue of a peptide or protein. In the present invention, the above parent glycopeptide is first reacted with an isothiocyanate of the formula SCN-R^5, to obtain an intermediate N^{LEU}-(thiocarbamoyl)-A82846B compound of the formula



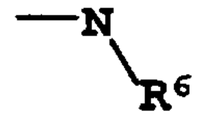
In the foregoing formula, R^5 represents

alkyl of C_1-C_{10},

phenyl,

naphthyl, or

phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, benzyloxy, nitro, or



wherein each R^6 is independently loweralkyl of C_1-C_4.

This reaction is conveniently carried out in water with pyridine, at a temperature of 25° to 30°C, employing a

slight excess of the isothiocyanate reactant. The N^{LEU}-(thiocarbamoyl)A82846B intermediate can be separated in conventional manner or can be employed after removal of reaction solvent in the second step of the Edman degradation.

In the second step, the N^{LEU}-(thiocarbamoyl)A82846B is reacted with an organic acid, preferably trifluoroacetic acid, in a non-polar solvent such as dichloromethane. The reaction proceeds at temperatures of from 0°C to 35°C but is preferably carried out at temperatures of from 0°C to 25°C. The reaction is generally complete in several hours. The resulting hexapeptide product is separated and purified if desired in conventional procedures. When it is desired to employ a salt, the product is reacted in standard procedures.

This second step of the Edman degradation can in some instances result in loss of the disaccharide epivancosamine. Longer reaction times can be used to prepare the N^{DISACC}-des-epivancosaminyl (R^1=hydrogen) compound of the present invention.

The following examples illustrate the preparation of the compounds of the present invention.

Preparation of N^{DISACC}-(p-(p-chlorophenyl)benzyl)-N^{LEU}-(phenylthiocarbamoyl)A82846B

N^{DISACC}-(p-(p-Chlorophenyl)benzyl)A82846B trihydrochloride (100.0 mg, 0.0526 mmol) was dissolved in 10 ml H_2O - pyridine (1:1 v/v) and treated with phenyl isothiocyanate (0.010 ml, 0.083 mmol). The resulting mixture was stirred at room temperature for 1 hr at which time HPLC analysis indicated complete consumption of the starting material. The reaction mixture was concentrated *in*

vacuo and the crude product was purified by preparative HPLC to give 76.6 mg (76% yield) of N^{DISACC}-(p-(p-chlorophenyl)benzyl)-N^{LEU}-(phenylthiocarbamoyl)A82846B. FAB-MS: calc. For $C_{93}H_{102}Cl_3N_{11}O_{26}S$ 1925.5, obtained 1928.5 (M+3).

Preparation of Compound of Example 6, from Isolated Thiourea

A sample of purified N^{DISACC}-(p-(p-chlorophenyl)benzyl)-N^{LEU}-(phenylthiocarbamoyl)A82846B (63.3 mg, 0.0327 mmol) was suspended in 10 ml CH_2Cl_2, cooled to 0 °C, then treated with trifluoroacetic acid (0.10 ml). After 1 hr the reaction mixture was warmed to room temperature and stirred an additional 2 hr. The solvent was removed *in vacuo* and the crude product was purified by preparative HPLC to give 25.3 mg (46% yield) of N^{DISACC}-(p-(p-chlorophenyl)benzyl)desleucyl-A82846B as a white powder. FAB-MS: calc. for $C_{79}H_{84}Cl_3N_9O_{25}$ 1663.5, obtained 1666.4 (M+3).

Preparation of Compound of Example 4, from Parent Antibiotic without Isolation of Thiourea Intermediate

N^{DISACC}-(p-Phenylbenzyl)A82846B (41.0 mg, 0.0233 mmol) was dissolved in 4 ml H_2O - pyridine (1:1 v/v) and treated with phenyl isothiocyanate (0.0040 ml, 0.033 mmol). The resulting mixture was stirred at room temperature for 3 hr at which time HPLC analysis indicated complete consumption of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude thiourea intermediate as a white solid. The thiourea derivative was then suspended in 10 ml CH_2Cl_2, cooled to 0 °C, then treated with trifluoroacetic acid (0.25 ml). After 30 minutes the reaction mixture was warmed to room temperature and stirred

an additional 1 hr. The solvent was removed *in vacuo* and the crude product was purified by preparative HPLC to give 14.0 mg (37% yield) of N^{DISACC}-(p-phenylbenzyl)desleucyl-A82846B as a white powder. FAB-MS: calc. for $C_{79}H_{85}Cl_2N_9O_{25}$ 1629.5, obtained 1632.5 (M+3).

The HPLC procedures reported in these examples were as follows:

Analytical: Reactions were monitored by analytical HPLC using a Waters C_{18} μBondapak or Novapak C_{18} column (3.9x300 mm) and UV detection at 280 nm. Elution was accomplished with a linear gradient of 5% CH_3CN - 95% buffer to 80% CH_3CN - 20% buffer over 30 minutes. The buffer used was 0.5% triethylamine in water, adjusted to pH 3 with H_3PO_4.

Preparative: Crude reaction mixtures were purified by preparative HPLC using a Waters C_{18} Nova-Pak column (40x300 mm) and UV detection at 280 nm. Elution was accomplished with a linear gradient of 5% CH_3CN - 95% buffer to 80% CH_3CN - 20% buffer over 30 minutes. The buffer used was 0.5% triethylamine in water, adjusted to pH 3 with H_3PO_4. The desired fractions were subsequently desalted with a Waters C_{18} Sep-Pak (35 cc) followed by lyophilization.

Compounds were desalted as follows. A Waters Sep-Pak cartridge was pre-wet with methanol (2-3 column volumes) then conditioned with water (2-3 column volumes). The sample, dissolved in a minimum volume of water, was loaded onto the Sep-Pak column which was then washed with water (2-3 column volumes) to remove the unwanted salts. The product was then eluted with an appropriate solvent system, typically 1:1 CH_3CN/H_2O, CH_3CN, and/or methanol. The organic solvent component was removed *in vacuo* and the resulting aqueous solution lyophilized to give the final product.

Representative compounds of the present invention are listed in the following table.

TABLE I

Ex #	Name	FAB-MS	M+X	Analytical HPLC, min
1	DESLEUCYL-A82846B	1466.7	3	4.6*
2	N^{DISACC}-(p-CHLOROBENZYL)-DESLEUCYL-A82846B	1590.5	3	14.3*
3	N^{DISACC}-(p-PHENOXYBENZYL)-DESLEUCYL-A82846B	1648.3	3	17.4*
4	N^{DISACC}-(p-PHENYLBENZYL)-DESLEUCYL-A82846B	1632.5	3	17.1*
5	N^{DISACC}-(p-n-BUTYLBENZYL)-DESLEUCYL-A82846B	1612.5	3	13.6**
6	N^{DISACC}-(p-(p-CHLOROPHENYL)-BENZYL)DESLEUCYL-A82846B	1666.4	3	14.2**
7	N^{DISACC}-DES-EPIVANCOSAMINYL-DESLEUCYL-A82846B	1323.4	2	8.5**
8	N^{DISACC}-(8-PHENYL-n-OCTYL)DESLEUCYL-A82846B	1654.6	3	16.1**

*Waters C_{18} NOVA column

**Waters C_{18} μBondapak

The compounds of the present invention are useful for the treatment of bacterial infections. Therefore, in another embodiment, the present invention is directed to a method for controlling a bacterial infection in a host animal, typically a warm-blooded animal, which comprises administering to the host animal an effective, antibacterial amount of a compound of the present invention. In this embodiment, the compounds can be used to control and treat infections due to various bacteria, but especially gram-positive bacteria. In a preferred embodiment, the compounds are used to control and treat infections due to bacteria

resistant to existing antibacterials. For example, certain bacteria are resistant to methicillin, and yet others are resistant to vancomycin and/or teicoplanin. The present compounds provide a technique for controlling and treating infections due to such resistant bacterial species.

In carrying out this embodiment of the invention, the compounds of the present invention can be administered by any of the conventional techniques, including the oral route and parenteral routes such as intravenous and intramuscular. The amount of compound to be employed is not critical and will vary depending on the particular compound employed, the route of administration, the severity of the infection, the interval between dosings, and other factors known to those skilled in the art. In general, a dose of from about 0.5 to about 100 mg/kg will be effective; and in many situations, lesser doses of from about 0.5 to about 50 mg/kg will be effective. A compound of the present invention can be administered in a single dose, but in the known manner of antibacterial therapy, a compound of the present invention is typically administered repeatedly over a period of time, such as a matter of days or weeks, to ensure control of the bacterial infection.

Also in accordance with known antibacterial therapy, a compound of the present invention is typically formulated for convenient delivery of the requisite dose. Therefore, in another embodiment, the present invention is directed to a pharmaceutical formulation comprising a compound of the present invention, in combination with a pharmaceutically-acceptable carrier. Such carriers are well known for both oral and parenteral routes of delivery. In general, a formulation will comprise a compound of the present invention in a concentration of from about 0.1 to about 90% by weight, and often from about 1.0 to about 3%.

The antibacterial efficacy of the present compounds is illustrated by the table. The minimal inhibitory concentrations (MICs) were determined using a standard broth micro-dilution assay.

TABLE II. Antibacterial Activity, Minimal Inhibitory Concentration (MIC) against Various Organisms*

Ex #	Resistant	Sensitive	SA 446	SA 489	SA 447	SH 105	SH 415	SE 270	SPY C203	SPN P1
1	>128	>128	64	32	64	>64	>64	64	4	
2	>81	5.2	4	0.5	2	2	4	2	≤0.06	
3	45	14	2	1	1	2	1	0.5	≤0.06	≤.06
4	16	3.4	2	0.25	1	1	1	1	≤0.06	0.125
5	108	9.2	1	1	2	1	2	1	0.125	0.25
6	6.7	2.6	2	2	1	0.5	1	0.5	≤.06	0.25
7	>128	56	32	8	64	64	>64	>64	4	2
8	5.7	0.76	4	1	2	0.25	2	1	0.125	0.125

*

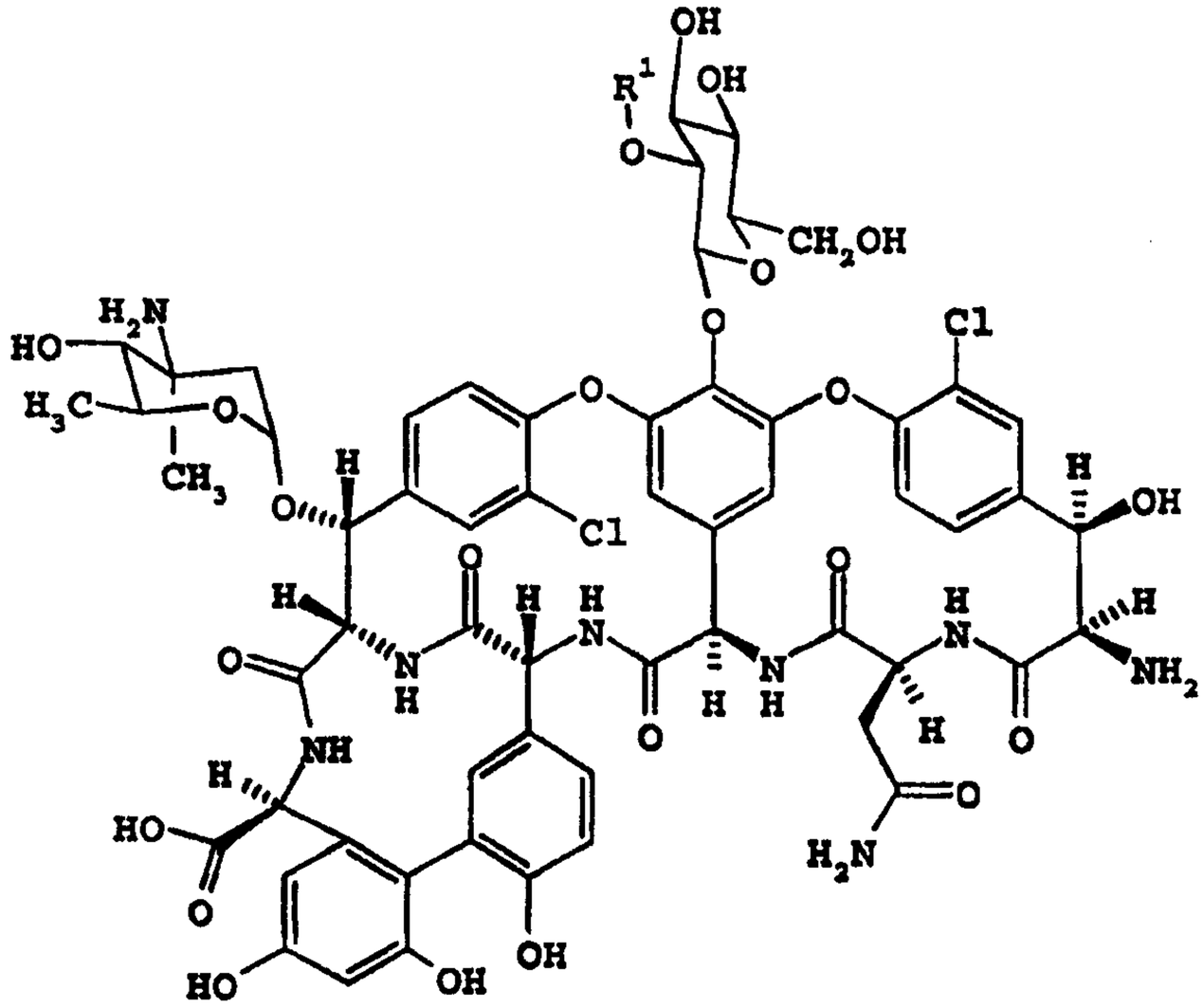
ABBREVIATIONS	ORGANISM
RESISTANT	*Enterococcus faecium* and *faecalis* (geometric mean of 4-6 isolates)
SENSITIVE	*Enterococcus faecium* and *faecalis* (geometric mean of 4-6 isolates)
SA446	*Staphylococcus aureus* 446
SA489	*Staphylococcus aureus* 489
SA447	*Staphylococcus aureus* 447
SH 105	*Staphylococcus haemolyticus* 105
SH 415	*Staphylococcus haemolyticus* 415
SE 270	*Staphylococcus epidermidis* 270
SPY C203	*Streptococcus pyogenes* C203
SPN P1	*Streptococcus pneumoniae* P1

The compounds of the present invention can also be employed as starting materials to other antibacterial compounds. More particularly, the present hexapeptides can be reacted to introduce an alkyl group on the free "N^1" amine.

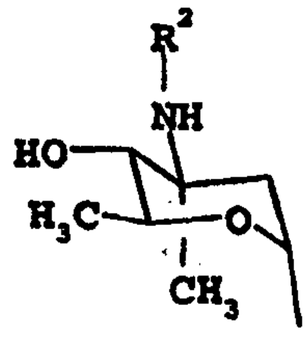
Alkylation is achieved by reacting the hexapeptide with an aldehyde to form a Schiff's base, which is then reduced to obtain the N^1-alkylhexapeptide. Both reactions are carried out in a polar solvent, such as DMF, and at temperatures of 0-100°C, preferably 60-70°C. The preferred reducing agent is sodium cyanoborohydride. In one embodiment, the reducing agent is added at the same time as the hexapeptide and aldehyde. The resulting N^1-alkylated hexapeptides are useful as antibacterials and can be employed as described above for the present compounds.

WE CLAIM:

1. A compound of the formula



wherein R^1 represents an epivancosaminyl radical of the formula



wherein R^2 represents $-CH_2-R^3$; and

wherein R^3 represents

alkyl of C_1-C_{11},

alkyl of C_1-C_{11}-R^4,

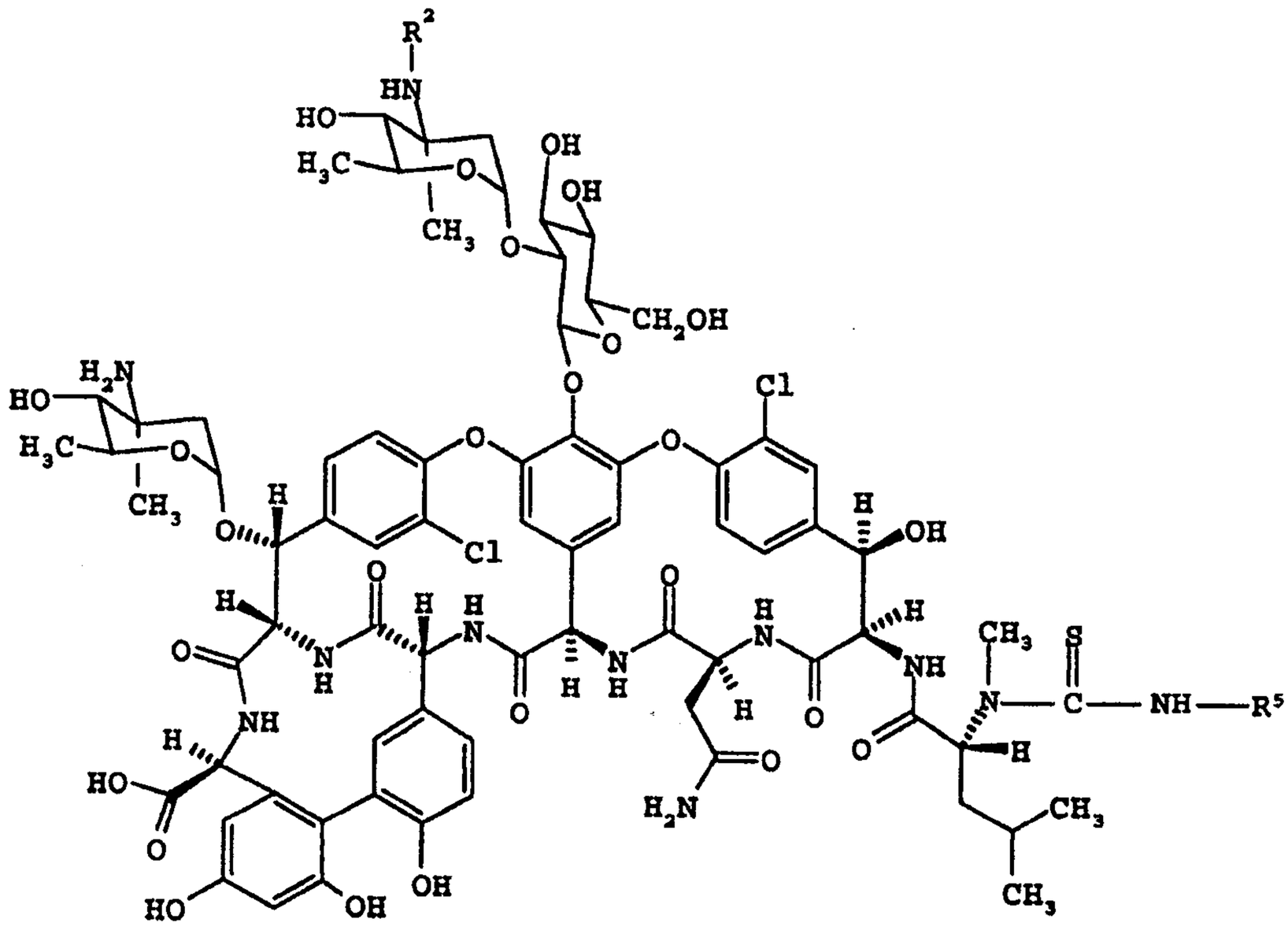
R^4- ($0_{(0 \text{ or } 1)}$-R^4) $_{0 \text{ or } 1}$,

wherein each R^4 is independently phenyl or phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, or loweralkylthio of C_1-C_4, or a pharmaceutically acceptable salt thereof.

2. The compound of Claim 1 in which $-CH_2-R^3$ is p-(p-chlorophenyl)benzyl.

3. A pharmaceutical formulation comprising the compound of Claim 1 or 2 in combination with a pharmaceutically acceptable diluent or carrier.

4. A process for the preparation of the compound as claimed in Claim 1 which comprises treating a compound of the formula



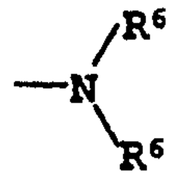
wherein R^2 is as defined in Claim 1, and R^5 represents

alkyl of C_1-C_{10},

phenyl,

naphthyl, or

phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, benzyloxy, nitro, or



wherein each R^6 is independently loweralkyl of C_1-C_4, with an organic acid in a non-polar solvent, and if desired, thereafter forming a pharmaceutically acceptable salt.

5. Use of an effective amount of the formulation of Claim 3 for treating a gram-positive bacterial infection in a host in need thereof.

6. The use of Claim 5, wherein the gram-positive bacterial infection is attributable to a vancomycin-resistant-enterococcus.

7. The use of Claim 5, wherein the gram-positive bacterial infection is attributable to an *Enterococcus* or to a *Staphylococcus* species.

8. Use of an effective amount of the compound of Claim 1 or 2, for treating a gram-positive bacterial infection in a host in need thereof.

9. The use of Claim 8, wherein the gram-positive bacterial infection is attributable to a vancomycin-resistant-enterococcus.

10. The use of Claim 8, wherein the gram-positive bacterial infection is attributable to an *Enterococcus* or to a *Staphylococcus* species.