

PATENT SPECIFICATION

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(54) NOVEL PSEUDOTRISACCHARIDES AND METHODS FOR THEIR PRODUCTION

(71) We, SCHERICO LTD., of Topferstrasse 5, Lucerne, Switzerland, a body corporate constituted under the laws of Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel pseudotrisaccharides and methods for their production, and to methods for their use as antibacterial agents.

More specifically, this invention relates to novel, antibacterially active 1-*N*-substituted derivatives of 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, to methods for their production, to pharmaceutical compositions comprising said 1-*N*-substituted derivatives of 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols and to methods for their use in treating bacterial infections.

Known in the art are broad spectrum antibacterial agents which may be classified chemically as 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols. Valuable antibacterial agents of this group are those wherein the aminocyclitol is 2-deoxystreptamine or a derivative thereof having amino functions at positions 1 and 3. Particularly valuable antibacterials of the 4,6-di-(aminoglycosyl)-2-deoxystreptamines are those wherein the aminoglycosyl group at the 6-position is a garosaminyl radical. Within the class of 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines are antibiotics such as gentamicins B, B₁, C₁, C_{1a}, C₂, C_{2a}, C_{2b} and X₂; sisomicin, verdamycin, Antibiotic G-418, Antibiotic G-52, Antibiotic JI-20A and Antibiotic JI-20B.

This invention relates to compounds, wherein the amino group at the 1-position of the 2-deoxystreptamine or derivative thereof in a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol is selectively *N*-substituted. The 1-*N*-substituted derivatives of the 4,6-di-(aminoglycosyl)-2-deoxystreptamines or derivatives thereof are valuable broad spectrum antibacterial agents.

In one of its product aspects, the invention relates to 1-*N*-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamycin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6; wherein the substituent is —CH₂X with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzy or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and the pharmaceutically acceptable acid addition salts thereof.

Included among the substituents contemplated for the moiety CH₂X in the novel compounds are straight and branched chain alkyl groups such as ethyl, *n*-propyl, *n*-butyl, β -methylpropyl, *n*-pentyl, β -methylbutyl, γ -methylbutyl and β , β -dimethylpropyl; *n*-hexyl, δ -methylpentyl, β -ethylbutyl, γ -ethylbutyl, *n*-heptyl, ϵ -methylheptyl, β -ethylpentyl, γ -ethylpentyl, δ -ethylpentyl, γ -propylbutyl, *n*-octyl, *iso*-octyl, β -ethylhexyl, δ -ethylhexyl, ϵ -ethylhexyl, β -propylpentyl, γ -propylpentyl; alkenyl groups such as β -propenyl, β -methylpropenyl, β -butenyl, β -methyl- β -butenyl, β -ethyl- β -hexenyl;

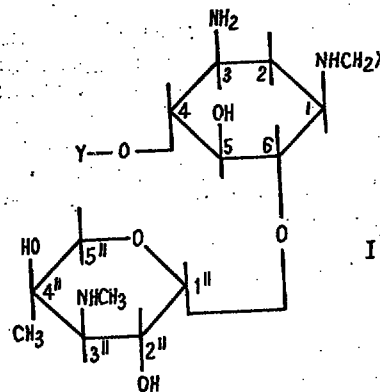
cyclic groups such as cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl and cyclopentylethyl; aromatic groups such as *o*-, *m*-, *p*-methylbenzyl;

hydroxy substituted straight and branched chain alkyl groups such as ϵ -hydroxypentyl, β -hydroxy- γ -methylbutyl, β -hydroxy- β -methylpropyl, δ -hydroxybutyl, β -hydroxypropyl, γ -hydroxypropyl, ω -hydroxyoctyl; amino substituted straight and branched chain alkyl groups such as ϵ -aminopentyl, β -aminopropyl, γ -aminopropyl, δ -aminobutyl, β -amino- γ -methylbutyl and ω -aminooctyl and mono-*N*-alkylated derivatives thereof such as the *N*-methyl, *N*-ethyl and *N*-propyl derivatives, e.g. ϵ -methylaminopentyl, β -methylaminopropyl, β -ethylaminopropyl, δ -methylaminobutyl, β -methylamino- γ -methylbutyl and ω -methylaminobutyl; amino and hydroxy disubstituted straight and branched chain alkyl groups such as β -hydroxy- ϵ -aminopentyl, γ -hydroxy- γ -methyl- δ -aminobutyl, β -hydroxy- δ -aminobutyl, β -hydroxy- γ -aminopropyl, and β -hydroxy- β -methyl- γ -aminopropyl; and mono-*N*-alkylated derivatives thereof such as β -hydroxy- ϵ -methylaminopentyl, γ -hydroxy- γ -methyl- δ -methylaminobutyl, β -hydroxy- δ -methylaminobutyl, β -hydroxy- γ -ethylaminopropyl and β -hydroxy- β -methyl- γ -methylaminopropyl.

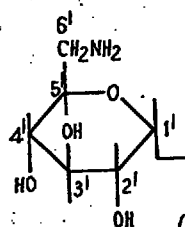
The compounds are preferably 1-*N*-CH₂X-derivatives containing garosaminyl as 6-aminoglycoside radical and, in a more preferred feature, 2-deoxystreptamine as 1,3-diaminocyclitol.

The 2-deoxystreptamine is present in all the above listed compounds of the invention except the mutamicins. The 1,3-diaminocyclitol nucleus in each of the 1-*N*-CH₂X-mutamicins 1, 2, 3, 4, 5 and 6 are streptamine, 2,5-dideoxystreptamine, 2-*epi*-streptamine, 5-amino-2,5-dideoxystreptamine and 5-*epi*-2-deoxystreptamine, respectively.

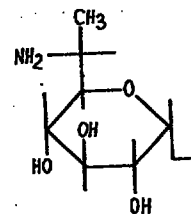
The 1-*N*-CH₂X-4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines of this invention are the derivatives from gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B and Antibiotic G-52 which compounds are defined by the following structural formula I:



wherein X is as defined hereinabove, and wherein Y is an aminoglycosyl function selected from



(in 1-*N*-CH₂X-gentamicin B)



(in 1-*N*-CH₂X-gentamicin B₁)

hyl and

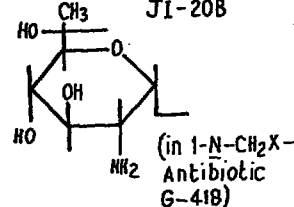
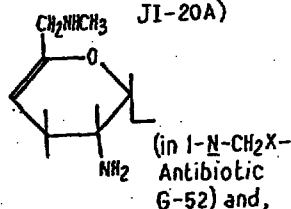
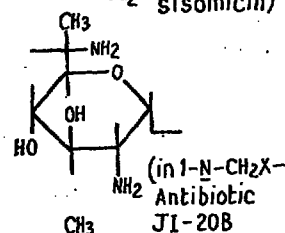
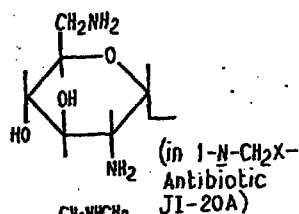
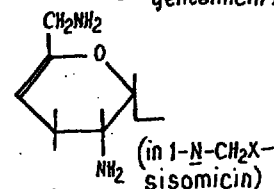
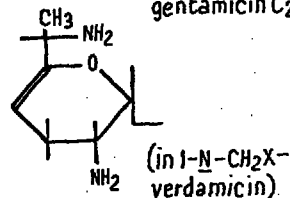
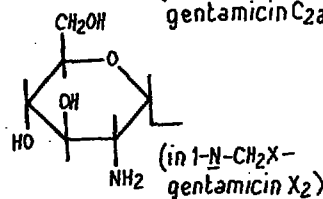
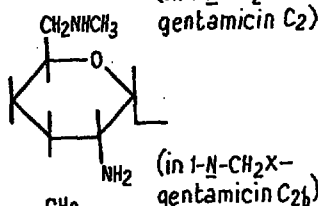
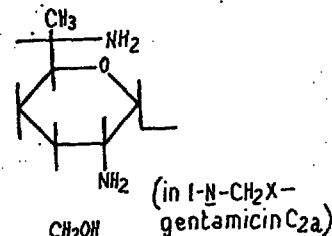
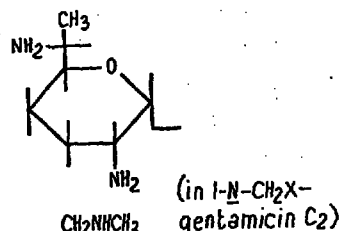
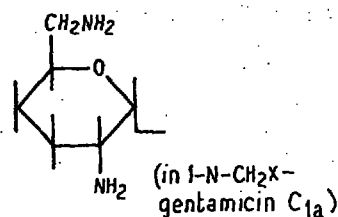
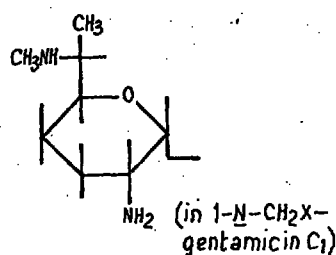
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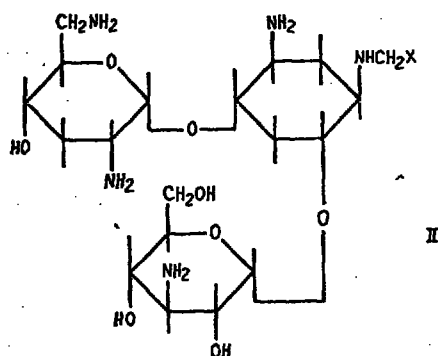
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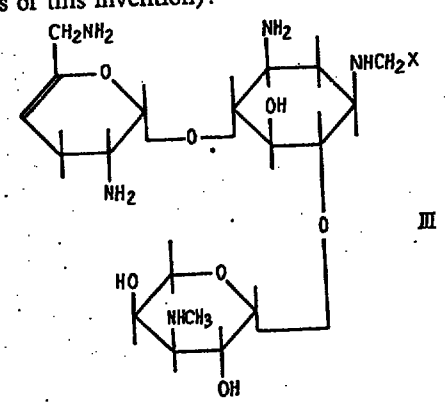
Other useful 1-N-CH₂X-4,6-di(aminoglycosyl)-2-deoxy-streptamines of this invention include 1-N-CH₂X-tobramycin of the following formula II:



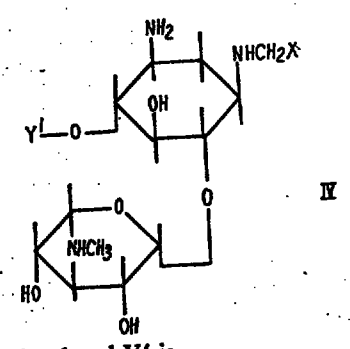
wherein X is as hereinabove defined;

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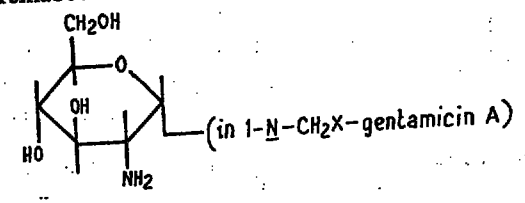
1-N-CH₂X-Antibiotic 66-40D of the following formula III (which are among the preferred compounds of this invention):



5 wherein X is as hereinabove defined; and 1-N-CH₂X-gentamicin A and 1-N-CH₂X-Antibiotic 66-40B of the following formula IV:

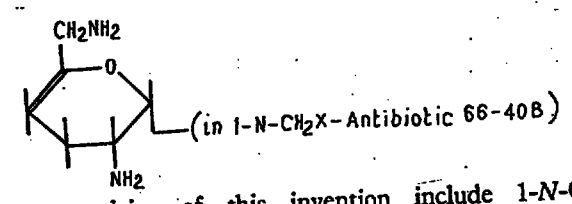


wherein X is as hereinabove defined and Y' is

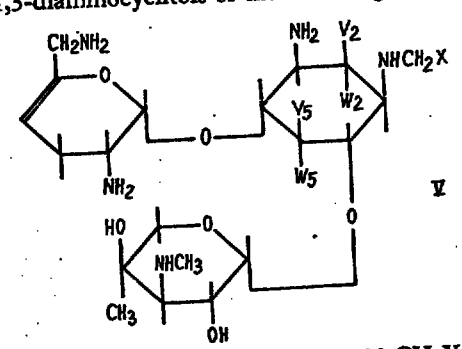


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and Y' is



The 1-N-CH₂X-mutamicins of this invention include 1-N-CH₂X-4-amino-glycosyl-6-garosaminyl-1,3-diaminocyclitols of the following formula V:



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wherein X is as hereinabove defined, and in 1-N-CH₂X-mutamicin 1, V₂ and W₂ are hydroxy and W₅ and V₅ are hydroxy

are among

in 1-N-CH₂X-mutamicin 2, W₂, V₂, W₃ and V₃ are hydrogen;
in 1-N-CH₂X-mutamicin 4, W₂ and W₃ are hydrogen and V₂ and V₃ are hydroxy;
in 1-N-CH₂X-mutamicin 5, W₂, V₂ and W₃ are hydrogen and V₃ is amino; and
in 1-N-CH₂X-mutamicin 6, W₂, V₂ and V₃ are hydrogen while W₃ is hydroxy.

(In the structural formulae set forth herein, the undesignated substituents at the bond terminals are understood to be hydrogen atoms).

Also included with in the invention are pharmaceutically acceptable acid addition salts of the 1-N-CH₂X-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols such as defined by formulae I, II, III, IV and V, which salts are made according to known procedures such as by neutralizing the free base with the appropriate acid usually to about pH 5. Suitable acids for this purpose include acids such as hydrochloric, sulfuric, phosphoric, nitric, hydrobromic, acetic, propionic, maleic, ascorbic, citric and the like. The physical embodiments of the acid addition salts of the 1-N-CH₂X-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols of this invention are characterized by being white solids which are soluble in water, sparingly soluble in most polar solvents and insoluble in non-polar organic solvents.

The 1-N-CH₂X-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols of this invention such as defined by formulae I, II, III, IV and V and their non-toxic pharmaceutically acceptable acid addition salts, in general, exhibit broad spectrum antibacterial activity. Particularly the 1-N-lower alkyl derivatives possess improved antibacterial activities compared to the parent antibiotics which is specifically manifest in the enhanced activity of the compounds against organisms resistant to the parent compound. Thus, for example, the compounds are more active against organisms resistant to the parent compound. Thus, for example, the compounds are more active against organisms which inactivate the parent antibiotics by acetylation of the 3-amino group and/or by adenylation of the 2''-hydroxyl group. Of these, some also exhibit anti-protozoal, antiamebic and anthelmintic properties.

A preferred group of compounds of the invention are the 1-N-substituted derivatives of the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin X₂, sisomicin, verdamicin, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, and Antibiotic G-418, of which the derivatives of gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamicin and Antibiotic G-52 are most preferred. Other particularly useful compounds are the 1-N-substituted derivatives of Antibiotic 66-40D.

In the 1-N-substituent X is preferably selected from hydrogen, alkyl, hydroxy-alkyl, aminoalkyl, aminohydroxyalkyl, phenyl or benzyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms. Of those the preferred radicals are hydrogen, alkyl, aminoalkyl, and hydroxyalkyl with up to seven carbon atoms and amino-hydroxyalkyl with up to three carbon atoms and bearing the substituents on different carbon atoms.

Particularly useful compounds of the invention are those wherein X is hydrogen, methyl, ethyl and propyl and preferably methyl and ethyl. A particularly valuable group are the 1-N-CH₂X-4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines of formula I wherein X is a lower alkyl having 1 to 3 carbon atoms, particularly the 1-N-lower alkyl derivatives of gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, sisomicin, verdamicin, and Antibiotic G-52 as well as 1-N-lower alkyl-Antibiotic 66-40D of formula III, which derivatives are broad spectrum antibacterial agents, being active against gram-positive bacteria (e.g. *Staphylococcus aureus*) and gram-negative bacteria (e.g. *Escherichia coli* and *Pseudomonas aeruginosa*) as determined by standard dilution tests, including bacteria resistant to the 1-N-unsubstituted precursors. Particularly useful are 1-N-ethylverdamicin, and 1-N-lower alkylsisomicins, e.g. 1-N-methylsisomicin, 1-N-(n-propyl)-sisomicin, 1-N-(n-butyl)-sisomicin and, preferably, 1-N-ethylsisomicin which exhibit activity against gram-negative organisms which are resistant to their 1-N-unsubstituted precursors. Other particularly useful compounds are 1-N-ethylgentamicin C_{1a}, 1-N-ethylgentamicin C₁, 1-N-ethylantibiotic G-52, 1-N-(n-propyl)-verdamicin, 1-N-(8-aminobutyl)-sisomicin, 1-N-methylverdamicin, 1-N-(n-butyl)verdamicin, 1-N-(S-2-hydroxy-4-aminobutyl)-gentamicin C₁, 1-N-(S-2-hydroxy-4-aminobutyl)-sisomicin and 1-N-(S-2-hydroxy-4-aminobutyl)-verdamicin.

In another of its product aspects the invention relates to 1-N-acylated derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, i.e. compounds of formulae I, II, III, IV and V, wherein in position 1 the substituent

he following

CH₂X-4-amino-

nicin 1, V₂ and



is substituted for substituent $-\text{CH}_2\text{X}$. The amide compounds are intermediates in the preparation of the compounds of formulae I to V and exhibit broad spectrum antibacterial activity. The amide compounds are 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamycin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl, or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, with the proviso that Z together with the carbonyl groups to which it is attached is other than S-3-amino-2-hydroxypropionyl or S-4-amino-2-hydroxybutyryl and, in case of tobramycin and gentamicin C₂, also other than S-5-amino-2-hydroxyvaleryl.

In a more limited manner the amides may be defined as 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols listed above wherein Z is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl with at least 5 carbon atoms, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms.

Included among the substituents contemplated for the moiety



in the novel amide compounds are most of the radicals listed above for the moiety $-\text{CH}_2\text{X}$, wherein the terminal methylene group is replaced by carbonyl, such as formyl, acetyl, propionyl, propenoyl, butyryl, isobutyryl, cyclopropylcarbonyl, benzoyl, hydroxyacetal, aminoacetyl, 4-hydroxybutyryl, 4-aminobutyryl and the like.

A preferred group of amide compounds are derivatives of the 4-aminoglycosyl-6-garoaminyl-2-deoxystreptamines gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamycin, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B and Antibiotic G-52 of which the more preferred derivatives are those of gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamycin and Antibiotic G-52.

On account of their antibacterial activity, the derivatives of sisomicin, verdamycin and Antibiotic 66-40D and especially the acetyl, propionyl and butyryl derivatives are most preferred. 1-N-Acetylsisomicin, 1-N-acetylverdamycin, 1-N-acetylantibiotic G-52 and 1-N-acetylgentamicin C_{1a} are examples of particularly useful compounds.

The invention also embraces the pharmaceutically acceptable acid addition salts of the 1-N-acylated compounds such as those formed with hydrochloric, sulfuric, phosphoric, propionic, maleic, tartaric, benzoic, phenylacetic, cyclopropane carboxylic acids or the like. These salts may be prepared by dissolving the free nitrogen base in water, adjusting the solution of the antibacterial agent to pH 4.0 and lyophilizing the resultant solution.

Most of the aforementioned 1-N-unsubstituted 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol antibiotics from which the 1-N-substituted derivatives of the invention may be made are known. Of the gentamicins, the starting compound referred to herein as gentamicin X₂ is also known in the art as gentamicin X. The starting compound referred to herein as gentamicin C_{2a} is isolated and characterized as set forth herein in Preparation 1.

The starting compound referred to herein as gentamicin C_{2b} is isolated and

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ramicin C₂,
antibiotic 66-
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wherein the

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glycosyl)-1,3-di-
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is isolated and

characterized as set forth in Preparation 2, and having the structural formula shown herein, is named in some prior art as gentamicin C_{2a}.

The isolation, properties, and planar configuration of gentamicin C₂ is described in U.S. Patent No. 3,651,042.

Antibiotics 66-40B and 66-40D, their preparation, isolation, properties, and configuration, are described in Belgian Patent No. 811,370. The antibiotics are co-produced with sisomicin, the major product of fermentation of *Micromonospora inyoensis* (described in British Patent No. 1,274,518) and may be separated from the fermentation medium by applying special chromatographic separation techniques.

Mutamycins 1, 2, 4, 5, and 6, the configuration of which is shown above, may be prepared by cultivating a mutant strain of *Micromonospora inyoensis* herein designated *Micromonospora inyoensis* strain 1550F-1G in an aqueous nutrient medium. This mutant strain is incapable of producing an antibiotic when cultivated under submerged aerobic conditions in an aqueous nutrient medium absent the 1,3-diaminocyclitol building block. However, when certain of such compounds are added to the fermentation medium, the mutamycins are produced. When 2-deoxystreptamine is added to the fermentation, the known antibiotic sisomicin is produced.

The requisite 1,3-diaminocyclitols which must be present during fermentation in order to obtain the mutamycins are as follows:

- streptamine for mutamicin 1
- 2,5-dideoxystreptamine for mutamicin 2
- 2-epistreptamine for mutamicin 4
- 2,5-dideoxy-5-aminostreptamine for mutamicin 5
- 5-epi-2-deoxystreptamine for mutamicin 6.

Streptamine is a known compound, 2,5-dideoxystreptamine may be prepared according to the procedure set forth in Preparation 6 by substituting hydrazine for monomethylhydrazine. 2-Epistreptamine may be prepared by the method described by Tetsue Suami et al. in the Journal of Organic Chemistry 33, No. 7, 2831-2834 (1968). 2,5-Dideoxy-5-aminostreptamine may be prepared by

- i) cleaving an N,N-diacyl derivative of 2-deoxystreptamine with periodate,
- ii) treating the so-obtained dialdehyde with nitromethane under alkaline conditions which effects ring closure and the insertion of a nitro group at the 5-position,
- iii) reducing the nitro group to an amino group followed by hydrolysis of the N,N-diacyl functions to free amine.

5-Epi-2-deoxystreptamine may be prepared from 1,3,4,6-tetraacetyl-5-epi-2-deoxystreptamine (prepared by the method of Hasagawa and Sable, Tetrahedron, 25, 35-67 (1969)) by hydrolyzing the ester and amide groups with 6N hydrochloric acid. The preparation of the mutamycins is set forth herein in Preparation 10.

Taxonomical, Biochemical and Morphological Properties of *Micromonospora inyoensis* Strain 1550F-1G (NRRL 5742)

The parent microorganism (*Micromonospora inyoensis*) is deposited with U.S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois, where it was assigned the numerical designation NRRL 3292, and is described in the Journal of Antibiotics (Japan) Vol. XXIII, No. 11, pages 551-558 (1970) in a publication by M. J. Weinstein, et al.

Micromonospora inyoensis strain 1550F-1G exhibits growth characteristics that are similar to *Micromonospora inyoensis* (the microorganism from which it is derived). A culture of *M. inyoensis* strain 1550F-1G is also deposited with the above-mentioned depository under the numerical designation NRRL 5742.

The following tables set forth a number of taxonomical, biochemical and morphological properties of the microorganism. In describing the color formations the following system and references are employed: The color designation consists of two designates. The first is a color name taken from the "Descriptive Color Name Dictionary," by Taylor, Knoche and Granville, published by the Container Corporation of America, 1950 (U.S.A.), with a color chip number corresponding to the color name; said chip number taken from the "Color Harmony Manual", 4th edition, 1958, published by the Container Corporation of America, U.S.A. The second designate, consists of a color-name and number which refers to the synonym or near synonym found in the National Bureau of Standards, Circular 553, November 1, 1955 (U.S.A.).

When cultivated on an agar medium comprising 3% NZ amine Type A (a peptone resulting from the enzymatic digestion of casein and serving as a source of nitrogen), 1% dextrose and 1.5% agar the microorganism *M. inyoensis* strain 1550F-1G NRRL 5742 shows such a poor growth that it is insufficient for characterization.

TABLE I

Colony Description of Micromonospora Inyoensis Strain 1550F-1G NRRL 5742 on Various Media or conditions	
Medium or Condition	Observations
Sucrose	Utilised
Temperature	Grows well at 28° and 37° No growth at 50°C
Aerobic or Anaerobic	Aerobic
Czapeks Medium (Glucose)	Growth fair to poor, plicate — membranous, no diffusible pigment g3ic light amber — dark orange yellow 72
Asparagine-Glucose Medium	Growth fair to poor, flat to membranous, no diffusible pigment g3ic light amber — dark orange yellow 72
Calcium Malate Agar	Growth poor, flat, no diffusible pigment g3ic light amber — dark orange yellow 72
Nitrate Reduction	Variable
Ordinary Agar (Water Agar)	Growth poor, insufficient for description
Nutrient Agar	Growth fair to poor, flat to slightly wrinkled g4ne luggage tan — strong brown 55 to g4pn chocolate brown — dark brown 59
Löffler's Serum Medium (Difco)	Growth fair, substrate partially liquefied g5pe terracotta — strong brown 55

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(17)

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TABLE 1 (Continued)

Medium or Condition	Observations
Potato Plug	No growth
Peptone Glucose Agar	Growth fair to poor, flat to slightly furrowed, no diffusible pigment produced g4ne luggage tan - strong brown 55
Egg Agar (Dorset Egg Medium-Difco)	Growth poor, insufficient for characterization
Gelatin Medium	Growth fair to poor, flat to slightly ridged, no diffusible pigment produced, gelatin weekly hydrolysed g4le turf tan - light brown 57
Starch Agar	Growth fair, flat, no diffusible pigment, starch weakly hydrolyzed only directly under colony g3le yellow maple - strong yellowish brown 74 to black
Tyrosine Medium	Growth fair to poor, flat, slight darkening of medium g4le turf tan - light brown 57
Litmus Milk (Difco)	Peptonized, acid reaction
Cellulose Medium	Cellulose poorly decomposed (hydrolysis of cellulose weak)
Bennett's Agar	Growth good, membranous - plicate, no diffusible pigment black
Emerson's Agar	Growth good, membranous, no diffusible pigment g3ni Clove brown, dark yellowish brown 78

TABLE 1 (Continued)

	Medium or Condition	Observations
5	Tomato Paste Oatmeal Agar	Growth fair, raised, ridged, no diffusible pigment g4nc russet orange, strong orange 50
	Glucose Yeast Extract Agar	Growth good, membranous, no diffusible pigment black
10	Potato Slice	+ CaCO ₃ , +++ growth, black - CaCO ₃ , no growth
	Tyrosine - Agar Yeast Ext.	Growth good, membranous, crystals dissolved, light brown diffusible pigment produced
15	Tyrosine - Beef Extract Growth fair to poor crystals weakly dissolved, brownish diffusible pigment produced only on cross-hatched method	Observations at 2, 7 and 14 days (after Gordon and Smith, J. Bact. 69:147 (1955))
20	Peptone Iron Agar Observations at 2, 7 and 14 days	No growth, no reaction

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TABLE II

Utilization of Nitrogen Sources by *Micromonospora Inyoensis*
Strain 1550F-G NRRL 5742

Nitrogen Source
+ 1% glucose

Observations

0.5% Difco
Yeast Extract

Growth good, membranous, no
diffusible pigment produced
black

1.0% NZ Amine
Type A

Growth good, membranous, plicate
g4nc russet orange, strong
orange 50 to black

1% Asparagine

Growth poor, insufficient for
description

1% Glutamic Acid

Growth poor, insufficient for
description

1% Sodium Nitrate

Growth poor, insufficient for
description

1% Ammonium Nitrate

Growth poor, insufficient for
description

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TABLE III

Utilization of Carbohydrates by <i>Micromonospora Inyoensis</i> Strain 1550F-1G NRRL 5742	
	Growth
5 Control	± poor
D-Arabinose	± poor
L-Arabinose	± poor
Dulcitol	± poor
D-Galactose	+ fair
10 D-Glucose	++ good
Glycerol	± poor
I-Inositol	± poor
D-Lactose	± poor
D-Levulose	+ fair
15 D-Mannitol	± poor
Mannose	+++ good
Melibiose	— ± poor
Melizitose	± poor

TABLE III (Continued)

	Growth
Raffinose	± poor
L-Rhamnose	± poor
D-Ribose	+ fair
Salicin	± poor
Sucrose	+ fair
D-Xylose	++ good

In one of its process aspects the invention embraces a process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with X being as defined above, and of the pharmaceutically acceptable acid addition salts thereof, which process comprises treating one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which may have amino-protecting groups at any position other than position 1, with an aldehyde of the formula



with X' being a group as defined for X above wherein any amino or hydroxy group present may be protected, in the presence of a hydride donor-reducing agent, and, if required removing all protecting groups present in the molecule the last process step being followed by isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

This process, whereby the 1-amino function in a 1-N-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol is selectively condensed with an aldehyde and concomitantly reduced *in situ* to form a 1-N-alkyl-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol antibacterial agent, is usually carried out at room temperature in the presence of air, although it may be advantageously carried out under an inert atmosphere (e.g. argon or nitrogen). Advantageously, the reaction is completed within a short time, usually less than 30 minutes, as determined by thin layer chromatography.

Hydride-donor reducing agents useful in this process include dialkylaminoboranes (e.g. dimethylaminoborane, diethylaminoborane and preferably morpholinoborane), tetraalkylammonium cyanoborohydride (e.g. tetrabutylammonium cyanoborohydride), alkali metal borohydride (e.g. sodium borohydride) and preferably, alkali metal cyanoborohydride (e.g. lithium cyanoborohydride and sodium cyanoborohydride).

The process is conveniently carried out in an inert solvent. By "inert solvent" is meant any organic or inorganic solvent in which the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol starting compounds and the reagents are soluble, and which will not interfere with the process under the reaction conditions thereof so there are produced a minimum of competing side reactions. Although anhydrous aprotic solvents may sometimes advantageously be employed in the process (such as tetrahydrofuran when

utilizing morpholinoborane as hydride donor-reducing agent) the process is usually carried out in protic solvents, e.g. in a lower alkanol or, preferably, in water or in an aqueous lower alkanol (e.g. aqueous methanol, aqueous ethanol), although other water-miscible co-solvent systems may be employed such as aqueous dimethylformamide, aqueous hexamethylphosphoramide, aqueous tetrahydrofuran and aqueous ethylene glycol dimethyl ether.

The process is conveniently carried out at a pH in the range of from 1 to 11, preferably from 2 to 5 and proceeds best in the range of from 2.5 to 3.5. The acidic medium which is preferred may be obtained by adding an organic or inorganic acid to the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol. Thereby acid addition salts of the compounds are formed. Any organic acid such as acetic acid, trifluoroacetic acid, or *p*-toluene-sulfonic acid or inorganic acid such as hydrochloric acid, sulfuric acid, phosphoric acid, or nitric acid may be used. It is most convenient to use sulfuric acid. In the preferred feature of the process, it is also convenient to prepare the acid addition salt starting compound *in situ* by adding the desired acid (e.g. sulfuric acid) to a solution or suspension of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol (e.g. sisomicin) in a protic solvent (e.g. water) until the pH of the solution is adjusted to the desired pH.

Typical aldehydes of the formula $X'CHO$ wherein X' is as above defined which are useful in the process include straight and branched chain alkyl aldehydes such as formaldehyde, acetaldehyde, *n*-propanal, *n*-butanal, 2-methylpropanal, *n*-pentanal, 2-methylbutanal, 3-methylbutanal, 2,2-dimethylpropanal, *n*-hexanal, 2-ethylbutanal, *n*-heptanal and *n*-octanal; alkenyl aldehydes such as propenal, 2-methylpropenal, 2-butenal, 2-methyl-2-butenal, 2-ethyl-2-hexenal; cyclic aldehydes such as cyclopropanecarbaldehyde, cyclopentanecarbaldehyde, cyclopentaneacetaldehyde, cyclohexanecarbaldehyde; benzaldehyde, *o*, *m*, and *p*-toluenecarbaldehydes and phenylacetaldehyde; hydroxy substituted straight and branched chain alkyl aldehydes such as 5-hydroxypentanal, 2-hydroxy-3-methylbutanal, 2-hydroxy-2-methylpropanal, 4-hydroxybutanal, 2-hydroxypropanal and 8-hydroxyoctanal; amino substituted straight and branched chain alkyl aldehydes such as 5-aminopentanal, 2-aminopropanal, 3-aminopropanal, 4-aminobutanal, 2-amino-3-methylbutanal, 8-aminooctanal and mono-*N*-alkyl derivatives thereof; and amino and hydroxy disubstituted straight and branched chain alkyl aldehydes such as 2-hydroxy-5-aminopentanal, 3-hydroxy-3-methyl-4-aminobutanal, 2-hydroxy-4-aminobutanal, 2-hydroxy-3-aminopropanal, 2-hydroxy-2-methyl-3-aminopropanal, 2-amino-3-hydroxyoctanal, and mono-*N*-alkyl derivatives thereof.

In this process, if the aldehyde possesses a chiral center, one can use each enantiomer separately or together as a racemate and there will be obtained the respective diastereoisomers or a mixture thereof, respectively.

The aldehyde reagents useful in the process are either known compounds or are easily prepared from known compounds utilizing procedures well known in the art. Thus, for example, alkylaldehydes substituted by both hydroxyl and amino functions (e.g. 2-hydroxy-5-aminopentanal) may be prepared from an aminoaldehyde acetal (e.g. 4-aminobutanal diethylacetal) by protecting the amino function therein as an acetamido or phthalimido group utilizing known procedures followed by removal of the acetal function by acid hydrolysis thereby obtaining an *N*-protected aminoaldehyde (e.g. by converting 4-aminobutanal diethylacetal to the corresponding *N*-phthalimido derivative which upon acid hydrolysis yields 4-phthalimidobutanal). Treatment of the *N*-protected aminoaldehyde with hydrocyanic acid yields the corresponding *N*-protected aminoalkyl hydroxynitrile (e.g. 2-hydroxy-5-phthalimidovaleronitrile) which upon catalytic reduction (e.g. hydrogen in the presence of palladium) or by hydride reduction (e.g. with di-isobutylaluminum hydride) yields an *N*-protected amino-hydroxy aldehyde (e.g. 2-hydroxy-5-phthalimidopentanal) which is an aldehyde reagent used in this process.

When carrying out the process whereby a 1-*N*-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol is treated with a hydride donor and an aldehyde, to obtain the corresponding 1-*N*-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, in order to minimize competing side reactions when an aminoaldehyde is used as a reagent, it is preferable to protect the amino function in the aldehyde, e.g. with an acyl blocking group such as acetamido, phthalimido, or the like, prior to carrying out the process, and then removing the *N*-protecting group in the compound thereby produced. It may also be advantageous to protect the hydroxyl group in hydroxyl-containing aldehydes when carrying out the process; however, it is not generally necessary.

It is also possible to use the acetal or hemiacetal of the aldehyde reagent in acidic medium which gives rise to *in situ* formation of the required aldehyde.

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butanal, n-
ropenal, 2-
clopropane-
hexanecarb-
etaldehyde;
5-hydroxy-
oxybutanal,
d branched
propanal, 4-
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A convenient method of carrying out the process comprises preparing a solution of a 1-N-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol antibacterial agent (e.g. sisomicin or verdamycin) in a protic solvent, (preferably water), and adjusting the pH of the solution to from about pH 2 to about pH 5 with an acid (usually dilute sulfuric acid) thereby preparing the requisite acid addition salt of the starting compound. When the pH of the solution is at about pH 5, the acid addition salt thereby produced usually contains about one equivalent of acid for each amino function in the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol (e.g. per mole of sisomicin there is present 2.5 moles of sulfuric acid). After the acid addition salt solution is prepared, there is added at least a molar equivalent, and preferably a large molar excess of the desired aldehyde (e.g. acetaldehyde, propanal or butanal) followed within a short time (usually in about 5 minutes) by the addition of about a molar equivalent (based upon the starting 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol) of a hydride donor-reducing reagent, preferably an alkali metal cyanoborohydride, usually sodium cyanoborohydride. The reaction is frequently completed in less than 30 minutes as determined by thin layer chromatography and there is obtained the corresponding 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol (e.g. 1-N-ethylsisomicin or 1-N-ethylverdamycin). Isolation and purification of the derivative thereby produced is effected utilizing known techniques such as precipitation, extraction and, preferably, chromatographic techniques.

The process thus provides a novel, convenient, one-vessel process whereby an aminoglycoside is reacted *in situ* with an aldehyde (preferably in excess quantities) and with a hydride donor-reducing agent to produce as the major product, a mono-N-substituted derivative (e.g. 1-N-ethylsisomicin), in which process the 1-amino group attached to a secondary carbon atom is usually alkylated preferentially over other amino groups attached to primary and other secondary carbon atoms in the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol starting material.

It is also possible to use partially N-protected 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols as starting materials in this process. In general, those 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols possessing a $-\text{CH}_2\text{NH}_2$ group as the 6' moiety may be N-protected at this position since in the blocking procedure this group is most reactive. Sisomicin may be protected in position 6' or in positions 2' and 6' or in positions 2', 3, and 6'. Other amino protecting groups may be bridging groups in positions 3'', 4'', such as carbonyl, in those 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols having the 3''-amino and 4''-hydroxy group in position *cis* to each other. Thus, for example, one may utilize as starting compound a 1-N-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol wherein the amino function at the 6'-carbon is N-protected (e.g. 6'-N-t-butoxycarbonylsisomicin) or a 1-N-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol wherein the amino functions at C-2' and C-3 are N-protected (e.g. 2',3-di-N-trifluoroacetylgentamicin C_1) and there will be formed the corresponding partially N-protected 1-N-substituted derivative (e.g. 1-N-ethyl-6'-N-t-butoxycarbonylsisomicin and 1-N-ethyl-2',3-di-N-trifluoroacetylgentamicin C_1 , respectively) which upon removal of the N-protecting groups, according to known methods, yields the 1-N- CH_2X -compounds of this invention, e.g. 1-N-ethylsisomicin and 1-N-ethylgentamicin C_1 respectively.

The requisite starting compounds wherein amino groups are protected may be prepared according to procedures similar or identical to those set forth hereinbelow in Preparation 3. As used herein, the terms "blocking group" or "protecting group" refers to groups which render the blocked or protected amino groups inert to subsequent chemical manipulation, but which can easily be removed after the chemical manipulation is carried out. Exemplary of such amino protecting groups are benzyl, 4-nitrobenzyl, triphenylmethyl, 2,4-dinitrophenyl; acyl groups such as acetyl, propionyl and benzoyl; alkoxycarbonyl groups such as methoxycarbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, t-butoxycarbonyl and 2-iodoethoxycarbonyl; and arylalkoxy-carbonyl groups such as carbobenzyloxy and 4-methoxybenzyloxycarbonyl groups.

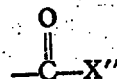
In the blocking process, the protecting group is usually employed in the form of an acid imidazole derivative, and acid azide or as active esters such as ethylthioltrifluoroacetate, N-benzoyloxycarbonyloxy succinimide or p-nitrophenyltrichloroethyl carbonate. Thus, blocking groups may be described as being derived from a compound BgLg, wherein Bg becomes the blocking group such as the acid portion of an active ester, and Lg is a leaving group such as imidazole.

Alternatively, the above described process may be carried out in a manner allowing the formation of a 1-N-Schiffs base derivative and reducing the so-obtained Schiffs base. The process for the preparation of the 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols listed above wherein the substituent is

—CH₂X and X is as defined above comprises reducing the N,C-double bond in a 1-N=CHX'-substituted derivative of one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, wherein all NH₂-groups are protected and NHCH₃ groups may be protected with X' being as defined above, removing all protecting groups present in the molecule and isolating the desired derivative as such or as a pharmaceutically acceptable acid addition salt.

Those 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols wherein the 6-aminoglycosyl radical is garosaminyl usually possess a 3''-N-4''-O-protecting group which is identical with the 1-N-substituent of the starting material because the oxazolidine ring is formed simultaneously with the 1-N-Schiffs base group. Thus, for example, 2',3-di-N-trifluoroacetylgentamicin C₁ upon reaction with an aldehyde (e.g. benzaldehyde, phenylacetaldehyde or acetaldehyde) is converted to the corresponding 3'',4''-oxazolidine-1-ylidene Schiff base starting material of this process (e.g. 1-N-3''-N-4''-O-di-benzylidene-2',3-di-N-trifluoroacetylgentamicin C₁, 1-N-3''-N-4''-O-di-phenethylidene-2',3-di-N-trifluoroacetylgentamicin C₁ and 1-N-3''-N-4''-O-diethylidene-2',3-di-N-trifluoroacetylgentamicin C₁) which, upon reduction with sodium borohydride and methanolic sodium methoxide yields the corresponding 1-N-CH₂X-3'',4''-oxazolidine (e.g. 1-N-benzyl-3''-N-4''-O-benzylidenegentamicin C₁, 1-N-phenethyl-3''-N-4''-O-phenethylidenegentamicin C₁ and 1-N-ethyl-3''-N-4''-O-ethylidenegentamicin C₁, respectively) which upon treatment with acid yields a 1-N-CH₂X compound of the invention, (e.g. 1-N-benzylgentamicin C₁, 1-N-phenethylgentamicin C₁ and 1-N-ethylgentamicin C₁, respectively).

The novel 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols such as defined by formulae I, II, III, IV and V may also be prepared by a process which comprises treating a 1-N-substituted derivative of one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, wherein one or more amino-groups may be protected and the 1-N-substituent is



with X'' being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkyl aminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl, tolyl or hydrocarbyloxy, said aliphatic radicals having up to seven carbon atoms, and if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and wherein any amino or hydroxy group present may be protected, with an amide-reducing hydride reagent and, if required, removing all protecting groups present in the molecule; the last process step being followed by isolating the desired derivative as such or as a pharmaceutically acceptable acid addition salt.

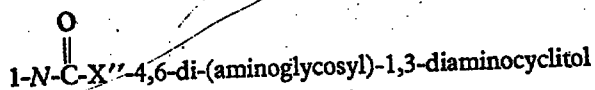
The process is usually carried out in a non-reactive organic solvent which is contemplated to be a solvent in which the starting compounds and the amide-reducing reagent are soluble and which will not react with the reagent so there is produced a minimum of competing side reactions. Non-reactive organic solvents which are most useful in the reduction process are ethers such as dioxane, tetrahydrofuran, diethylene-glycol dimethyl ether and the like.

Preferred amide-reducing hydride reagents are aluminum hydrides and borohydrides including lithium aluminum hydride, lithium trimethoxy aluminum hydride, aluminum hydride, diborane, di-isoamylborane, and 9-borabicyclo [3.3.1] nonane.

In general, it is preferred to use diborane as the amide-reducing agent except when the starting compound possesses a double bond, e.g. as in 1-N-acylisomycin, 1-N-acylverdamicin, 1-N-acyl-Antibiotic 66-40B, 1-N-acyl-Antibiotic 66-40D, and 1-N-acyl-Antibiotic G-52, which compounds are conveniently reduced by means of lithium aluminum hydride.

When X'' represents hydrocarbyloxy, such as *t*-butoxy, and the amide is subjected to reduction, the corresponding 1-N-methyl compound is formed.

In this process whereby a



is reduced to the corresponding 1-N-CH₂X derivative of the invention, if the acyl side chain of the 1-N-acyl-intermediate possesses a chiral center, one can use each

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groups may
aps present
naceutically

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is identical
ine ring is
, 2',3-di-N-
nzaldehyde,
ing 3'',4''-
-3''-N-4''-
-O-di-phen-
liethylidene-
dium boro-
-X-3'',4''-
-phenethyl-
-ethylidene-
1-N-CH₂X
ylgentamicin

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prepared by
of the above
mino-groups

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alkyl, phenyl,
seven carbon
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be protected,
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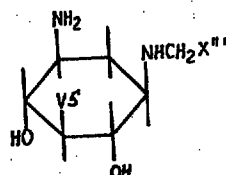
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stereoisomer separately or a mixture thereof, and there will be obtained the correspond-
ing diastereoisomers or a mixture thereof, respectively.

Another process of this invention, for the preparation of 1-N-substituted deriva-
tives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols sisomicin and mutamicin 2,
wherein the substituent is —CH₂X''' with X''' being hydrogen, methyl, ethyl or
propyl, and of the pharmaceutically acceptable acid addition salts thereof comprises
cultivating a mutant strain of the species *Micromonospora inyoensis*, namely *Micro-
monospora inyoensis* strain 1550F-1G or a strain of the same species corresponding
thereto in its capability of producing one of the above defined antibiotics, in a nutrient
medium comprising a compound of the formula



wherein V₅ is hydroxy or hydrogen, and X''' is as defined above, until substantial
antibiotic activity is imparted to the medium and isolating the so-obtained compound
as such or in the form of a pharmaceutically acceptable acid addition salt.

The microorganism *Micromonospora inyoensis* strain 1550F-1G is deposited with
U.S. Department of Agriculture, Northern Utilization Research and Development
Division, Peoria, Illinois under the numerical designation NRRL 5742; several
taxonomical, biochemical, and morphological properties thereof being listed herein-
above.

Some of the requisite 1-N-alkyl-2-deoxy-D-streptamine aminocyclitols are known,
e.g. 1-N-methyl-2-deoxy-D-streptamine (also identified as (—)-hyosamine) which,
when cultivated with *Micromonospora inyoensis* strain 1550F-1G in the manner des-
cribed in Example 16 hereinbelow produces 1-N-methylsisomicin.

Other requisite starting aminocyclitols (e.g. 1-N-ethyl and 1-N-propyl-2-deoxy-D-
streptamine are prepared from known aminocyclitols (e.g. (1S)-1-acetamido-3-amino-
1,3-dideoxy-myco-inositol) or from known pseudodisaccharides (e.g. garamine) via
chemical transformations known in the art as exemplified in Preparations 7—9 herein-
below. Cultivation of each of 1-N-ethyl-2-deoxy-D-streptamine and 1-N-propyl-2-
deoxy-D-streptamine with *Micromonospora inyoensis* strain 1550F-1G leads to 1-N-
ethyl-sisomicin and 1-N-propylsisomicin, respectively.

Other aminocyclitol starting compounds (e.g. (±)-mono-N-methyl-2,5-dideoxy-
streptamine) are prepared from cis-4,8-dioxatricyclo[5.1.0.0^{3,5}]octane by treatment
thereof with a mono-N-lower alkyl hydrazine (e.g. N-methylhydrazine) and hydro-
genolizing the thereby produced (±)-mono-N-lower alkyl-6,7-diaza-2,4-dihydroxy-
bicyclo[3.2.1]octane. The (±)-mono-N-lower alkyl-2,5-dideoxystreptamines thereby
produced may be separated by optical resolution procedures known in the art to
obtain 1-N-lower alkyl-2,5-dideoxy-D-streptamine and 3-N-lower alkyl-2,5-dideoxy-D-
streptamine, respectively. Alternatively, the racemate of the precursor, i.e. (±)-mono-
N-lower alkyl 6,7-diaza-2,4-dihydroxybicyclo[3.2.1]octane may be separated by optical
resolution procedures to obtain 6-N-lower alkyl-6,7-diaza-2,4-dihydroxybicyclo[3.2.1]-
octane and 7-N-lower alkyl-6,7-diaza-2,4-dihydroxybicyclo[3.2.1]octane. Hydrogeno-
lysis of the aforementioned 6-N-lower alkyl derivative leads to 1-N-lower alkyl-2,5-
dideoxy-D-streptamine while hydrogenolysis of the 7-N-lower alkyl derivative leads to
3-N-lower alkyl-2,5-dideoxy-D-streptamine.

Similarly, mono-N-alkyl-2-deoxystreptamine prepared from (±)-1,3-dicarbo-
benzyloxy-4,5-O-isopropylidene-2-deoxystreptamine, according to procedures des-
cribed in Preparation 9, may be separated by optical resolution procedures to obtain
1-N-lower alkyl-2-deoxy-D-streptamine and 3-N-lower alkyl-2-deoxy-D-streptamine.

To obtain a 1-N-alkyl-4,6-di-(aminoglycosyl)-1,3-diaminocyclitol of this invention,
a 1-N-lower alkyl-2-deoxy-D-streptamine (e.g. 1-N-methyl-2-deoxy-D-streptamine and
1-N-methyl-2,5-dideoxy-D-streptamine) is cultivated with *Micromonospora inyoensis*
strain 1550-1G, to obtain a 1-N-lower alkylsisomicin derivative (e.g. 1-N-methyl-
sisomicin and 1-N-methyl-5-deoxy-sisomicin). Optionally, the racemic mixture of a
mono-N-alkyl-streptamine, (e.g. (±)-mono-N-methyl-2-deoxystreptamine and (±)-
mono-N-methyl-2,5-dideoxystreptamine) may be cultivated with *Micromonospora
inyoensis* strain 1550F-1G to produce a mixture of 1- (and 3-) N-alkylsisomicins (e.g.
a mixture of 1-N-methylsisomicin and 3-N-methylsisomicin) and a mixture of 1-N-

25 methyl -5-deoxysisomicin and 3-N-methyl-5-deoxysisomicin which may be separated via chromatographic techniques to obtain 1-N-alkylsisomicin of this invention, e.g. 1-N-methylsisomicin and 1-N-methyl-5-deoxysisomicin.

5 Fermentation is effected in the following manner. A lyophilized culture or cells from a slant culture of *Micromonospora inovoensis* strain 1550F-1G NRRL 5742 is transferred to a sterile inoculum medium. The medium is an aqueous one containing assimilable sources of nitrogen, carbohydrates and the usual complement of trace metals. The inoculated medium is permitted to incubate under aerobic conditions at from about 24° to about 49°C., preferably about 35°C for from about 2 to about 10 5, preferably about 3 days. The pH is maintained in the range of from about 6.0 to about 8.0, preferably from about 6.8 to about 7.4.

15 The so-produced inoculum is aseptically transferred to a fermentation medium which medium may be the same as or different from the inoculum medium. An aminocyclitol may be added to the fermentation medium before sterilization, at the time of inoculation or up to 48 hours after inoculation. The aminocyclitol is usually dissolved in water, sterile filtered and added to the fermentation medium. In general, the concentration of the compound is from about 100 to about 1500 mcg/ml. of fermentation broth. The fermentation is conducted under aerobic conditions, and under about the same conditions of temperature and pH as in the inoculum. 20 Peak antibiotic production is determined by the assay used for sisomicin [see J. Antibiotics (Japan) Vol. XXIII]. The sisomicin derivatives thereby produced are isolated from the fermentation and from co-produced minor components having antibacterial activity by methods generally used in the art for aminoglycoside antibiotics.

25 Another process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols listed above wherein the substituent is straight chain alkyl having up to five carbon atoms and of the pharmaceutically acceptable acid addition salts thereof comprises reacting one of these 4,6-di-(amino-glycosyl)-1,3-diaminocyclitols, which possesses amino-protecting groups at any position other than position 1, and wherein the 1-amino group may be activated, with an alkylating agent 30 containing the straight chain alkyl group having up to five carbon atoms and a leaving group, removing the protecting groups and, if required, the activating group or groups present in the molecule, and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

35 Examples of alkylating agents advantageously used in this process are alkyl iodide, alkyl bromide, dialkyl sulfate, alkyl fluorosulphonate and alkyl *p*-toluenesulfonate wherein the alkyl group is the required straight chain alkyl group having up to five carbon atoms. Other alkylating agents, wherein the alkyl group preferably has one or two carbon atoms, are trialkylanilinium hydroxide, trialkyloxonium fluoroborate, trialkylsulfonium fluoroborate, or trialkylsulfoxonium fluoborate. All of these alkylating agents 40 contain a good leaving group, such as Br⁻, I⁻, OSO₂F⁻, di-alkylanilin or dialkylether.

45 The amino group in position 1 of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol can be free or activated. An example of an activating group is trifluoromethylsulfonyl. These activating groups may be introduced into the molecule by reacting a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol which possesses amino-protecting groups at any position other than position 1, e.g. 3''-N-4''-O-carbonyl-2',3,6'-tri-N-*t*-butoxycarbonyl-sisomicin, with a compound providing the activating group, such as trifluoromethylsulfonyl chloride.

50 The 1-amino group can also be alkylated by way of the corresponding di-(2-cyanoethyl)-derivative which is derived from treatment with acrylonitrile of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol which possesses amino protecting groups at any position other than position 1. The 1-N-di-(2-cyanoethyl)-derivative thus prepared is then alkylated with one of the above listed alkylating agents followed by removal of the cyanoethyl groups.

55 The process of the invention is carried out under conditions similar to those employed in the well-known direct alkylation procedures of amines.

60 Yet other processes for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols listed above, wherein the substituent is methyl, and of the pharmaceutically acceptable acid addition salts thereof comprise reacting one of these 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which possesses amino-protecting groups at any position other than position 1, either with formaldehyde and a cyclic imide, preferably succinimide, and treating the so-obtained compound with a 65 hydride-donor reducing agent, preferably sodium borohydride, or with formaldehyde in the presence of formic acid, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition

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salt. The formation of the 1-N-methyl substituent with formaldehyde and formic acid is well known as Eschweiler-Clarke reaction.

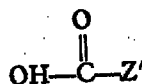
A process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols listed above, wherein the substituent is 2-hydroxyethyl, and of the pharmaceutically acceptable acid addition salts thereof comprises reacting one of these 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which possesses amino protecting groups at any position other than position 1, with ethylene oxide, removing all protecting groups present in the molecule and isolating the derivatives as such or as a pharmaceutically acceptable acid addition salt.

In another of its process aspects the invention relates to the preparation of the amide compounds which are useful as intermediates in the preparation of the 1-N-CH₂X-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols and which possess broad spectrum antibacterial activity.

The process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamycin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamycin 1, mutamycin 2, mutamycin 4, mutamycin 5 and mutamycin 6, wherein the substituent is



with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, with the proviso that Z together with the carbonyl group to which it is attached is other than S-3-amino-2-hydroxypropionyl or S-4-amino-2-hydroxybutyryl, and, in the case of tobramycin, also other than S-5-amino-2-hydroxyvaleryl, and of the pharmaceutically acceptable acid addition salts thereof, comprises treating one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which may have amino protecting groups at any position other than position 1, with an acid of the formula



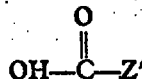
with Z' being a group as defined for Z above, wherein any amino or hydroxy group present may be protected, in the presence of a carbodiimide such as dicyclohexylcarbodiimide, or with a reactive derivative thereof, and, if required, removing all protecting groups present in the molecule, the last process step being followed by isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

The 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol starting compounds of this process may have free amino groups or protected amino groups. If amino groups are protected in the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin B, gentamicin C_{1a}, Antibiotic JI-20A, Antibiotic 66-40B, Antibiotic 66-40D, sisomicin, mutamycin 1, mutamycin 2, mutamycin 4, mutamycin 5 and mutamycin 6, it is usually the 6'-amino group being protected. Gentamicin C₁ may be protected at positions 2' and 3'. The 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol starting compounds may be used as a free nitrogen-base (with or without N-protecting groups) or, in an inventive modification of the process, as a compound wherein 1 to n amino groups of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol are neutralized by formation of an acid addition salt whereby n is the number of amino groups in the molecule. The acid addition salt may also contain N-protecting groups. In a preferred feature of the inventive modification of the acylating process (n-1) amino groups are neutralized by formulation of an acid addition salt. For example, one equivalent of gentamicin C₁ having five amino groups (n=5) requires five equivalents of acid to form the "per" acid addition salt. In the preferred feature, an acid addition salt of gentamicin C₁ is used having (n-1), i.e. four, amino groups which are protonated. The term "acid addition salt" embraces such salts as may be formed between the basic antibiotic and an acid without regard to whether the acid may be termed inorganic or organic. Exemplary of acids embraced by the term are sulfuric, hydrochloric, phosphoric, nitric, acetic, propionic, succinic,

oxalic, cyclopropylcarboxylic, trimethylacetic, maleic, benzoic, phenylacetic, trifluoroacetic or the like.

If it is desired to use as a starting material an acid addition salt, wherein (n-1) amino groups are protonated, this compound is advantageously produced *in situ* thereby reacting a "per" acid addition salt with an equivalent of strong base, e.g. triethylamine.

In general, the use of reactive derivatives of the acid



as acylating agents is preferred. Reactive derivatives of the acid comprise esters, azides, imidazole derivatives or anhydrides. In those instances wherein Z' is unsubstituted, one of the preferred reactive derivatives is the anhydride of the requisite acid. In other instances it may be preferable to use the N-hydroxysuccinimidyl ester of the acid.

When carrying out the process whereby a reactive derivative of an acid containing an amino function is used, it is preferable to protect the amino function prior to carrying out the process and then removing the N-protecting group in the compound thereby formed. It may also be advantageous to protect a hydroxy group present in the acylating agent, however, it is not generally necessary.

It is obvious to those skilled in the art that the amide compounds excluded from the scope of the present invention, i.e. those compounds wherein Z together with the carbonyl group to which it is attached is S-3-amino-2-hydroxy-propionyl or S-4-amino-2-hydroxy-butyryl and, in case of tobramycin, also S-5-amino-2-hydroxy-valeryl, can be prepared in a manner similar to that described above for the preparation of the amide compounds of this invention. Furthermore, the preparation of some of these compounds is set forth hereinbelow in Preparation 4.

The following Preparations exemplify the manufacture of a multiplicity of requisite starting materials and the following Examples illustrate the invention.

PREPARATION 1

Gentamicin C_{2a}

Separation of Gentamicin C_{2a} from Co-produced Antibiotics

Dissolve 96 gms. of gentamicin base (prepared from the sulfate salt obtained by the procedure of Example 4 of U.S. Patent 3,091,572) in 400 ml. of the upper phase which results when methanol, chloroform and 17% ammonium hydroxide are mixed in the volume ratio of 1:2:1. Add one tenth of the solution to each of the first ten tubes in a 500×80 ml. tube counter current extractor. Fill all of the tubes including the first ten to capacity with the lower phase of the above-described solvent mixture. Set the solvent reservoir to deliver 40 ml. of upper phase to tube one (1) for each transfer. Set the apparatus for 500 transfers. When the transfers are complete, sample every eighth tube for chromatography (in duplicate) on Schleicher and Schuell paper No. 589 using the lower phase of the above-described solvent mixture. Permit the chromatograms to develop for about 16 hours and then dry the papers. Plate one paper on an agar plate seeded with *Staphylococcus aureus* (A.T.C.C. 6538P), spray the duplicate with the conventional ninhydrin solution and heat to develop. Incubate the agar plate at 37°C overnight and combine the solution from tubes containing the material that migrates like gentamicin C₁ (i.e. tubes 290—360).

Replace tubes 290—360 with fresh tubes containing 40 ml. of upper phase and 40 ml. lower phase. Re-set the apparatus for an additional 2800 transfers and repeat the chromatographic procedure performed above. Combine tubes 1—16 and concentrate *in vacuo* to obtain 1.3 gms. of gentamicin C_{2a}, having the following

(a) a molecular weight of 463 as determined by mass spectrometry which is consistent with an empirical formula of C₂₀H₄₁N₅O₇;

(b) a specific optical rotation as measured by the D line of sodium at 26°C of +114° ± 5° in water at 0.3% concentration; and

(c) a proton magnetic resonance (pmr) spectrum as follows: pmr(ppm) (D₂O): δ 0.99 (3H, d, J=6.5 Hz, CH—CH₃); 1.17 (3H, s, C—CH₃); 2.47 (3H, s, N—CH₃); 2.51 (1H, d, J=10.5 Hz, H—3''); 3.75 (1H, q, J=10.5, 4 Hz, H—2''); 4.00 (1H, d, J=12 Hz, H—5'' eq); 5.04 (1H, d, J=4 Hz, H—1''); 5.13 (1H, d, J=3.5 Hz, H—1').

Irradiation of the secondary methyl group at δ 0.99 ppm reveals H—6' as a doublet (J=6.5 Hz) at δ 2.81 ppm.

PREPARATION 2

Gentamicin C_{2b}

Separation of Gentamicin C_{2b} from Co-produced Antibiotics

Separate the major gentamicin C components (C₁, C₂ and C_{1a}) as described in U.S. Patent 3,651,042, Example 2, and combine those fractions containing predominantly overlaps of gentamicins C₁ and C₂ free base (500 g. of gentamicin C mixture gives 53.4 g. of overlaps). Apply 1.5 g. of this gentamicin C₁ and C₂ mixture to a column containing 50 g. of silica gel made up in a solvent system comprising chloroform:methanol:15% ammonium hydroxide (1:2:1). Elute the column with the same solvent system and monitor the eluted fractions by thin layer chromatography on silica gel plates using the solvent system chloroform:methanol:22% ammonium hydroxide (1:2:1) as developer. Combine those fractions containing a mixture of gentamicins C₁ and C₂ together with gentamicin C_{2b} (Fractions 39—57 [410 mg.]). Recchromatograph fractions 39—57 over silica gel using a chloroform:methanol:7% ammonium hydroxide (1:2:1) solvent system and combine those fractions (98—130) containing pure gentamicin C_{2b} as determined by thin layer chromatography (yield 45 mg.) having the following constants $[\alpha]_D^{20} + 165.5^\circ$ (c=0.3%, H₂O); Mass spectrum m/e 463 (M+1)⁺, 446, 445, 433, 350, 332, 322, 304, 333 305, 287, 191, 173, 163, 145, 160, 142, 118, 143; pmr(ppm) (D₂O): δ 1.25 (3H, s, C—CH₃); 2.40 (3H, s, N—CH₃); 2.55 (3H, s, N—CH₃), 5.12 (1H, d, J=4 Hz, H—1''); 5.22 (1H, d, J.3 Hz H—1').

Pure gentamicin C_{2b} can be differentiated from gentamicin C₁ and C₂ by its mobility on thin layer chromatography using silica gel plates and a chloroform:methanol:22% ammonium hydroxide (1:2:1) solvent system as developer. The approximate R_f values in this system are as follows:

gentamicin C ₁	0.47
gentamicin C ₂	0.47
gentamicin C _{2b}	0.35

PREPARATION 3

Selectively blocked Di-(aminoglycosyl)-1,3-diaminocyclitols

A. 2',3,6'-Tri-N-butoxycarbonyl-3''-N-4''-O-carbonylsisomicin

1. Penta-N-carbobenzoxysisomicin

Dissolve 25 g. of sisomicin and 13 g. of sodium carbonate in 625 ml. of distilled water. Add 100 ml. of carbobenzoxychloride to the stirred solution at 25°C and stir the mixture for sixteen hours. Filter off the solid, wash thoroughly with water, dry *in vacuo*, and then wash with hexane to obtain penta-N-carbobenzoxysisomicin (62 g.) as a colorless amorphous solid. m.p.=165—173°C $[\alpha]_D^{20} + 96.2^\circ$ (CH₃OH) IR: ν max (CHCl₃) 3400, 1720, 1515, 1215, 1050, 695, cm⁻¹ NMR: δ (CDCl₃) 1.03 (3H, broad singlet, 4''—C—CH₃); 3.02 (3H, broad singlet, 3''—NCH₃); 5.02 (10H, broad singlet, CH₂C₆H₅); 3.28, 3.30 ppm (25H, broad singlets, —CH₂C₆H₅).

2. Tetra-N-carbobenzoxy-3''-N-4''-O-carbonyl-sisomicin

Dissolve 5 g. of penta-N-carbobenzoxy-sisomicin in 50 ml. of dimethylformamide, add 250 mg. of sodium hydride to the stirred solution, and stir the reaction mixture under argon at room temperature for two hours. Filter and add glacial acetic acid (2 ml.) to the filtrate which is then concentrated *in vacuo*. Extract the residue with chloroform (200 ml., previously passed through basic alumina), wash the extract with water and dry over sodium sulfate. The solution is evaporated to give tetra-N-carbobenzoxy-3''-N-4''-O-carbonyl-sisomicin as an amorphous powder (3.5 g.) m.p.=210—213°C $[\alpha]_D^{20} + 68.8^\circ$ (C=0.22) IR: ν max (Nujol) 3550, 1840, 1760, 1580 cm⁻¹ NMR: δ (CDCl₃) 1.34 (3H, singlet, 4''—CH₃); 2.68 (3H, singlet, 3''—N—Me); 5.04 (8H, broad singlet, —CH₂C₆H₅). "Nujol" is a Registered Trade Mark.

3. 3''-N-4''-O-Carbonyl-sisomicin

To a solution of 1,3,2',6'-tetra-N-benzyloxycarbonyl-3''-N-4''-O-carbonyl-sisomicin (10.1 g.) in tetrahydrofuran (200 ml.) add 1 litre of liquid ammonia (redistilled from sodium). To the stirred solution add 6 grams of sodium in small pieces. After stirring for 3 hours destroy the excess sodium by addition of ammonium chloride. Allow the solvents to evaporate under a stream of nitrogen. Dissolve the residue in water and pass through a medium of Amberlite IRC-50 resin (H⁺ form) and wash the resin well with water then elute the product with 2N ammonium hydroxide solution. "Amberlite" is a Registered Trade Mark. Evaporate the ammonia eluate in

vacuo to give the title product (3''-N-4''-O-carbonyl-sisomicin). Yield ca 4 gms. IR: ν max (Nujol) 1745 cm^{-1} . The product may be used in subsequent steps without further purification. However a very pure sample may be obtained by chromatography of the product over silica gel using the lower phase of a chloroform:methanol:conc. ammonium hydroxide (1:1:1) solvent system as eluant.

4. 2',3,6'-Tri-N-t-butoxycarbonyl-3''-N-4''-O-carbonyl-sisomicin

Dissolve 3''-N-4''-O-carbonyl-sisomicin (1.4 g., 3 mmoles) in 10 ml. of 50% aqueous methanol containing triethylamine (3.5 mmoles). With stirring, add t-butoxycarbonyl azide (3.5 mmoles) dropwise. Stir the mixture for two days at room temperature. Add 5 ml. of Amberlite IRA-401S (OH-) ion exchange resin along with 5 ml. methanol and stir for 1/2 hour. Remove the resin by filtration and wash with methanol. Concentrate the filtrate and chromatograph the residue on a column of silica gel (60—100 mesh, 20.0 g.) using chloroform:methanol:ammonium hydroxide (30:10:0.4) as the solvent system. Pool the homogeneous fractions containing the title material and remove the solvent by evaporation *in vacuo*. Dissolve the residue in methanol and precipitate with excess ether. Isolate the solid product by filtration and dry.

B. 2',3-Di-N-Trifluoroacetyl Gentamicin C_1

1. 2'-N-Trifluoroacetyl Gentamicin C_1

Dissolve 1.7 g. of gentamicin C_1 in 20 ml. of methanol, cool the mixture of 4°C and add 0.46 ml. (0.563 g.) of ethyl thioltrifluoroacetate with stirring. Allow the reaction to continue for 2 hours and concentrate the solution to a residue *in vacuo*. Chromatograph the product on 80 g. of silica gel G using the lower phase of a mixture of chloroform:methanol:water:ammonium hydroxide in the volume ratio of 10:5:4:1 as eluant. Combine the fractions containing the major component and concentrate to obtain 1.4 g. of the title compound, m.p. 108—111°C, $[\alpha]_D^{20} = +128^\circ$ (c=0.3%, H_2O). Analysis for $\text{C}_{23}\text{H}_{42}\text{N}_5\text{O}_8\text{F}_3 \cdot \text{H}_2\text{O}$ requires C=46.69%; H=7.50%; N=11.84%; F=9.63%. Found: C=46.66%; H=7.65%; N=11.60%; F=9.24%.

2. 2',3-Di-N-Trifluoroacetyl Gentamicin C_1

Dissolve 0.66 g. of the product of Step 1 in 10 ml. of methanol, cool the mixture to 4°C and add 0.148 ml. (0.182 g.) of ethyl thioltrifluoroacetate dissolved in 3 ml. of methanol. Stir the reaction mixture for about 16 hours and concentrate to a residue *in vacuo*. Chromatograph the product on 30 g. of silica gel as described in Step 1. Monitor the column by thin layer chromatography, combine the appropriate fractions and concentrate to obtain 0.32 g. of the title compound, m.p. 121—129°C $[\alpha]_D^{20} = 121^\circ$ (c=0.3%; H_2O). Analysis for $\text{C}_{23}\text{H}_{41}\text{N}_5\text{O}_8\text{F}_3$ requires C=44.84%; H=6.17%; N=10.46%. Found: C=44.94%; H=6.35%; N=10.17%.

C. 6'-N-Trifluoroacetyl-sisomicin

Dissolve 20 g. of sisomicin in 1.2 liters of anhydrous methanol and add dropwise a solution of 6 ml. of ethyl thioltrifluoroacetate in 60 ml. of methanol over a 3 hour period with stirring. Allow the reaction to proceed for 18 hours at room temperature and remove the solvent *in vacuo* to give a residue of 23.8 g. of product of approximately 95% purity having the following physicochemical properties: Mass Spectral data: m/e 543 M^+ , other definitive peaks at m/e 413, 395, 385, 362, 223 and 126. NMR (60 MHz, D_2O) δ 5.37 (doublet, J=2 Hz, H-1'); 5.12 (doublet J=4 Hz, H-1''); 4.96 (broad singlet, H-4'); 2.57 (singlet, N- CH_3); 1.26 (singlet, C- CH_3).

D. 6'-N-t-butoxycarbonyl Gentamicin C_{1a}

Dissolve 2.69 g. of gentamicin C_{1a} in 60 ml. of methanol:water (1:1), cool to 5°C and add 1.815 ml. of triethylamine. Add with stirring 1.91 g. of t-butoxycarbonyl azide dropwise. Stir the mixture at 5°C for 18 hours. Add 20 ml. of Amberlite IRA-401S resin ($\ominus\text{OH}$ form), stir for 30 minutes, filter and evaporate the filtrate to dryness *in vacuo*. Chromatograph the crude product over silica gel (350 g.) using the lower phase of a 2:1:1, chloroform:methanol:concentrated ammonium hydroxide solvent system as eluant. Take 3 ml. fractions and monitor their contents by TLC. Combine fractions containing the major reaction product and evaporate to obtain the title compound of this example (0.42 g., 13%), $[\alpha]_D^{20} = +137^\circ$ (CH_3OH), UMR δ 1.23 (3H, s, C- CH_3); 1.45 (9H, s, C-(CH_3)₃); 2.53 (3H, s, N- CH_3); δ 5.08 (2H, overlapping doublets, J $\approx$ 3.5 Hz, H-1' and H-1'') PPM. Mass spectrum m/e 550 [(M+1) $^+$] and m/e 549 (M^+).

E. 6'-N-*t*-butoxycarbonyl Gentamicin B

Dissolve 1 g. of gentamicin B in 30 ml. of 50% aqueous methanol and cool to 5°C. Add 0.297 g. of *t*-butoxycarbonyl azide dropwise with stirring followed by 0.186 ml. of triethylamine and stir the resulting solution for 18 hours. Evaporate the reaction mixture *in vacuo* to a residue and chromatograph the residue on 100 g. of silica gel using the lower phase of a 2:1:1, chloroform:methanol:concentrated ammonium hydroxide solvent system as eluant. Collect 2 ml. fractions and monitor the column effluent by TLC. Combine fractions containing like material (fracts. 180—230) and evaporate to obtain 0.830 g. 6'-N-*t*-butoxycarbonyl gentamicin B having the following physical constants: PMR (60 MHz, D₂O) δ 1.21 (3H, s, C—CH₃); 1.42 (9H, s, C(CH₃)₃); 2.53 (3H, s, N—CH₃); 5.2 (1H, d, J=4.5 Hz, H—1''); 5.23 (1H, d, J=3.0 Hz, H—1') PPM.

PREPARATION 4

1-N-(Aminohydroxyacyl)-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols

A. 1-N-(S-4-Amino-2-hydroxybutyryl) Gentamicin C_{1a}1. 1-N-(S-4-Benzoyloxycarbonylamino-2-hydroxybutyryl)-Gentamicin C_{1a}

Dissolve 2.8 g. (4 mmoles) of gentamicin C_{1a} sulfate in 30 ml. of water and add 15 ml. of methanol. Add 0.56 ml. (4 mmoles) of triethylamine and stir for 10 minutes. Add a solution containing 4 mmoles of N-(S-4-benzoyloxycarbonylamino-2-hydroxybutyryloxy)succinimide in 20 ml. of dry dimethylformamide dropwise with stirring to the antibiotic solution. Stir the mixture overnight (16 hours) at ambient temperature. Thin layer chromatography of the reaction mixture via TLC on silica gel using the lower phase of a solvent system consisting of chloroform:methanol:ammonium hydroxide (1:1:1), shows the presence of a plurality of minor components and one major component. Concentrate the reaction mixture to a residue *in vacuo* and triturate the residue with methanol to yield 3.2 g. of white solids containing all the components previously observed by chromatography.

Chromatograph 150 mg. of the product on 50 g. of silica gel using the lower phase of a solvent system consisting of chloroform:methanol:ammonium hydroxide (2:1:1). Pool the fractions containing the major component and lyophilize to give 1-N-(S-4-benzoyloxycarbonylamino-2-hydroxybutyryl) gentamicin C_{1a}. NMR (D₂O) δ 1.15 (3H, s, C—CH₃); 2.49 (3H, s, NCH₃); 4.10 (1H, dd, J=8.0, 4.0 Hz, side-chain H—2); 7.36 (5H, m, phenyl).

2. 1-N-(S-4-Amino-2-hydroxybutyryl) Gentamicin C_{1a}

Dissolve the product of Preparation 4A (1) in a mixture consisting of 12 ml. of methanol and 3 ml. of water, add 20 mg. of 10% palladium on carbon and hydrogenate at 4 atmospheres at room temperature. After 3 hours the reaction is essentially complete. Remove the catalyst by filtration and lyophilize the filtrate and obtain 46 mg. of 1-N-(S-4-amino-2-hydroxybutyryl) gentamicin C_{1a}. NMR (D₂O) δ 1.17 (3H, s, C—CH₃); 2.48 (3H, s, NCH₃); 4.22 (1H, dd, J=9.5 Hz, 4.0 Hz, side chain CHOH); 5.04 (2H, m, H—1', and H—1''). Mass Spectral data: M—H₂O) m/e 532.

B. 1-N-(S-4-Amino-2-hydroxybutyryl) Gentamicin B

1. 1-N-(S-4-benzoyloxycarbonylamino-2-hydroxybutyryl) Gentamicin B

Dissolve 3.39 g of gentamicin B sulfate in 48.4 ml. of water and dilute with 23.7 ml. of methanol. Add 0.7 ml. of triethylamine dropwise with stirring. Dissolve 1.67 g. of N-(S-4-benzoyloxycarbonylamino-2-hydroxybutyryloxy)succinimide in dimethylformamide and add the solution dropwise with stirring to the antibiotic solution. Stir the resulting solution at room temperature for 18 hours then concentrate to a residue *in vacuo*. Dissolve the residue in water and treat with dilute barium hydroxide solution with stirring until the pH reaches about 8.0. Remove the precipitated barium sulfate by filtration using a filter aid. Wash the precipitate with water, combine the filtrate and washings and concentrate to dryness *in vacuo*. Chromatograph the residue on a column containing 600 g. of silica gel using the lower phase of a solvent system consisting of chloroform:methanol:ammonium hydroxide (1:1:1) as the eluant. Pool the material which is eluted *immediately ahead* of gentamicin B and concentrate the pooled fractions to dryness and obtain thereby 1-N-(S-4-benzoyloxycarbonylamino-2-hydroxybutyryl) gentamicin B as an amorphous solid. Yield=0.2 g. NMR (D₂O): δ 1.27 (3H, s, C—CH₃); 2.51 (3H, s, NCH₃); 5.08 (1H, d, J=4 Hz); 5.25 (1H, d, J=3.5 Hz).

2. 1-*N*-(*S*-4-amino-2-hydroxybutyryl) gentamicin B

Dissolve the product of Preparation 4B (1) in a mixture consisting of 20 ml. of water and 8 ml. of methanol. Hydrogenate the product in the presence of 60 mg. of 5% palladium-on-carbon at 3.5 atmospheres and room temperature for 3 hours. Remove the catalyst by filtration through a filter aid. Wash the filter pad with water and combine the filtrate and washings. Concentrate the combined filtrate and washings to dryness *in vacuo*. Chromatograph the residue on a silica gel column containing 10 g. of silica gel using a solution consisting of chloroform:methanol:ammonium hydroxide (1:2:1) as the eluant. Fractions containing the most polar component are pooled, concentrated and lyophilized to give 1-*N*-(*S*-4-amino-2-hydroxybutyryl) gentamicin B.

C. 1-*N*-(*S*-4-Amino-2-hydroxybutyryl)-verdamicin1. 1-*N*-(*S*-4-Phthalimido-2-hydroxybutyryl)-verdamicin

Dissolve 5.00 g. of verdamicin sulphate in 50 ml. of water and add 25 ml. of methanol. Add 0.50 ml. of triethylamine and stir for 10 minutes. Add a solution containing 2.5 g. of *N*-(*S*-4-phthalimido-2-hydroxybutyryloxy)succinimide in 10 ml. of dimethylformamide dropwise with stirring. Stir the mixture overnight at ambient temperature then concentrate to a residue *in vacuo*. Chromatograph the residue over 160 g. of silica gel, eluting with the lower phase of a chloroform:methanol:concentrated ammonium hydroxide (1:1:1) solvent mixture. Combine and evaporate fractions containing the major component of the reaction (determined by TLC on silica gel plates) and obtain thereby the compound of this example as a white amorphous solid.

2. 1-*N*-(*S*-4-Amino-2-hydroxybutyryl)-verdamicin

Dissolve the product of Preparation 4C (1) in 40 ml. of ethanol and add 0.2 g. of hydrazine hydrate. Reflux the solution for 3 hours, then evaporate to dryness *in vacuo*. Chromatograph the residue over 160 g. of silica gel, eluting the lower phase of a chloroform:methanol:concentrated ammonium hydroxide (1:1:1) solvent mixture. Combine and evaporate fractions containing the major component of the reaction (determined by TLC on silica gel plates) and obtain thereby the compound of this example as a white amorphous solid.

D. In the procedures of Preparations 4A to 4C, the sulfate salts of other 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols may be substituted for gentamicin C_{12a}, gentamicin B and verdamicin and other *N*-(*S*-benzyloxycarbonylamino-2-hydroxybutyryloxy)succinimides may be substituted for *N*-(*S*-4-benzyloxycarbonylamino-2-hydroxybutyryloxy)succinimide or *N*-(*S*-4-phthalimido-2-hydroxybutyryloxy)succinimide and there will be obtained the corresponding 1-*N*-(aminohydroxyacyl)-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols.

PREPARATION 5

Preparation of Aldehyde Intermediates

A. 2-Acetamido-3-hydroxyoctanal

Protect the amino function in the 2-amino-3-hydroxyoctanoic acid by conversion thereof to an acetamido function, then esterify the resultant 2-acetamido-3-hydroxyoctanoic acid with methanol; reduce the resultant 2-acetamido-3-hydroxyoctanoic acid methyl ester with di-isobutylaluminum hydride according to known procedures to obtain 2-acetamido-3-hydroxyoctanal.

B. 2-Acetoxy-4-(*N*-methylacetamido)butanal

Treat the diethylacetal of 2-hydroxy-4-aminobutanal with acetic anhydride in pyridine followed by treatment of the resulting diethylacetal of 2-acetoxy-4-acetamidobutanal with sodium hydride and methyl iodide to obtain the diethylacetal of 2-acetoxy-4-(*N*-methylacetamido)butanal. Remove the acetal protecting group by means of acid to obtain 2-acetoxy-4-(*N*-methylacetamido)butanal.

PREPARATION 6

(±)-Mono-*N*-Methyl-2,5-Dideoxystreptamine

Prepared from *Cis*-4,8-Dioxatricyclo[5.1.0.0^{3,5}]Octane

A. (±)-2,4-Dihydroxy-mono-*N*-methyl-6,7-diazabicyclo[3.2.1]octane

1. Mix 2 gm. of *cis*-4,8-dioxatricyclo[5.1.0.0^{3,5}]octane, 0.8 gm. of monomethylhydrazine, and 1.4 gm. of anhydrous magnesium sulfate with 200 ml. of 2-methoxyethanol. Heat the mixture at reflux temperature under an inert atmosphere, e.g.

	24	25	1,473,733	25
of of rs. iter and on- ol: m- xy-	5 10	5 10	argon, for about 40 hours. Remove the solvent <i>in vacuo</i> and extract the resultant solid residue with 100 ml of hot methanol, filter, and evaporate the filtrate <i>in vacuo</i> to a residue of about 20 ml. and cool. Filter the resultant precipitate comprising ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane (yield=2.1 gm.); m.p.=177—178°C. 2. In the above procedure, by substituting for monomethylhydrazine equivalent amounts of other monoalkylhydrazines, e.g. monoethylhydrazine and monopropylhydrazine, there is obtained the corresponding monoalkyl derivative, e.g. ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -ethyl-6,7-diazabicyclo[3.2.1]octane or ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -propyl-6,7-diazabicyclo[3.2.1]octane, respectively. 3. Separate ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane by optical resolution procedures known in the art to obtain 2,4-dihydroxy-6- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane and 2,4-dihydroxy-7- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane, respectively. In similar manner, the ($\pm$)-mono- <i>N</i> -ethyl and ($\pm$)-mono- <i>N</i> -propyl derivatives, prepared as described in Preparation 6A (2), are separated by optical resolution procedures to obtain 2,4-dihydroxy-6- <i>N</i> -ethyl-6,7-diazabicyclo[3.2.1]octane, 2,4-dihydroxy-7- <i>N</i> -ethyl-6,7-diazabicyclo[3.2.1]octane, 2,4-dihydroxy-6- <i>N</i> -propyl-6,7-diazabicyclo[3.2.1]octane and 2,4-dihydroxy-7- <i>N</i> -propyl-6,7-diazabicyclo[3.2.1]octane, respectively. B. ($\pm$)-Mono- <i>N</i> -methyl-2,5-dideoxystreptamine 1. Mix 2.1 gm. of ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane and 0.5 gm. of Raney nickel in 90 ml. of water and hydrogenate at room temperature with an initial hydrogen pressure of 4 atmospheres, until hydrogenation uptake ceases (36 hours). Remove the catalyst by filtration and lyophilize the filtrate to obtain ($\pm$)-mono- <i>N</i> -methyl-2,5-dideoxystreptamine as a white hygroscopic solid (yield=2.12 gms.); m.p. 156—157°C. 2. In similar manner, treat each of ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -ethyl-6,7-diazabicyclo[3.2.1]octane and ($\pm$)-2,4-dihydroxy-mono- <i>N</i> -propyl-6,7-diazabicyclo[3.2.1]octane with hydrogen in the presence of Raney nickel. Isolate and purify each of the resultant products in a manner similar to that described to obtain, respectively, ($\pm$)-mono- <i>N</i> -ethyl-2,5-dideoxystreptamine and ($\pm$)-mono- <i>N</i> -propyl-2,5-dideoxystreptamine. C. 1- <i>N</i> -Methyl-2,5-dideoxy- <i>D</i> -streptamine 1. In the procedure of Preparation 6B (1), by using 2,4-dihydroxy-6- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane as starting compound in place of the racemic mixture of 2,4-dihydroxy-mono- <i>N</i> -methyl-6,7-diazabicyclo[3.2.1]octane, there is obtained 1- <i>N</i> -methyl-2,5-dideoxy- <i>D</i> -streptamine. 2. Similarly, by hydrogenating each of 2,4-dihydroxy-6- <i>N</i> -ethyl-6,7-diazabicyclo[3.2.1]octane and 2,4-dihydroxy-6- <i>N</i> -propyl-6,7-diazabicyclo[3.2.1]octane in the manner of Preparation 6B (1) and isolating each of the resulting products in a manner similar to that described, there is obtained, respectively, 1- <i>N</i> -ethyl-2,5-dideoxy- <i>D</i> -streptamine and 1- <i>N</i> -propyl-2,5-dideoxy- <i>D</i> -streptamine. PREPARATION 7 1- <i>N</i> -Alkyl-2-Deoxy- <i>D</i> -Streptamine Prepared from (1 <i>S</i>)-1-Acetamido-3-amino-1,3-dideoxy- <i>myo</i> -inositol A. (1 <i>S</i>)-1-Amino-3-(ethylamino)-1,3-dideoxy- <i>myo</i> -inositol To a solution of 10 gm. of (1 <i>S</i>)-1-acetamido-3-amino-1,3-dideoxy- <i>myo</i> -inositol in 100 ml. of ethanol add 6 gm. of acetaldehyde. Allow the solution to stand at room temperature for 16 hours, then to the reaction mixture containing the (1 <i>S</i>)-1-acetamido-3- <i>N</i> -ethylidene derivative thereby produced, cautiously add 5 gm. of sodium borohydride. Allow the mixture to stand for a further 24 hours, then evaporate to a small volume <i>in vacuo</i> . Add 6 N hydrochloric acid and reflux for 6 hours to hydrolyze the 1-acetamido group. Cool, evaporate <i>in vacuo</i> , dissolve the resultant residue in water, bring the aqueous solution to neutrality with 1 N ammonium hydroxide, then add the solution to a column of Amberlite IRC-50 resin (NH ₄ ⁺ cycle). Wash the column with water to remove inorganic salts, then elute the column with 2 N ammonium hydroxide. Concentrate the eluate <i>in vacuo</i> and chromatograph the resultant residue over silica gel (350 gms.) eluting with a mixture of chloroform: methanol: 7% ammonium hydroxide (1:2:1). Combine like fractions as determined by thin layer chromatography and evaporate the combined fractions to a residue comprising (1 <i>S</i>)-1-amino-3-ethylamino-1,3-dideoxy- <i>myo</i> -inositol.	5 10 15 20 25 30 35 40 45 50 55 60

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26B. 1-*N*-Ethyl-2-deoxy-*D*-streptamine

To 1 gm. of (1*S*)-1-amino-3-(ethylamino)-1,3-dideoxy-*myo*-inositol which has been carefully dried and finely powdered, add 10 ml. of acetyl bromide and 10 ml. of acetic anhydride. Seal the mixture in a glass pressure bottle and heat at 150°C for 6 hours. Cool, pour into 100 ml. of ethanol, evaporate to dryness, then treat the resultant residue with 10 ml. of acetic anhydride and 20 ml. of pyridine at room temperature overnight. Evaporate the reaction mixture to dryness and chromatograph the resultant residue over silica gel eluting with chloroform containing 1.5% methanol. Combine the fractions containing the major products of the reaction as determined by thin layer chromatography, evaporate the combined eluates to a residue, dissolve the foregoing residue in 100 ml. of 50% aqueous ethanol, add Raney nickel T-4 and Amberlite IR-401S resin (OH⁻ form) and hydrogenate for 2 days at 3.5 atmospheres hydrogen pressure. Filter the solution, evaporate, dissolve the resultant residue in 6 *N* hydrochloric acid and heat at reflux temperature for 6 hours. Cool, evaporate the solution *in vacuo*, redissolve the residue in water, neutralize the aqueous solution with ammonium hydroxide and apply the solution to an excess of Amberlite IRC-50 resin (NH₄⁺ form). Wash the column with water, then elute the product with 2 *N* ammonium hydroxide. Combine the like eluates as determined by thin layer chromatography and evaporate the combined eluates *in vacuo* to a residue comprising 1-*N*-ethyl-2-deoxy-*D*-streptamine. Purify by chromatography over silica gel eluting with the solvent system chloroform:methanol:7% ammonium hydroxide (1:2:1). Combine the like fractions as determined by thin layer chromatography and evaporate to a residue of 1-*N*-ethyl-2-deoxy-*D*-streptamine as a white amorphous solid [α]_D²⁰ -40° (water), mass spectrum *m/e* 191 (*M*+1)⁺.

C. Other 1-*N*-Alkyl-2-deoxy-*D*-streptamines

By substituting propionaldehyde for acetaldehyde in the procedure of Preparation 7A and by treating the corresponding resulting (1*S*)-1-amino-3-alkyl-amino-1,3-dideoxy-*myo*-inositol thereby formed according to the procedure described in Preparation 7B, there is obtained the corresponding 1-*N*-alkyl-2-deoxy-*D*-streptamine, e.g. 1-*N*-propyl-2-deoxy-*D*-streptamine.

PREPARATION 8

1-*N*-Alkyl-2-deoxy-*D*-streptamines

Prepared from Garamine

A. 1-*N*-Carbobenzyloxy-3,4;3',4'-di-*N,O*-carbonylgaramine

To a solution of 1 gm. of 1,3,3'-tri-*N*-carbobenzyloxygaramine in 50 ml. of dry dimethylformamide add 200 mg. of sodium hydride. Stir vigorously under a nitrogen atmosphere for 24 hours then dilute the solution cautiously with water. Add Amberlite IRC-50 resin (H⁺ form) to the solution until neutral, filter off the resin, and wash the resin well with ethanol. Evaporate the combined filtrate and washings *in vacuo* to a residue to obtain the bis-oxazolidinone derivative, i.e. 1-*N*-carbobenzyloxy-3,4;3',4'-di-*N,O*-carbonylgaramine.

B. 1-*N*-Propyl-2-deoxy-*D*-streptamine

Dissolve the bis-oxazolidinone prepared in Preparation 8A in 50 ml. of ethanol and hydrogenate for 16 hours at 3.5 atmospheres over a 10% palladium-on-carbon catalyst. Remove the catalyst by filtration and to the ethanol solution of the 3,4;3',4'-di-*N,O*-carbonylgaramine thereby formed add 0.5 gms. of propionaldehyde. Allow the solution to stand at room temperature for 6 hours, then add 0.5 gms. of sodium borohydride. Allow the foregoing reaction mixture to stand for a further 2 hours then evaporate *in vacuo* to a residue comprising 1-*N*-propyl-3,4;3',4'-di-*N,O*-carbonylgaramine. To this residue add 75 ml. of 6 *N* hydrochloric acid and heat the solution at reflux temperature for 6 hours. Evaporate *in vacuo* and redissolve the resultant residue in water. Neutralize with ammonium hydroxide and apply the solution to a column of Amberlite IRC-50 resin (NH₄⁺ form). Wash the column with water to remove the inorganic salts, then elute with 2 *N* ammonium hydroxide. Evaporate the ammonium hydroxide eluate *in vacuo* to a residue comprising 1-*N*-propyl-2-deoxy-*D*-streptamine. Purify by chromatography over silica gel in a manner similar to that described for the purification of 1-*N*-ethyl-2-deoxy-*D*-streptamine in Preparation 7B to obtain 1-*N*-propyl-2-deoxy-*D*-streptamine as a white amorphous solid.

C. Other 1-*N*-Alkyl-2-deoxy-*D*-streptamines

In the procedure of Preparation 8B by substituting acetaldehyde for propionaldehyde and by isolating and purifying each of the resultant products, there is

obtained the corresponding 1-*N*-alkyl-2-deoxystreptamine, e.g. 1-*N*-ethyl-2-deoxy-*D*-streptamine.

PREPARATION 9

(±)-Mono-*N*-alkyl-2-deoxystreptamine from

(±)-1,3-Di-*N*-Carbobenzyloxy-4,5-*O*-isopropylidene-2-deoxystreptamine

A. (±)-1-*N*-Carbobenzyloxy-3,4-*N,O*-carbonyl-2-deoxystreptamine

To a solution of 4.7 gms. of carefully dried (±)-1,3-di-*N*-carbobenzyloxy-4,5-*O*-isopropylidene-2-deoxystreptamine in 75 ml. of dry dimethylformamide add 0.5 gms. of sodium hydride and stir at room temperature under a dry nitrogen atmosphere for 24 hours. Evaporate the reaction mixture to a small volume, cautiously add aqueous ethanol to destroy any excess sodium hydride then immediately dilute the reaction product with a mixture comprising 30 ml. of ethanol, 50 ml. of acetic acid and 20 ml. of water. Allow the solution to stand at room temperature for 4 hours, then evaporate in vacuo and extract the resultant residue with hot ethanol. Concentrate the combined ethanol extracts and dilute the resultant residue with a small amount of water. Cool the solution and separate the resultant precipitate by filtration to obtain (±)-1-*N*-carbobenzyloxy-3,4-*N,O*-carbonyl-2-deoxystreptamine as a white crystalline solid.

B. (±)-1-*N*-Ethyl-3,4-*N,O*-Carbonyl-2-deoxystreptamine

1. Dissolve 2 gms. of (±)-1-*N*-carbobenzyloxy-3,4-*N,O*-carbonyl-2-deoxystreptamine in 60 ml. of acetic acid and hydrogenate at 3.5 atmospheres hydrogen pressure for 16 hours over a 10% palladium-on-carbon catalyst. Remove the catalyst by filtration, wash the catalyst with acetic acid then evaporate the combined filtrate and washing in vacuo to a residue comprising an oxazolidinone derivative, i.e. (±)-3,4-*N,O*-carbonyl-2-deoxystreptamine.

2. Dissolve the foregoing oxazolidinone derivative in 20 ml. of ethanol, add 0.5 ml. of acetaldehyde and allow the mixture to stand at 0°C for 4 hours. Add about an equimolar quantity of sodium cyanoborohydride relative to the starting oxazolidinone derivative and allow the mixture to stand for 24 hours at room temperature. Evaporate to a residue comprising (±)-1-*N*-ethyl-3,4-*N,O*-carbonyl-2-deoxystreptamine.

C. (±)-Mono-*N*-ethyl-2-deoxystreptamine

Dissolve the (±)-1-*N*-ethyl-3,4-*N,O*-carbonyl-2-deoxystreptamine obtained in Preparation 9B in 50 ml. of 6 *N* hydrochloric acid and heat at reflux temperature for 6 hours. Evaporate in vacuo, redissolve the resultant residue in water and neutralize with ammonium hydroxide. Apply the solution thereby obtained to a column of Amberlite IRC-50 resin (NH₄⁺ cycle) and wash the column with water to remove inorganic salts, then elute with 2 *N* ammonium hydroxide. Evaporate the eluate in vacuo to a residue comprising (±)-mono-*N*-ethyl-2-deoxystreptamine. Purify by chromatography over 150 gms. silica gel eluting with chloroform:methanol:7% ammonium hydroxide (1:1.5:1) solvent system.

D. Other (±)-Mono-*N*-alkyl-2-deoxystreptamines

In the procedure of Preparation 9B by substituting other aldehydes, e.g. propionaldehyde and formaldehyde for acetaldehyde and isolating and purifying the resultant product in a manner described, there is obtained the corresponding (±)-mono-*N*-alkyl-2-deoxystreptamine, e.g. (±)-mono-*N*-propyl-2-deoxystreptamine and (±)-mono-*N*-methyl-2-deoxystreptamine, respectively.

E. 1-*N*-Alkyl-2-deoxy-*D*-streptamine

Separate (±)-mono-*N*-ethyl-2-deoxystreptamine by optical resolution procedures known in the art to obtain 1-*N*-ethyl-2-deoxy-*D*-streptamine and 3-*N*-ethyl-2-deoxy-*D*-streptamine respectively. In similar manner separate each of (±)-mono-*N*-propyl-2-deoxystreptamine and (±)-mono-*N*-methyl-2-deoxystreptamine by optical resolution procedures known in the art to obtain 1-*N*-propyl-2-deoxy-*D*-streptamine and 3-*N*-propyl-2-deoxy-*D*-streptamine, and 1-*N*-methyl-2-deoxy-*D*-streptamine and 3-*N*-methyl-2-deoxy-*D*-streptamine, respectively.

PREPARATION 10

Preparation of mutamycin

A. Fermentation of *Micromonospora inyoensis* strain 1550F-1G NRRL 5742

Inoculum Preparation

Germination Stage 1:

Under aseptic conditions, add a lyophilized culture (or cells obtained from a

slant culture) of *M. inyoensis* strain 1550F-1G to a 300 ml. shake flask containing 100 ml. of the following sterile medium:

	Beef extract	3 g.
	Tryptose	5 g.
5	Yeast extract	5 g.
	Dextrose	1 g.
	Starch	24 g.
	Calcium carbonate	2 g.
	Tap water	1000 ml.

10 Incubate the flask and its contents for 2—5 days at 35°C on a rotary shaker (280 r.p.m., 2 inch stroke).

Germination Stage 2:

15 Aseptically transfer 25 ml. of the fermentation medium of germination stage 1 to a two-liter shake flask containing 500 ml. of the aforescribed sterile germination medium. Incubate the flask and its contents for three days at 28°C on a rotary shaker (280 r.p.m., 2 inch stroke).

Fermentation Stage:

Aseptically transfer 500 ml. of the inoculum obtained from germination stage 2 to a 14 l. fermentation tank containing 9.5 l. of the following sterile medium:

20	Dextrin	50 g.
	Dextrose	5 g.
	Soybean meal	35 g.
	Calcium carbonate	7 g.
	Cobalt chloride	10 ⁻⁶ molar
25	Tap water	1000 ml.
	Antifoam (GE 60)	10 ml.

30 Prior to sterilizing the aforescribed medium, adjust the pH to 8 and add an aqueous solution containing 8.0 gms. of streptamine. Ferment the contents under aerobic conditions for 48—240 hours, with stirring at 250 r.p.m., with air input at 4.5 liters per l./minute and at 2.5 atmospheres. The pH of the fermentation medium changes slightly during the antibiotic production, varying in the range of about 6.8 to about 7.3.

35 Monitor the antibiotic production using the assay procedure described for sisomicin and when peak production is attained, harvest the product (the fermentation is usually complete in about 7 days).

Peak production for mutamincins 1, 2, 4, 5 and 6 is in the order of magnitude of from about 5 to about 50 mcg/ml.

B. Isolation of Mutamicin 1

40 Add 7.0 g. of oxalic acid to the whole broth from Preparation A with stirring. Acidify the broth to pH 2.0 using 6 N sulfuric acid. Stir the mixture for about 15 minutes and filter using a suitable filter aid. Neutralize (pH 7.0) the filtrate with 6 N ammonium hydroxide. Pass the filtrate through a 1.0 liter cation exchange resin column in the ammonium form (e.g., Amberlite IRC-50, Rohm and Haas, Philadelphia, Pa.). Discard the spent broth and eluate the column with 2 N ammonium hydroxide collecting fractions of about 100 ml. Monitor the column eluate by disc testing each fraction against *Staphylococcus aureus* ATCC 6538P. Combine the active fractions and evaporate to about 100 ml. *in vacuo* and lyophilize to obtain a solid product. Triturate the product several times with warm methanol, filter and evaporate the filtrate to a residue. Chromatograph the product on silica gel (25 g.) using the lower phase of a chloroform:methanol:concentrated ammonium dioxide (1:1:1) system as the eluant. Combine and evaporate the fractions containing antibiotic activity to obtain thereby mutamicin 1. Mass Spectrum: (M+1)⁺ m/e 464 also m/e 118, 142, 160, 127, 161, 179, 189, 207, 315, 333, 320, 338, 348, 366, 378, 446.

55 C. In a similar manner, by substituting an equivalent quantity of 2,5-dideoxystreptamine, 2-*epi*-streptamine, 2,5-dideoxy-5-aminostreptamine or 5-*epi*-2-deoxystreptamine for streptamine, and by following the procedure of Preparations A and B, mutamincins 2, 4, 5 and 6, respectively, may be produced.

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containing

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Mass Spectral Data:

Mutamycin 2: (M+1)⁺ m/e 432 also m/e 127, 118, 142, 160, 129, 147, 157, 175, 255, 274, 283, 301, 288, 306, 316, 336, 346, 414, 431.

Mutamycin 4: (M+1)⁺ m/e 464 also m/e 127, 118, 142, 160, 161, 179, 189, 207, 315, 333, 320, 338, 348, 366, 378, 446.

Mutamycin 5: (M+1)⁺ m/e 447 also m/e 127, 118, 142, 160, 144, 162, 190, 270, 288, 316, 303, 321, 331, 349, 446.

Mutamycin 6: (M+1)⁺ m/e 448 also m/e 145, 163, 173, 191, 127, 118, 142, 160, 271, 289, 299, 317, 304, 322, 332, 350, 447.

EXAMPLE 1
1-N-substituted-sisomicin

A. 1-N-Ethylsisomicin

To a solution of 5 gm. of sisomicin in 250 ml. of water add 1 N sulfuric acid until the pH of the solution is adjusted to about 5. To the solution of sisomicin sulfuric acid addition salt thereby formed, add 2 ml. of acetaldehyde, stir for 10 minutes, then add 0.85 gm. of sodium cyanoborohydride. Continue stirring at room temperature for 15 minutes, then concentrate solution *in vacuo* to a volume of about 100 ml., treat the solution with a basic ion exchange resin (e.g. Amberlite IRA 401S (OH⁻)), then lyophilize to a residue comprising 1-N-ethylsisomicin.

Purify by chromatographing on 200 gm. of silica gel, eluting with lower phase of a chloroform-ethanol-7% aqueous ammonium hydroxide (2:1:1) system. Combine the like eluates as determined by thin layer chromatography and concentrate the combined eluates of the major component *in vacuo* to a residue comprising 1-N-ethylsisomicin (yield 1.25 gm.). Further purify by again chromatographing on 100 gm. of silica gel eluting with a chloroform-methanol-3.5% ammonium hydroxide (1:2:1) system. Pass the combined, like eluates (as determined by thin layer chromatography) through a column of basic ion exchange resin and lyophilize the eluate to obtain 1-N-ethylsisomicin (yield 0.54 gm.): $[\alpha]_D^{20} + 164^\circ$ (0.3%, H₂O); pmr (ppm) (D₂O): δ 1.05 (3H, t, J=7 Hz, —CH₂CH₃); 1.19 (3H, s, —C—CH₃); 2.5 (3H, s, N—CH₃); 4.85 (1H, m, =CH—); 4.95 (1H, d, J=4 Hz, H₁''); 5.33 (1H, d, J=2.5 Hz, H₁'). Mass Spectrum: (M+1)⁺ m/e 476 also m/e 127, 154, 160, 173, 191, 201, 219, 256, 299, 317, 332, 345, 350, 360, 378, 390, 400.

B. In the procedure of above Example 1A, instead of adding 1N sulfuric acid to the solution of sisomicin in water until it reaches a pH of about 5, other acids may be used, such as acetic acid, trifluoroacetic acid, p-toluene sulfonic acid, hydrochloric acid, phosphoric acid, or nitric acid. The acidified aqueous solution is then treated with acetaldehyde and sodium cyanoborohydride and the resultant product is purified as described above, whereby is obtained 1-N-ethylsisomicin.

C. Alternatively, in the procedure of Example 1A replace sodium cyanoborohydride with an equivalent quantity of other hydride donor-reducing agents. For example, with one of morpholinoborane, tetrabutylammonium cyanoborohydride or sodium borohydride, and there is obtained 1-N-ethylsisomicin.

D. 1-N-Methylsisomicin

To a solution of 4.64 gms. of sisomicin in 180 ml. of water add 1 N sulfuric acid until the solution is at a pH of about 5. Add 1.2 ml. of 37% aqueous formaldehyde, stir for 10 minutes then add 460 mg. of sodium cyanoborohydride. Pass the reaction solution through a column of a basic ion exchange resin (e.g. Amberlite IRA 401S (OH⁻ form)) and lyophilize. Chromatograph the resultant residue on silica gel in the lower phase of a chloroform-methanol-7% aqueous ammonium hydroxide (2:1:1) solvent mixture. Combine the like eluates containing substantially 1-N-methylsisomicin as determined by thin layer chromatography. Evaporate *in vacuo* to a residue of 1-N-methylsisomicin; $[\alpha]_D^{20} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

E. 1-N-(n-propyl)sisomicin

In a manner similar to that described in Example 1A treat the sulfuric acid addition salt of sisomicin in water with propanal and sodium cyanoborohydride. Isolate and

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purify the resultant product in a manner similar to that described to obtain 1-*N*-(*n*-propyl)sisomicin; $[\alpha]_D^{20} + 140^\circ$ (0.3%, H_2O); pmr (ppm) (D_2O): δ 0.83 (3H, t, $J=7$ Hz, $-CH_2CH_3$); 1.14 (3H, s, $-C-CH_3$); 2.45 (3H, s, $-N-CH_3$); 4.82 (1H, m, $=CH-$); 4.90 (1H, d, $J=4$ Hz, H_1''); 5.78 (1H, d, $J=2$ Hz, H_1'); Mass Spectrum: $(M+1)^+$ m/e 490 also 127, 160, 168, 187, 205, 215, 233, 256, 313, 331, 346, 359, 364, 374, 404, 414.

F. 1-*N*-(*n*-butyl)sisomicin

To a solution of 3 gms. of sisomicin in 200 ml. of water add 1 N sulfuric acid until the solution is at a pH of about 3.5. Add 1.5 ml. of *n*-butanal, stir for 10 minutes then add 450 mg. of sodium cyanoborohydride. Continue stirring for one hour then concentrate the solution *in vacuo* to a volume of about 100 ml. Pass this solution through a column of a basic ion exchange resin (e.g. Amberlite IRA 401S (OH⁻)) and lyophilize. Chromatograph the resultant residue on 140 gm. of silica gel in the lower phase of a chloroform-methanol-7% aqueous ammonium hydroxide (2:1:1) solvent mixture. Combine the like fractions containing 1-*N*-(*n*-butyl)sisomicin as determined by thin layer chromatography and evaporate the combined eluates *in vacuo* to a residue comprising 1-*N*-(*n*-butyl)-sisomicin; $[\alpha]_D^{20} + 129^\circ$ (0.3%, H_2O), pmr (ppm) (D_2O): δ 0.82 (3H, t, $J=7$ Hz, $-CH_2CH_3$); 1.15 (3H, s, $-C-CH_3$); 2.46 (3H, s, $-N-CH_3$); 4.82 (1H, m, $=CH-$); 4.92 (1H, d, $J=4$ Hz, H_1''); 5.29 (1H, d, $J=2$ Hz, H_1'). Mass Spectrum: $(M+1)^+$ m/e 504; also m/e 127, 160, 182, 201, 219, 229, 247, 256, 327, 345, 360, 373, 388, 418, 428.

G. Other 1-*N*-Alkyl, 1-*N*-Alkenyl and 1-*N*-Aralkyl Sisomicins

In the procedure of Example 1A instead of acetaldehyde substitute equivalent amounts of each of the following alkyl aldehydes:

1. 2-methylpropanal,
2. *n*-pentanal,
3. 3-methylbutanal,
4. 2-methylbutanal,
5. 2,2-dimethylpropanal,
6. 2-ethylbutanal,
7. *n*-octanal,
8. propenal,
9. 2-ethyl-2-hexenal,
10. benzaldehyde, and
11. phenylacetaldehyde.

In each case carry out the reaction in a manner similar to that described in Example 1A and isolate and purify each of the resultant products in a manner similar to that described to obtain, respectively,

1. 1-*N*-(β -methylpropyl)sisomicin,
2. 1-*N*-(*n*-pentyl)sisomicin,
3. 1-*N*-(γ -methylbutyl)sisomicin,
4. 1-*N*-(β -methylbutyl)sisomicin,
5. 1-*N*-(β,β -dimethylpropyl)sisomicin,
6. 1-*N*-(β -ethylbutyl)sisomicin $[\alpha]_D^{20} + 121^\circ$ ($c=0.4\%$, H_2O); pmr (PPm) D_2O : δ 0.75 (6H, t, 6.5Hz, CH_2-CH_3); 2.4 (3H, s, $N-CH_3$); 4.78 (1H, m, $=CH-$); 4.88 (1H, d, 4.0Hz, H_1''); 5.22 (1H, d, 2.0Hz, H_1'); Mass Spectrum: $(M+1)^+$ m/e 532 also m/e 127, 160, 210, 229, 256, 275, 355, 373, 388, 401, 406, 416, 446, 456,
7. 1-*N*-(*n*-octyl)sisomicin,
8. 1-*N*-(β -propenyl)sisomicin,
9. 1-*N*-(β -ethyl- β -hexenyl)sisomicin,
10. 1-*N*-benzylsisomicin, and
11. 1-*N*-phenylethylsisomicin.

H. 1-*N*-(Hydroxyalkyl)sisomicins

In the procedure of Example 1A, instead of acetaldehyde, substitute equivalent amounts of each of the following aldehydes:

1. 5-hydroxypentanal,
2. 2-hydroxypropanal,
3. 2-hydroxy-3-methylbutanal,

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4. 2-hydroxy-2-methylpropanal,
5. 4-hydroxybutanal,
6. 8-hydroxyoctanal, and
7. 2-hydroxy-4-pentenal.

In each case carry out the reaction in a manner similar to that described in Example 1A, and isolate and purify each of the resultant products in a manner similar to that described to obtain respectively,

1. 1-N-(ε-hydroxypentyl)sisomicin,
2. 1-N-(β-hydroxypropyl)sisomicin,
3. 1-N-(β-hydroxy-γ-methylbutyl)sisomicin,
4. 1-N-(β-hydroxy-β-methylpropyl)sisomicin,
5. 1-N-(δ-hydroxybutyl)sisomicin,
6. 1-N-(ω-hydroxyoctyl)sisomicin,
7. 1-N-(β-hydroxy-δ-pentenyl)sisomicin.

I. 1-N-(δ-Aminobutyl)sisomicin

Add 1 N sulfuric acid dropwise to a solution of 3 gm. of sisomicin in 120 ml. of water until the pH of the solution is adjusted to about 5. To the aqueous solution of the sulfuric acid addition salt of sisomicin thereby formed, add 60 ml. of dimethylformamide followed by a solution of 2 gm. of 4-phthalimidobutanal in 10 ml. of dimethylformamide. Continue stirring for 10 minutes then add 420 mg. of sodium cyanoborohydride. After about 20 minutes add the reaction solution to 1 liter of anhydrous methanol with stirring and collect by filtration the resultant precipitate comprising the sulfuric acid addition salt of 1-N-(δ-phthalimidobutyl)sisomicin.

Purify by dissolving the precipitate in water and passing the aqueous solution over a basic ion exchange resin. Evaporate *in vacuo* to a residue, chromatograph the residue over silica gel eluting with the lower phase of a chloroform-methanol-7% aqueous ammonium hydroxide (2:1:1) solvent mixture, and evaporate the combined, like eluates to a residue comprising 1-N-(δ-phthalimidobutyl)-sisomicin.

To 0.5 gm. of 1-N-(δ-phthalimidobutyl)sisomicin, add 5 ml. of 5% ethanolic hydrazine hydrate and heat under reflux for 3 hours. Pour the reaction solution into a large volume of tetrahydrofuran and collect by filtration the resulting precipitate comprising 1-N-(δ-aminobutyl)sisomicin.

Alternatively, the compound of this example is prepared as follows:

(1) 4-Acetamidobutyraldehyde

Dissolve 5 gms. of 4-acetamidobutyraldehyde diethyl acetal in 75 ml. of distilled water and 5 ml. of 1 N sulfuric acid. Allow the solution to stand at room temperature until the hydrolysis is complete as determined by thin layer chromatography. Neutralize the solution with sodium bicarbonate then saturate the solution with sodium chloride and extract with chloroform. Distil the combined chloroform extracts to a residue comprising 4-acetamidobutyraldehyde, which can be used without further purification in the following procedure.

(2) 1-N-(δ-Acetamidobutyl)sisomicin

To 3 gms. of sisomicin in 120 ml. of distilled water add 0.1 N sulfuric acid until the solution is at about pH 5. Add 6 gms. of δ-acetamidobutyraldehyde prepared as described in the preceding procedure followed, after 10 minutes, with 600 gms. of solid sodium cyanoborohydride. After 2 hours, concentrate the solution to a small volume and pour into methanol. Collect the resultant precipitate by filtration, dissolve in water and pass the aqueous solution through a column of Amberlite IRA 401-S (OH⁻) ion exchange resin. Evaporate the eluant and chromatograph the resultant residue on silica gel eluting with the lower phase of a chloroform:methanol:7% ammonium hydroxide solvent mixture. Evaporate the eluant to a residue comprising 1-N-(δ-acetamidobutyl)sisomicin.

(3) 1-N-(δ-aminobutyl)sisomicin

Treat 1-N-(δ-acetamidobutyl)sisomicin obtained in the preceding example with 10% aqueous potassium hydroxide at 100°C for 3 hours, then neutralize with Amberlite IRC-50 ion exchange resin and elute with 2 N aqueous ammonium hydroxide. Concentrate the eluant and dissolve the resultant residue in water and lyophilize to obtain 1-N-(δ-aminobutyl)-sisomicin. $[\alpha]_D^{25} + 109^\circ$ (c=0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 519 also 127, 160, 197, 216, 234, 244, 256, 262, 342, 360, 375, 388, 393, 403, 443.

J. Other 1-*N*-(Aminoalkyl)sisomicins and 1-*N*-(Hydroxyaminoalkyl)sisomicins

In a manner similar to that described in Example 1I, treat the sulfuric acid addition salt of sisomicin in aqueous dimethylformamide with sodium cyanoborohydride, and with each of the following amino substituted aldehydes:

- 5 1. 3-phthalimidopropanal,
2. 5-phthalimidopentanal,
3. 2-phthalimidopropanal,
4. 2-hydroxy-5-phthalimidopentanal,
- 10 5. 3-methyl-3-hydroxy-4-phthalimidobutanal,
6. 2-hydroxy-4-phthalimidobutanal,
7. 2-phthalimido-3-methylbutanal,
8. 2-hydroxy-3-phthalimidopropanal,
9. 2-hydroxy-2-methyl-3-phthalimidopropanal, and
10. 8-phthalimidoctanal.

15 In each case carry out the reaction in a manner similar to that described in Example 1A, and isolate and purify each of the resultant products in a manner similar to that described to obtain, respectively,

1. 1-*N*-(γ -phthalimidopropyl)sisomicin,
2. 1-*N*-(ϵ -phthalimidopentyl)sisomicin,
- 20 3. 1-*N*-(β -phthalimidopropyl)sisomicin,
4. 1-*N*-(β -hydroxy- ϵ -phthalimidopentyl)sisomicin,
5. 1-*N*-(γ -methyl- γ -hydroxy- δ -phthalimidobutyl)sisomicin,
6. 1-*N*-(β -hydroxy- δ -phthalimidobutyl)sisomicin,
7. 1-*N*-(β -phthalimido- γ -methylbutyl)sisomicin,
- 25 8. 1-*N*-(β -hydroxy- γ -phthalimidopropyl)sisomicin,
9. 1-*N*-(β -hydroxy- β -methyl- γ -phthalimidopropyl)sisomicin, and
10. 1-*N*-(ω -phthalimidoctyl)sisomicin.

Treat each of the foregoing *N*-phthalimidoalkyl sisomicin derivatives with ethanolic hydrazine hydrate as described in Example 1I to obtain, respectively,

- 30 1. 1-*N*-(γ -aminopropyl)sisomicin,
2. 1-*N*-(ϵ -aminopentyl)sisomicin,
3. 1-*N*-(β -aminopropyl)sisomicin,
4. 1-*N*-(β -hydroxy- ϵ -aminopentyl)sisomicin,
5. 1-*N*-(γ -methyl- γ -hydroxy- δ -aminobutyl)sisomicin,
- 35 6. 1-*N*-(β -hydroxy- δ -aminobutyl)sisomicin,
7. 1-*N*-(β -amino- γ -methylbutyl)sisomicin,
8. 1-*N*-(β -hydroxy- γ -aminopropyl)sisomicin,
9. 1-*N*-(β -hydroxy- β -methyl- γ -aminopropyl)sisomicin, and
10. 1-*N*-(ω -aminooctyl)sisomicin.

40 K. Alkylaminoalkylsisomicins and Other Hydroxyaminoalkylsisomicins

(1) 1-*N*-(β -methylaminoethyl)sisomicin

In a manner similar to that described in Example 1A treat sisomicin in water with 1 N sulfuric acid and 2-(*N*-methylacetamido)acetaldehyde followed by sodium cyanoborohydride. Isolate the resultant product in a manner similar to that described to obtain 1-*N*-(β -(*N*-methylacetamido)ethyl)sisomicin.

45 Treat the foregoing *N*-acetylated intermediate with 10% aqueous sodium hydroxide for 3 hours at 100°C, pour the foregoing reaction solution onto Amberlite IRC 50 ion exchange resin, elute with 2 M ammonium hydroxide, concentrate the combined eluates *in vacuo* to a volume of about 100 ml., then lyophilize to a residue comprising 1-*N*-(β -methylaminoethyl)sisomicin.

50 (2) In the procedure described in above Example 1K(1) substitute for 2-(*N*-methylacetamido)acetaldehyde other aldehydes such as 2-acetamido-3-hydroxyoctanal or 2-acetamido-4-pentenal as aldehyde reagents to obtain the corresponding 1-*N*-(aminoalkyl)sisomicin, e.g. 1-*N*-(β -amino- γ -hydroxyoctyl)sisomicin and 1-*N*-(β -amino- δ -pentenyl)sisomicin, respectively.

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L. 1-*N*-(β -Aminoethyl)sisomicin and 1-*N*-(γ -Aminopropyl)sisomicin

In a manner similar to that described in the alternate procedure of Example 1-I, treat an aqueous solution of sisomicin to which 0.1 N sulfuric acid has been added

until the solution is at about pH 5, with β -acetamidoacetaldehyde followed by solid sodium cyanoborohydride. Isolate and purify the resultant product in a manner similar to that described to obtain 1-N-(β -acetamidoethyl)sisomicin. Hydrolyze the 1-N-(β -acetamidoethyl)sisomicin with 10% aqueous potassium hydroxide and isolate and purify the resultant product in a manner similar to that described in Paragraph (3) of the alternate procedure of Example 1-I to obtain 1-N-(β -aminoethyl)sisomicin. Mass Spectrum: (M+1)⁺ m/e 491, also 160, 169, 187, 206, 216, 234, 256, 283, 314, 325, 334, 347, 360, 370, 375, 405, 415.

In the above procedure, by substituting γ -acetamidopropanal for β -acetamidoacetaldehyde, there is formed 1-N-(γ -acetamidopropyl)sisomicin, which when hydrolyzed with 10% aqueous potassium hydroxide and isolated and purified in the described manner yields 1-N-(γ -aminopropyl)sisomicin.

EXAMPLE 2

1-N-substituted-Gentamicin C_{1a}

A. 1-N-Ethylgentamicin C_{1a}

To 5 gm. of gentamicin C_{1a} in 125 ml. of water add 1 N sulfuric acid until the pH of the solution is about 5.2. Then add 2 ml. of acetaldehyde. Stir the solution for 5 minutes, then add 1 gm. of sodium cyanoborohydride. Continue stirring at room temperature for 30 minutes, concentrate the solution *in vacuo* to a volume of about 75 ml., pass the solution through a column of a basic ion exchange resin, (e.g. Amberlite IRA 401S (OH⁻)), then lyophilize to a residue comprising 1-N-ethylgentamicin C_{1a}.

Purify by chromatographing on 200 gm of silica gel eluting with the lower phase of a chloroform-methanol-7% aqueous ammonium hydroxide (2:1:1) system. Combine the like eluates as determined by thin layer chromatography and concentrate the combined eluates of the major component *in vacuo* to a residue comprising 1-N-ethylgentamicin C_{1a} (yield—0.95 gm.). Further purify by again chromatographing the 1-N-ethylgentamicin C_{1a} on 100 gm. of silica gel eluting with chloroform-methanol-3.5% ammonium hydroxide (1:2:1) system. Treat the combined, like eluates (as determined by thin layer chromatography) with a basic ion exchange resin and lyophilize the eluate to obtain 1-N-ethylgentamicin C_{1a} (0.42 gm.), [α]_D²⁰ +118° (c=0.3%, H₂O); pmr (ppm) (D₂O): δ 1.06 (3H, t, J=7Hz, —CH₂—CH₃); 1.19 (3H, s, —C—CH₃); 2.51 (3H, s, —N—CH₃); 4.97 (1H, d, J=4Hz, H₁"); 5.16 (1H, d, J=3.5Hz, H₁'). Mass Spectrum (M+1)⁺ m/e 478 also m/e 129, 154, 160, 173, 191, 201, 219, 258, 301, 317, 319, 329, 332, 347, 350, 360, 378 and 402.

B. In the procedure of Example 2A instead of using 1 N sulfuric acid to adjust the pH to about 5.2, add other acids such as acetic acid, *p*-toluenesulfonic acid, trifluoroacetic acid, hydrochloric acid, phosphoric acid, or nitric acid. Treat the acidified aqueous solution of gentamicin C_{1a} thereby produced with acetaldehyde and sodium cyanoborohydride in a manner similar to that described in above Example 2A and isolate and purify the resultant product in a manner similar to obtain 1-N-ethylgentamicin C_{1a}.

C. In the procedure of Example 2A, by substituting for acetaldehyde other aldehyde reagents, e.g. formaldehyde, *n*-propanal, *n*-butanal, *n*-octanal, hydroxyacetaldehyde, 4-hydroxybutanal and phenylacetaldehyde there is obtained the corresponding 1-N-alkylgentamicin C_{1a}, e.g. 1-N-methylgentamicin C_{1a}, 1-N-(*n*-propyl)gentamicin C_{1a}, 1-N-(*n*-butyl)gentamicin C_{1a}, 1-N-(*n*-octyl)gentamicin C_{1a}, 1-N-(β -hydroxyethyl)gentamicin C_{1a}, 1-N-(δ -hydroxybutyl)gentamicin C_{1a}, and 1-N-(phenylethyl)gentamicin C_{1a}, respectively.

EXAMPLE 3

1-N-Substituted-Verdamicin

A. 1-N-Ethylverdamicin

To 0.5 gm. of verdamicin in 65 ml. of water add 1 N sulfuric acid until the pH of the solution is adjusted to about pH 4.9, then add 0.2 ml. of acetaldehyde. Stir the solution for 5 minutes, add 65 mg. of sodium cyanoborohydride, concentrate the solution *in vacuo* to a volume of about 10 ml. and pour the solution into 50 ml. of methanol with stirring. Collect by filtration the resultant precipitate comprising 1-N-ethylverdamicin. Purify by chromatographing on 100 gm. of silica gel eluting with a chloroform-methanol-3.5% ammonium hydroxide (1:2:1) system. Collect the like fractions as determined by thin layer chromatography and evaporate *in vacuo* the combined fractions containing the major component to a residue comprising 1-N-ethylverdamicin. Further purify by again chromatographing on 7 gm. of silica gel eluting with the lower phase of a chloroform-methanol-7% ammonium hydroxide

2:1:1) system. Combine the like eluates and evaporate *in vacuo* to a residue of 1-*N*-ethylverdamicin (yield 50 mg.); Mass Spectrum: $(M+1)^+$ m/e 490 also m/e 141, 154, 160, 173, 191, 201, 219, 270, 313, 317(w), 331, 332, 341, 350(w), 357, 359, 360, 378, 390, 414.

5 B. In the procedure of above Example 3A substitute for acetaldehyde other aldehyde intermediates, e.g. formaldehyde, *n*-propanal, *n*-butanal, *n*-octanal, hydroxyacetaldehyde, 4-hydroxybutanal and phenylacetaldehyde. Isolate and purify each of the resultant products in a manner similar to that to obtain, respectively, 1-*N*-methylverdamicin, 1-*N*-(*n*-propyl)verdamicin $[\alpha]_D^{20} + 122^\circ$ (*c*=0.3%, H₂O); pmr (ppm) (D₂O): δ 0.88 (3H, t, J=7Hz, CH₂CH₃); 1.19 (3H, s, C—CH₃); 1.16 (3H, d, J=6Hz, CH—CH₃); 4.81 (1H, m, =CH—); 4.97 (1H, d, J=4.0Hz, H₁''); 5.30 (1H, d, J=2.0Hz, =H₁'); Mass Spectrum: $(M+1)^+$ m/e 528 also m/e 141, 160, 168, 187, 205, 215, 233, 270, 346, 355, 373, 504.

10 1-*N*-(*n*-butyl)verdamicin $[\alpha]_D^{20} + 121^\circ$ (*c*=0.3%, H₂O); pmr (ppm) (D₂O): δ 0.8 (3H, t, J=6.5Hz, CH₂—CH₃); 2.45 (3H, s, NCH₃); 4.8 (1H, m, C=CH—); 4.92 (1H, d, J=4.0Hz, H₁''); 5.25 (1H, d, J=2.0Hz, H₁'); Mass Spectrum: $(M+1)^+$ m/e 518 also m/e 141, 160, 182, 201, 219, 229, 247, 270, 341, 360, 378, 387, 388, 418, 442.

15 1-*N*-(β -hydroxyethyl)verdamicin, 1-*N*-(δ -hydroxybutyl)verdamicin, and 1-*N*-phenylethylverdamicin.

EXAMPLE 4

1-*N*-Substituted-Gentamicin C₁

A. 1-*N*-Ethylgentamicin C₁

25 In a manner similar to that described in Example 1A treat 5 gm. of gentamicin C₁ in 250 ml. of water with 1 N sulfuric acid until the pH of the solution is about pH 5; then treat the acidified solution with acetaldehyde and sodium cyanoborohydride in a manner similar to that described and isolate and purify the resultant product to obtain 1-*N*-ethylgentamicin C₁, $[\alpha]_D^{20} + 114^\circ$ (*c*=0.3%, H₂O); pmr (ppm) (D₂O): δ 1.03 (3H, t, 7Hz, —CH₂CH₃); 1.03 (3H, d, J=6.5Hz, —CH—CH₃); 1.17 (3H, s, C—CH₃); 2.32 (3H, s, 6'-N—CH₃); 2.49 (3H, s, 3''—NHCH₃); 4.94 (1H, d, J=4Hz, H₁''); 5.13 (1H, d, J=3.5Hz, H₁'). Mass Spectrum. $(M+1)^+$ m/e 506 also m/e 154, 157, 160, 173, 191, 201, 219, 286, 317, 329(w), 347, 350, 360, 375, 430.

30 B. In the procedure of above Example 4A in place of acetaldehyde utilize other aldehyde reagents, e.g. formaldehyde, *n*-propanal, *n*-butanal, *n*-octanal, hydroxyacetaldehyde, 4-hydroxybutanal and phenylacetaldehyde. Isolate and purify each of the resultant products in a manner similar to that to obtain, respectively, 1-*N*-methylgentamicin C₁, 1-*N*-(*n*-propyl)gentamicin C₁, 1-*N*-(*n*-butyl)gentamicin C₁, 1-*N*-(*n*-octyl)gentamicin C₁, 1-*N*-(β -hydroxyethyl)gentamicin C₁, $[\alpha]_D^{20} + 98.0^\circ$ (*c*=0.3%, H₂O); pmr (ppm) (D₂O): δ 0.99 (3H, d, J=6.5Hz, 6'—CH₃); 1.13 (3H, s, 4''—CH₃); 2.28 (3H, s, 6'—NCH₃); 2.45 (3H, s, 3''—NCH₃); 4.97 (1H, d, J=4Hz, H₁'') and 5.11 (1H, d, J=3.5 Hz, H₁'); Mass Spectrum $(M+1)^+$ m/e 522 also m/e 446, 404, 394, 391, 376, 373, 366, 363, 348, 345, 333, 286, 235, 217, 207, 189, 160, 157; ν max (KBr) 3300, 1060 cm⁻¹.

45 1-*N*-(δ -hydroxybutyl)gentamicin C₁, and 1-*N*-(phenylethyl)gentamicin C₁ $[\alpha]_D^{20} + 99.4^\circ$ (*c*=0.3%, H₂O); pmr (ppm) (D₂O): δ 0.99 (3H, d, J=6.5 Hz, 6'—CH₃); 1.10 (3H, s, 4''—CH₃); 2.28 (3H, s, 6'—NCH₃); 2.43 (3H, s, 3''—NCH₃); 4.88 (1H, d, J=4Hz, H₁''), 5.08 (1H, d, J=3.5Hz, H₁') and 7.33 (5H, s, —C₆H₅); Mass Spectrum $(m+1)^+$ m/e 582 also m/e 506, 464, 454, 451, 436, 433, 426, 423, 408, 405(w), 393, 295, 286, 277, 267, 249, 160, 157; ν max (KBr) 3300, 1050, 1030 cm⁻¹, respectively.

EXAMPLE 5

1-*N*-Substituted-Antibiotic G-52

A. 1-*N*-Ethyl Antibiotic G-52

55 Dissolve 875 mg. of Antibiotic G-52 in 40 ml. of distilled water and add 1 N sulfuric acid until the pH of the solution is adjusted to about 3.5. Add 0.7 ml. of acetaldehyde, stir the reaction mixture for 10 minutes then add 100 mg. of sodium cyanoborohydride. Monitor the reaction solution by thin layer chromatography, and when the starting Antibiotic G-52 appears to have completely reacted (i.e. about 10 minutes) then concentrate the solution *in vacuo* at about 35 to 40°C to a volume of about 10 ml. Pass the concentrated solution through a basic ion exchange resin then lyophilize to a residue comprising 1-*N*-ethyl Antibiotic G-52. Purify by chromatographing on a silica gel column (4' by 1/2'' column) eluting with the lower phase of a

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chloroform-methanol-7% aqueous ammonium hydroxide (2:1:1) system. Combine the like eluates as determined by thin layer chromatography and concentrate the combined eluates of the major component *in vacuo* to a residue comprising 1-N-ethyl Antibiotic G-52 (yield 60 mg.). Further purify the overlap eluates from the foregoing chromatography by chromatographing on silica gel eluting with the lower phase of a chloroform-methanol-7% aqueous ammonium hydroxide (1:2:1) system to obtain an additional 35 mg. of residue comprising 1-N-ethyl Antibiotic G-52. Pass the combined residues of 1-N-ethyl Antibiotic G-52 through a column of basic ion exchange resin (e.g. Amberlite IRA 401S) and lyophilize the eluate to obtain 1-N-ethyl Antibiotic G-52 (yield 90 mg); $[\alpha]_D^{20} + 122.1^\circ$ (c=0.3%, H₂O), pmr (ppm) (D₂O): δ 1.06 (3H, t, J=6.5Hz, 1N—CH₂CH₃); 1.21 (3H, s, 4''—C—CH₃); 2.30 (3H, s, 3''—N—CH₃); 2.50 (3H, s, 6'—N—CH₃); 4.94 (1H, m, H₁'); 4.97 (1H, d, J=4.0Hz H₁''); 5.34 (1H, d, J=2.5Hz, H₁'). Mass Spectrum: (M+1)⁺ m/e 490 also m/e 141, 154, 160, 173, 191, 201, 219, 270, 313, 317(w), 331, 332, 341, 350, 359, 360, 378, 390, 414.

B. In the procedure of above Example 5A in place of acetaldehyde use other aldehyde reagents such as formaldehyde, *n*-propanal, *n*-butanal, *n*-octanal, hydroxyacetaldehyde, 4-hydroxybutanal and phenylacetaldehyde. Isolate and purify each of the resultant products in a manner similar to that described in Example 5A to obtain, respectively, 1-N-methyl-Antibiotic G-52, 1-N-(*n*-propyl)-Antibiotic G-52, 1-N-(*n*-butyl)-Antibiotic G-52, 1-N-(β -hydroxyethyl)-Antibiotic G-52, 1-N-(δ -hydroxybutyl)-Antibiotic G-52 and 1-N-phenylethyl-Antibiotic G-52.

EXAMPLE 6

A. In a manner similar to that described in Example 1A treat each of the following 4,6-diaminoglycosyl-1,3-diaminocyclitols in water with sulfuric acid followed by acetaldehyde and sodium cyanoborohydride:

1. gentamicin A,
2. gentamicin B,
3. gentamicin B₁,
4. gentamicin C₁,
5. gentamicin C_{1a},
6. gentamicin C₂,
7. gentamicin C_{2a},
8. gentamicin C_{2b},
9. gentamicin X₂,
10. Antibiotic G-418,
11. Antibiotic JI-20A,
12. Antibiotic JI-20B,
13. tobramycin,
14. Antibiotic 66-40B,
15. Antibiotic 66-40D,
16. mutamicin 1,
17. mutamicin 2,
18. mutamicin 4,
19. mutamicin 5,
20. mutamicin 6.

Isolate and purify each of the resultant products in a manner similar to that described in Example 1A to obtain the corresponding 1-N-ethyl compound, i.e.

1. 1-N-ethylgentamicin A,
2. 1-N-ethylgentamicin B,
3. 1-N-ethylgentamicin B₁,
4. 1-N-ethylgentamicin C₁,
5. 1-N-ethylgentamicin C_{1a},
6. 1-N-ethylgentamicin C₂,
7. 1-N-ethylgentamicin C_{2a},
8. 1-N-ethylgentamicin C_{2b},
9. 1-N-ethylgentamicin X₂,
10. 1-N-ethyl Antibiotic G-418,
11. 1-N-ethyl Antibiotic JI-20A,
12. 1-N-ethyl Antibiotic JI-20B,
13. 1-N-ethyltobramycin,

14. 1-N-ethyl Antibiotic 66-40B,
15. 1-N-ethyl Antibiotic 66-40D,
16. 1-N-ethylmutamicin 1,
17. 1-N-ethylmutamicin 2,
18. 1-N-ethylmutamicin 4,
19. 1-N-ethylmutamicin 5,
20. 1-N-ethylmutamicin 6.

B. In the procedure of Example 6A by utilizing propanal in place of acetaldehyde there is obtained the corresponding 1-N-(n-propyl) derivative of each of the 4,6-diaminoglycosyl-1,3-diaminocyclitols listed therein.

C. By treating each of the 4,6-diaminoglycosyl-1,3-diaminocyclitol starting compounds listed in above Example 6A in a manner similar to that described in any of Examples 1A through 1K there is obtained the corresponding 1-N-alkyl derivative thereof.

EXAMPLE 7

Preparation of 1-N-substituted-4,6-di-(Aminoglycosyl)-1,3-di-aminocyclitols via Selectively Blocked Intermediates

A. 1-N-Ethylgentamicin C₁ via a 2',3-di-N-substituted Intermediate

Dissolve 240 mg. of 2',3-di-N-trifluoroacetylgentamicin C₁ in 10 ml. of water-methanol (2:1) and adjust the pH of the solution to about 3.5 by adding 1 N sulfuric acid. Add 0.19 ml. of acetaldehyde, stir for 10 minutes, then add 27 mg. of sodium cyanoborohydride, and stir the reaction mixture for an additional 10 minutes. Evaporate the reaction mixture *in vacuo* to a residue comprising 1-N-ethyl-2',3-di-N-trifluoroacetylgentamicin C₁. Dissolve the foregoing residue in 50 ml. of concentrated ammonium hydroxide and allow the solution to stand at room temperature for 24 hours. Evaporate the mixture *in vacuo* and chromatograph the resultant residue over silica gel (12 g.) eluting with the lower phase of a mixture of chloroform-methanol-10% aqueous ammonium hydroxide (2:1:1). Combine the like fractions (as determined by thin layer chromatography) and evaporate the combined eluates *in vacuo* to a residue comprising 1-N-ethylgentamicin C₁. (Yield=80 mg.)

B. 1-N-Ethylsisomicin via a 6'-N-substituted intermediate

In a manner similar to that described in above Example 7A, treat 6'-N-t-butoxycarbonylsisomicin (prepared in a manner similar to that of Preparation 3D) in aqueous methanol with sulfuric acid, then acetaldehyde and sodium cyanoborohydride. Allow the reaction mixture to stand at room temperature for 30 minutes then evaporate *in vacuo* to obtain 1-N-ethyl-6'-N-t-butoxycarbonylsisomicin. Dissolve the foregoing residue in trifluoroacetic acid and allow the solution to stand for 10 minutes. Then add to an excess of anhydrous methanol, filter the resultant precipitate of the trifluoroacetic acid salt of 1-N-ethylsisomicin and chromatograph over silica gel using the lower phase of a chloroform-methanol-ammonium hydroxide solvent system in a manner similar to that described in Example 7A to obtain 1-N-ethylsisomicin.

C. 1-N-Methylsisomicin from 3''-N-4''-O-carbonyl-sisomicin

3''-N-4''-O-carbonyl-sisomicin (5 gm.) in distilled water (300 ml.) is treated with 1 N sulphuric acid until the pH of the solution is 2.5. Aqueous formaldehyde (37%) (2 ml.) is added and, after ten minutes, a solution of sodium cyanoborohydride (500 mg.) in water (5 ml.) is added dropwise. After 1 hour, the volume of the solution is reduced to one half by evaporation *in vacuo*, the pH of the concentrated solution is adjusted to 11 with addition of 1 N sodium hydroxide solution, and the solution is evaporated to dryness. The residue is extracted with methanol (3×100 ml.) and the combined methanol extracts are diluted with an equal volume of chloroform, filtered and evaporated to dryness to give crude 1-N-methyl-3''-N-4''-O-carbonyl-sisomicin.

The crude product is treated with 10% aqueous potassium hydroxide at 100°C for 5 hours. The cooled solution is passed down an Amberlite IRC-50 (H⁺) ion exchange resin and eluted with 2N aqueous ammonium hydroxide and the eluant is concentrated and lyophilized to give crude 1-N-methyl-sisomicin.

Chromatography of the crude material on silica gel (300 g.) in chloroform-methanol-7% ammonium hydroxide (2:1:1) gives 1-N-methylsisomicin. $[\alpha]_D^{20} +153^\circ$ (0.3%, H₂O; Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

37

D. 1-N-methylverdamicin via a 3'',4''-N-O-oxazolidone intermediate

(1) To an aqueous solution of 1 g. of verdamicin add sodium carbonate until the pH is in the range of about 8 to 9. Add a solution of 5 g. of *p*-nitrophenylchloro-carbonate in 25 ml. of dimethylformamide dropwise with stirring over a period of 3 hours while maintaining the pH of the reaction mixture in the range of about 8 to 9 by adding more sodium carbonate solution. After the addition is complete, continue stirring at pH 8 to 9 for 16 hours. Evaporate the mixture *in vacuo*, extract the resultant residue several times with hot chloroform, combine and evaporate the extracts and chromatograph the resultant residue over 100 g. of silica gel eluting with the lower phase of a (2:1:1) chloroform-methanol-concentrated ammonium hydroxide solvent system. Combine and evaporate the like fractions as determined by thin layer chromatography to obtain verdamicin 3'',4''-N,O-oxazolidone.

(2) In a manner similar to that described in Example 1D treat the foregoing oxazolidone derivative in water with sulfuric acid, formaldehyde, and sodium cyanoborohydride. Isolate and purify the resultant product in a manner similar to that described to obtain 1-N-methylverdamicin-3'',4''-N,O-oxazolidone.

(3) Dissolve 0.2 g. of 1-N-methylverdamicin-3'',4''-N,O-oxazolidone in 10 ml. of 2N sodium hydroxide solution. Heat under reflux for 5 hours, neutralize the reaction solution with acetic acid and evaporate to a residue. Chromatograph the residue over 10 g. of silica gel eluting with the lower phase of a (2:1:1) mixture of chloroform-methanol-15% ammonium hydroxide. Combine and evaporate like fractions as determined by thin layer chromatography to a residue to obtain 1-N-methylverdamicin.

EXAMPLE 8

1-N-Benzylgentamicin C₁ via a tri-N-protected-1-N-Schiff base Intermediate

(1) Dissolve 0.3 g. of 2',3-di-N-trifluoroacetylgentamicin C₁ in 12 ml. of ethanol and add 0.9 ml. of benzaldehyde. Stir the reaction mixture for 3 hours then evaporate *in vacuo*. Dissolve the resultant residue in 0.8 ml. of chloroform and add the solution dropwise to 25 ml. of hexane-ether (3:1). Separate the resultant precipitate by filtration and dry *in vacuo* to obtain 1-N-3''-N-4''-O-bisbenzylidene-2',3-di-N-trifluoroacetylgentamicin C₁. (Yield=0.38 g.); m.p. 128–134°C; $[\alpha]_D^{25} +74.6^\circ$ (c=0.26%, ethanol).

(2) Dissolve 1.37 g. of the product obtained in above Example 8(1) in 100 ml. of ethanol and add to a stirred mixture of 1.37 g. sodium methoxide and 1.94 g. of sodium borohydride in 100 ml. of ethanol. Stir for 3 hours, acidify the mixture to a pH of about 3 with hydrochloric acid then stir for an additional 16 hours. Extract the solution with ether, separate and discard the ether layer. Add ammonium hydroxide to the aqueous phase until it is basic, then evaporate *in vacuo* to a residue. Extract the residue with 35 ml. of hot ethanol. Combine the extracts and evaporate *in vacuo*. Chromatograph the resultant residue over 75 g. of silica gel eluting with the lower phase of a chloroform-methanol-ammonium hydroxide-water (2:1:0.2:0.8) solvent system. Combine like fractions as determined by thin layer chromatography and evaporate to a residue comprising 1-N-benzylgentamicin C₁, m.p. 83–88°C, $[\alpha]_D^{25} +90^\circ$ (c=0.3%, H₂O); pmr (ppm) (D₂O): δ 1.03 (3H, d, J=7Hz, HC-CH₃); 1.16 (3H, s, C-CH₃); 2.27 (3H, s, N-CH₃); 2.50 (3H, s, N-CH₃); 4.7 (D₂O+PhCH₂N-); 4.92 (1H, d, J=4Hz, H-1''); 5.08 (1H, d, J=3.5Hz, H-1'); 7.43 (5H, s, aromatic H); Mass Spectrum: (M+1)⁺ m/e 568 also m/e 440, 437, 412, 394, 379, 281, 263, 253, 235, 160, 157.

(3) In the above procedure by substituting other aldehydes, e.g. propionaldehyde or phenylacetaldehyde for benzaldehyde there is obtained 1-N-propylgentamicin C₁ and 1-N-phenylethylgentamicin C₁, respectively.

EXAMPLE 9

1-N-Subst.-4,6-Diaminoglycosyl-1,3-Diaminocyclitols Prepared by Hydride Reduction of the Corresponding 1-N-Acyl Derivatives

A. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C₁

(1) Suspend 98 mg. of 1-N-(S- δ -amino- β -hydroxybutyryl)gentamicin C₁ in 8 ml. of tetrahydrofuran. Add 14 ml. of 1N diborane in tetrahydrofuran and heat at reflux temperature for 6 hours under an atmosphere of nitrogen. Carefully add 2 ml. of water to decompose any excess diborane and evaporate. Dissolve the resultant residue in hydrazine hydrate and heat at reflux temperature under an atmosphere of nitrogen for 16 hours. Evaporate the solution and extract the residue with hot aqueous ethanol. Evaporate the combined ethanol extracts and chromatograph the resultant residue over 10 ml. of silica gel eluting with the lower phase of a chloroform-methanol-concentrated ammonium hydroxide (2:1:1) solvent system. Combine and

evaporate the like fractions as determined by thin layer chromatography to obtain 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C₁ (yield 14.5 mg), m.p. 93–98°C, $[\alpha]_D^{25} + 72.4^\circ$ (c=0.35%, H₂O); pmr (ppm) (D₂O) δ 1.18 (3H, d, J=7Hz, CH–CH₃); 1.24 (3H, s, C–CH₃); 2.49 (3H, s, N–CH₃); 2.54 (3H, s, N–CH₃); 5.07 (1H, d, J=3.5Hz, H-1''); 5.24 (1H, d, J=3.5Hz, H-1'). Mass Spectrum: (M+1)⁺ m/e 565 also m/e 528, 516, 490, 437, 434, 410, 397, 278, 250, 232, 160, 157.

(2) In the above procedure substitute 1-N-(S- γ -amino- β -hydroxypropionyl)gentamicin C₁ for 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C₁ to obtain 1-N-(S- γ -amino- β -hydroxypropionyl)gentamicin C₁.

(3) Treat each of the following 1-N-(S- δ -amino- β -hydroxybutyl)-4,6-diaminoglycosyl-1,3-diaminocyclitols with diborane in tetrahydrofuran in the manner described in Example 9A(1):

1. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin A,
2. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin B,
3. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin B₁,
4. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{1a},
5. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C₂,
6. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{2a},
7. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{2b},
8. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin X₂,
9. 1-N-(S- δ -amino- β -hydroxybutyl)toframycin,
10. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic G-418,
11. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic JI-20A,
12. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic JI-20B.

Isolate and purify each of the resultant products in a manner similar to that described in Example 9A(1) to obtain, respectively,

1. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin A,
2. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin B,
3. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin B₁,
4. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{1a},
5. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C₂,
6. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{2a},
7. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin C_{2b},
8. 1-N-(S- δ -amino- β -hydroxybutyl)gentamicin X₂,
9. 1-N-(S- δ -amino- β -hydroxybutyl)toframycin,
10. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic G-418,
11. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic JI-20A,
12. 1-N-(S- δ -amino- β -hydroxybutyl)Antibiotic JI-20B.

(4) In the procedure of Example 9A(3) hereinabove utilize as starting compounds the corresponding 1-N-(S- γ -amino- β -hydroxypropionyl) derivatives to obtain the corresponding 1-N-(S- γ -amino- β -hydroxypropyl)derivatives, i.e.

1. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin A,
2. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin B,
3. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin B₁,
4. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin C_{1a},
5. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin C₂,
6. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin C_{2a},
7. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin C_{2b},
8. 1-N-(S- γ -amino- β -hydroxypropyl)gentamicin X₂,
9. 1-N-(S- γ -amino- β -hydroxypropyl)toframycin,
10. 1-N-(S- γ -amino- β -hydroxypropyl)Antibiotic G-418,
11. 1-N-(S- γ -amino- β -hydroxypropyl)Antibiotic JI-20A,
12. 1-N-(S- γ -amino- β -hydroxypropyl)Antibiotic JI-20B.

B. 1-N-Ethylgentamicin C₁

(1) In a manner similar to that described in Example 9A(1) treat 1-N-acetyl-gentamicin C₁ with diborane in tetrahydrofuran. Isolate and purify the resultant products in a manner similar to that described in Example 9A(1) to obtain 1-N-ethyl-gentamicin C₁.

(2) Treat the following 1-*N*-acetyl-4,6-diaminoglycosyl-1,3-diaminocyclitols in the manner of above Example 9B(1):

1. 1-*N*-acetylgentamicin A,
2. 1-*N*-acetylgentamicin B,
3. 1-*N*-acetylgentamicin B₁,
4. 1-*N*-acetylgentamicin C_{1a},
5. 1-*N*-acetylgentamicin C₂,
6. 1-*N*-acetylgentamicin C_{2a},
7. 1-*N*-acetylgentamicin C_{2b},
8. 1-*N*-acetylgentamicin X₂,
9. 1-*N*-acetyltobramycin,
10. 1-*N*-acetyl Antibiotic G-418,
11. 1-*N*-acetyl Antibiotic JI-20A,
12. 1-*N*-acetyl Antibiotic JI-20B.

Isolate and purify each of the resultant products in the manner similar to that described to obtain, respectively,

1. 1-*N*-ethylgentamicin A,
2. 1-*N*-ethylgentamicin B,
3. 1-*N*-ethylgentamicin B₁,
4. 1-*N*-ethylgentamicin C_{1a},
5. 1-*N*-ethylgentamicin C₂,
6. 1-*N*-ethylgentamicin C_{2a},
7. 1-*N*-ethylgentamicin C_{2b},
8. 1-*N*-ethylgentamicin X₂,
9. 1-*N*-ethyltobramycin,
10. 1-*N*-ethyl Antibiotic G-418,
11. 1-*N*-ethyl Antibiotic JI-20A
12. 1-*N*-ethyl Antibiotic JI-20B.

C. 1-*N*-Ethyl sisomicin

(1) Suspend 1 gm. of 1-*N*-acetylsisomicin in 100 ml. of tetrahydrofuran. Add 1 gm of lithium aluminum hydride, then stir the resultant suspension at reflux temperature for 24 hours under an atmosphere of nitrogen. Cool and decompose the excess hydride by careful addition of ethyl acetate. Evaporate the reaction mixture to a small volume and dilute with water. Separate the insoluble solids by filtration and wash well with acetic acid. Evaporate the combined filtrate and washings and dissolve the resultant residue in water. Adjust the pH of the aqueous solution to about 7 by addition of ammonium hydroxide. Pass the solution through a column of Amberlite IRC 50 resin in the ammonium cycle and wash the column well with water. Elute with 0.5 N ammonium hydroxide, evaporate the eluate, and chromatograph the resultant residue over 20 gm. of silica gel eluting with the lower phase of a 2:1:1 chloroform-methanol-concentrated ammonium hydroxide solvent system. Combine and evaporate the like fractions as determined by thin layer chromatography to obtain 1-*N*-ethylsisomicin.

(2) Treat the following 4,6-diaminoglycosyl-1,3-diaminocyclitols in the manner described in the procedure of Example 9C(1):

1. 1-*N*-acetylverdamicin,
2. 1-*N*-acetyl Antibiotic 66-40B,
3. 1-*N*-acetyl Antibiotic 66-40D,
4. 1-*N*-acetyl Antibiotic G-52,
5. 1-*N*-acetylmutamicin 1,
6. 1-*N*-acetylmutamicin 2,
7. 1-*N*-acetylmutamicin 4,
8. 1-*N*-acetylmutamicin 5,
9. 1-*N*-acetylmutamicin 6.

Isolate and purify each of the resultant products to obtain, respectively,

1. 1-*N*-ethylverdamicin,
2. 1-*N*-ethyl Antibiotic 66-40B,
3. 1-*N*-ethyl Antibiotic 66-40D,

4. 1-N-ethyl Antibiotic G-52,
5. 1-N-ethylmutamycin 1,
6. 1-N-ethylmutamycin 2,
7. 1-N-ethylmutamycin 4,
8. 1-N-ethylmutamycin 5,
9. 1-N-ethylmutamycin 6.

D. 1-N-Methylgentamicin C₁

(1) Dissolve 1 gm. of 2',3-di-N-trifluoroacetylgentamicin C₁ in 30 ml. of 50% aqueous methanol. Cool to 5°C then add 0.25 gm. of *t*-butoxycarbonyl azide dropwise with stirring followed by 0.155 ml. of triethylamine. Stir the resultant solution for 18 hours, evaporate the reaction mixture *in vacuo* to a residue and chromatograph the residue on 100 gm. of silica gel eluting with the lower phase of 2:1:1, chloroform-methanol-concentrated ammonium hydroxide solvent system. Combine and evaporate the like fractions of the major product as determined by thin layer chromatography to obtain 1-N-*t*-butoxycarbonyl-2',3-di-N-trifluoroacetylgentamicin C₁.

(2) Dissolve the product of Example 9D(1) in a mixture comprising 30 ml. of methanol and 20 ml. of concentrated ammonium hydroxide. Allow the solution to stand for 3 days at room temperature, then evaporate to a residue comprising 1-N-*t*-butoxycarbonylgentamicin C₁.

(3) Dissolve 100 mg. of 1-N-*t*-butoxycarbonylgentamicin C₁ in 15 ml. of 1 M diborane in tetrahydrofuran. Reflux the resultant solution for 16 hours under an atmosphere of nitrogen. Add 2 ml. of water to decompose any excess diborane and evaporate the mixture to a residue. Dissolve the residue in 10 ml. of hydrazine hydrate and heat at reflux temperature under an atmosphere of nitrogen for 16 hours. Evaporate the solution, extract the resultant residue with hot aqueous ethanol then evaporate the combined extracts and chromatograph the resultant residue over 10 gm. of silica gel eluting with the lower phase of a 2:1:1 mixture of chloroform-methanol-15% ammonium hydroxide. Combine and evaporate the like fractions as determined by thin layer chromatography to obtain 1-N-methylgentamicin C₁.

EXAMPLE 10

1-N-Methylsisomicin from 3''-N-4''-O-carbonyl-2',3,6'-tri-*t*-butoxycarbonyl-sisomicin (0.77 gm.) Dissolve 3''-N-4''-O-carbonyl-2',3,6'-tri-*t*-butoxycarbonyl-sisomicin (0.77 gm.) in tetrahydrofuran (20 ml.) and cool in an ice-bath. Add methyl fluorosulphonate (0.12 gm.) and allow to warm to room temperature. Remove the solvent and dissolve the residue in trifluoroacetic acid. After five minutes at room temperature remove the trifluoroacetic acid *in vacuo* and treat the residue with 10% potassium hydroxide solution at 100°C for 5 hours.

Pass the cooled solution down a column of Amberlite IRC-50 (H⁺) ion exchange resin and elute with 2N aqueous ammonium hydroxide. Concentrate the eluant and lyophilize to obtain the crude title product.

Chromatograph the crude material on silica gel in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent mixture to obtain 1-N-methylsisomicin. $[\alpha]_D^{25} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

EXAMPLE 11

1-N-Methylsisomicin

2',3,6'-Tri-N-*t*-butoxycarbonyl-3''-N-4''-O-carbonyl-sisomicin (5 g.) in ethanol (100 ml.) is treated with 37% aqueous formaldehyde (1 ml.) and ammonium formate (5 gm.) and the solution is heated under reflux for 24 hours. The solution is diluted with water (200 ml.) and extracted with chloroform (3 × 100 ml.). The combined extracts are reduced to dryness and the residue containing 2',3,6'-Tri-N-*t*-butoxycarbonyl-3''-N-4''-O-carbonyl-1-N-methyl-sisomicin is dissolved in trifluoroacetic acid and after five minutes at room temperature, the solvent is removed *in vacuo*. The residue is treated with 10% potassium hydroxide (25 ml.) at 100° for 5 hours. The cooled solution is passed down a column of Amberlite IRC 50 (H⁺) ion exchange resin and the crude product is eluted with 2 N aqueous ammonium hydroxide. The combined eluant is reduced to dryness *in vacuo* and the residue chromatographed on silica gel (200 gm.) in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent system to give 1-N-methylsisomicin. $[\alpha]_D^{25} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 122, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

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EXAMPLE 12

1-N-Methylsisomicin

Treat 2',3,6'-Tri-N-t-butoxycarbonyl-3''-N-4''-O-carboxylsisomicin (0.77 gm.) in THF (25 ml.) with methylamine (101 mg.) and trifluoromethylsulphonic anhydride (290 mg.) at 0° for 18 hours. Reduce the solution to dryness and dissolve the residue in DMF (10 ml.) and stir with methyl iodide (300 mg.) and potassium carbonate (130 mg.) for a further 18 hours. Remove the solvent by evaporation and treat the residue with 10% aqueous potassium hydroxide at 100° for 12 hours. Pass the cooled solution through a column of Amberlite IRC 50 (H⁺) ion exchange resin. The crude product is eluted with 2 N aqueous ammonium hydroxide. The combined eluant is reduced to dryness *in vacuo* and the residue chromatographed on silica gel (200 gm.) in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent system to give 1-N-methylsisomicin. $[\alpha]_D^{25} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

EXAMPLE 13

1-N-Methylsisomicin

2',3,6'-Tri-N-t-butoxycarbonyl-3''-N-4''-O-carboxylsisomicin (0.77 gm.) in THF (20 ml.) is treated with 37% aqueous formaldehyde (3 ml.) and succinimide (170 mg.) at room temperature for 18 hours. The solution is dropped into a mixture of diethyl-ether-hexane (1:1) and the precipitate is collected by filtration. This material is treated with sodium borohydride (200 mg.) in ethanol (20 ml.) at room temperature for 5 hours. The ethanol is removed *in vacuo* and the residue is treated with 10% aqueous potassium hydroxide for 12 hours at 100°C. The cooled solution is passed down a column of Amberlite IRC 50 (H⁺) ion exchange resin and the crude product is eluted with 2N aqueous ammonium hydroxide. The combined eluant is reduced to dryness *in vacuo* and the residue chromatographed on silica gel (220 gm.) in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent system to give 1-N-methylsisomicin. $[\alpha]_D^{25} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

EXAMPLE 14

1-N-Methylsisomicin

Dissolve 2',3,6'-Tri-N-t-butoxycarbonyl-3''-N-4''-O-carboxylsisomicin (0.77 gm.) in dichloromethane (100 ml.) with acrylonitrile (0.25 gm.) and leave at room temperature for 24 hours. Remove the solvent *in vacuo* to leave a residue which is dissolved in dimethylformamide and treat with methyl iodide (180 mg.) at 50°C for 12 hours. Remove the solvent, and treat the residue with 10% aqueous potassium hydroxide at 100° for 8 hours. The cooled solution is passed down a column of Amberlite IRC 50 (H⁺) ion exchange resin and the crude product is eluted with 2 N aqueous ammonium hydroxide. The combined eluant is reduced to dryness *in vacuo* and the residue chromatographed on silica gel (200 gm.) in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent system to give 1-N-methylsisomicin. $[\alpha]_D^{25} + 153^\circ$ (0.3%, H₂O); Mass Spectrum: (M+1)⁺ m/e 462 also m/e 127, 140, 159, 160, 177, 187, 205, 256, 285, 303, 318, 331, 336(w), 346, 376, 386.

EXAMPLE 15

1-N-(Hydroxyethyl)sisomicin

Dissolve 2',3,6'-Tri-N-t-butoxycarbonyl-3''-N-4''-O-carboxylsisomicin (1.0 gm.) in methanol (30 ml.) in a stainless steel pressure reactor (500 ml. capacity) that is connected to an ethylene oxide cylinder through a shut-off valve and which is equipped with a heater and a pressure gauge. Fill with ethylene oxide until a pressure inside the reactor of 2 atmospheres is obtained. Heat the reactor to 60°C while rocking for 24 hours. Cool the reactor and depressurize. Remove the solvent to obtain a residue containing 1-N-(hydroxyethyl)-2',3,6'-tri-N-t-butoxycarbonyl-3''-N-4''-O-carboxylsisomicin.

Heat the residue with 10% aqueous potassium hydroxide (20 ml.) at 100°C for 8 hours and cool. The cooled solution is passed down a column of Amberlite IRC 50 (H⁺) ion exchange resin and the crude product is eluted with 2 N aqueous ammonium hydroxide. The combined eluant is reduced to dryness *in vacuo* and the residue chromatographed on silica gel (200 gm.) in the lower phase of a chloroform-methanol-7% ammonium hydroxide (2:1:1) solvent system to give 1-N-(hydroxyethyl)sisomicin.

EXAMPLE 16

Preparation of 1-N-Alkylisomicin and Derivatives thereof by Cultivation of *Micromonospora inyoensis* Strain 1550F-1G in the Presence of a 1-N-Alkyl-2-Deoxy-D-Streptamine or Derivatives Thereof

A. Preparation of Inoculum of *Micromonospora inyoensis* Strain 1550F-1G

Germination Stage 1:

Under aseptic conditions, add a lyophilized culture (or cells obtained from a slant culture) of *Micromonospora inyoensis* strain 1550F-1G to a 300 ml. shake flask containing 100 ml. of the following sterile medium:

Beef extract	3 gm.
Tryptose	5 gm.
Yeast extract	5 gm.
Dextrose	1 gm.
Starch	24 gm.
Calcium carbonate	2 g.
Tap Water	1000 ml.

Incubate the flask and its contents for 2—5 days at 35°C. on a rotary shaker (280 r.p.m., 2 inch stroke).

Germination Stage 2:

Aseptically transfer 25 ml. of the fermentation medium of germination stage 1 to a two-liter shake flask containing 500 ml. of the aforescribed sterile germination medium. Incubate the flask and its contents for three days at 28°C. on a rotary shaker (280 r.p.m., 2 inch stroke).

Fermentation Stage:

Aseptically transfer 500 ml. of the inoculum obtained from germination stage 2 to a 14 l. fermentation tank containing 9.5 l. of the following sterile medium:

Dextrin	50 gm.
Dextrose	5 gm.
Soybean meal	35 gm.
Calcium carbonate	7 gm.
Cobalt chloride	10 ⁻⁶ molar
Tap Water	1000 ml.
Antifoam (Ge 60)	10 ml.

B. Preparation of 1-N-Methylisomicin

(1) The requisite 1-N-methyl-2-deoxy-D-streptamine (also known as (—)-hyosamine) is prepared by hydrolysis of destomycin A according to the procedure of S. Kondo et. al, J. Antibiotics Ser. A., 18, 192, (1965).

(2) Prior to sterilization of the medium described in Example 16 A, adjust the pH to 8 and add an aqueous solution containing 8.0 gms. of 1-N-methyl-2-deoxy-D-streptamine. Ferment the contents under aerobic conditions for 48—240 hours, with stirring at 250 r.p.m., with air input at 4.5 liters per minute and at 2.5 atmospheres. The pH of the fermentation medium changes slightly during the antibiotic production, varying in the range of about 6.8 to about 7.3.

(3) Monitor the antibiotic production using the assay procedure described in J. antibiotics (Japan) Vol. XXIII, and when peak production is attained, harvest the product (the fermentation is usually complete in about 7 days).

C. Isolation of 1-N-Methylisomicin

Add 7.0 gm. of oxalic acid to the whole broth from Example 16B with stirring. Acidify the broth to pH 2.0 using 6N sulfuric acid. Stir the mixture for about 15 minutes and filter using a suitable filter aid. Neutralize (pH 7.0) the filtrate with 6 N ammonium hydroxide. Pass the filtrate through a 1.0 liter cation exchange resin column in the ammonium form (e.g., Amberlite IRC-50, Rohm and Haas, Philadelphia, Pa.). Discard the spent broth and elute the column with 2 N ammonium hydroxide collecting fractions of about 100 ml. Monitor the column eluate by disc testing each fraction against *Staphylococcus aureus* TACC 6538P. Combine the active fractions and evaporate to about 100 ml. *in vacuo* and lyophilize to obtain a solid product. Triturate the product several times with warm methanol, filter and evaporate the filtrate to a residue. Chromatograph the product on silica gel (25 gm.) using the

lower phase of a chloroform-methanol-concentrated ammonium hydroxide (1:1:1) system as the eluant. Combine and evaporate the fractions containing antibiotic activity to obtain thereby 1-*N*-methylsisomicin.

D. 1-*N*-Alkylsisomicins

(1) In a manner similar to that described in Example 16B by substituting for 1-*N*-methyl-2-deoxy-*D*-streptamine equivalent quantities of other mono-*N*-alkyl-2-deoxystreptamines, e.g. 1-*N*-ethyl-2-deoxy-*D*-streptamine, 1-*N*-propyl-2-deoxy-*D*-streptamine, 1-*N*-methyl-2,5-dideoxy-*D*-streptamine, there is obtained the corresponding 1-*N*-alkylsisomicin, e.g. 1-*N*-ethylsisomicin, 1-*N*-propyl-sisomicin, and 1-*N*-methyl-5-deoxysisomicin.

E. Preparation of Mono-*N*-Methyl-5-Deoxysisomicin

(1) Ferment *Micromonospora inyoensis* strain 1550F-1G in the presence of (±)-mono-*N*-methyl-2,5-dideoxystreptamine according to the procedure described in Example 16B and isolate the resultant antibiotic thereby formed in a manner similar to that described in Example 16C to obtain an antibacterial substance comprising a mixture of 1-*N*-methyl-5-deoxysisomicin and 3-*N*-methyl-5-deoxy-sisomicin.

By substituting (±)-mono-*N*-methyl-2-deoxystreptamine for (±)-mono-*N*-methyl-2,5-dideoxystreptamine in the above procedure there is obtained a mixture comprising 1-*N*-methylsisomicin and 3-*N*-methylsisomicin.

(2) Chromatograph the mono-*N*-methyl-5-deoxysisomicin mixture prepared in Example 16E (1) according to procedures known in the art, combine like fractions as determined by thin layer chromatography and evaporate each of the combined fractions to a residue to obtain 1-*N*-methyl-5-deoxysisomicin and 3-*N*-methyl-5-deoxysisomicin, respectively.

Similarly, chromatograph the mono-*N*-methylsisomicin mixture obtained as described in the second paragraph of Example 16E (1) to obtain 1-*N*-methylsisomicin and 3-*N*-methylsisomicin, respectively.

EXAMPLE 17

Acid Addition Salts

A. Sulfate Salts (Sulfuric Acid Addition Salts)

Dissolve 5.0 gms. of 1-*N*-ethylsisomicin in 25 ml. of water and adjust the pH of the solution to 4.5 with 1 N sulfuric acid. Pour into about 300 ml. of methanol with vigorous agitation, continue the agitation for about 10—20 minutes and filter. Wash the precipitate with methanol and dry at about 60°C *in vacuo* to obtain 1-*N*-ethylsisomicin sulfate.

In like manner, the sulfate salt of the compounds of Examples 1—16 may also be prepared.

B. Hydrochloride Salts

Dissolve 5.0 grams of 1-*N*-ethylverdamicin in 25 ml. of water. Acidify with 2 N hydrochloric acid to pH 5. Lyophilize to obtain 1-*N*-ethylverdamicin hydrochloride.

In like manner, the hydrochloride salt of the compounds of Examples 1—16 may also be prepared.

EXAMPLE 18

1-*N*-Acetylsisomicin

(A) Dissolve 1.25 g of sisomicin sulfate in 200 ml. of methanol-water (2:3, v/v) and chill the solution. Add 1.5 ml. of acetic anhydride and after approximately 10 minutes add 0.125 ml. of triethylamine in 10 ml. of methanol over a 15 minute interval. Allow the reaction mixture to warm to room temperature over a 2 hour interval then evaporate the solvent *in vacuo*. Dissolve the residue in water and convert the product to the free base by passage of an aqueous solution thereof through Amberlite IRA-401S resin in the hydroxide ion cycle. Lyophilize the column eluate and chromatograph the residue on 50 g of silica gel using the lower phase of (2:1:1) chloroform, methanol, 7% ammonium hydroxide solvent system as eluant. Monitor the fractions via TLC and combine like fractions to obtain thereby the title compound.

Yield—0.185 g., m.p. 128°—130°C. $[\alpha]_D^{25} = 159^\circ$ (0.3% H₂O), NMR: (D₂O) δ 1.22 (3H, s, —C—CH₃); 2.02 (3H, s, NH—CO—CH₃); 2.53 (3H, s, N—CH₃); 4.88 (1H, m, =CH—); 5.08 (1H, d, J=4 Hz, H₁''); 5.35 (1H, d, J=2 Hz, H₁'). Mass spectrum: (M+1)⁺ m/e 490, M+ m/e 489.

(B) In a similar manner, treat an equivalent quantity of the sulfate salt of the following antibiotics to the process of Example 18A:

5 gentamicin C₁,
gentamicin C_{1a},
gentamicin C₂,
gentamicin C_{2a},
tobramycin,
Antibiotic G-418,
Antibiotic 66-40B,
10 Antibiotic 66-40D,
Antibiotic JI-20A,
Antibiotic JI-20B,
Antibiotic G-52,
mutamicin 1,
15 mutamicin 2,
mutamicin 4,
mutamicin 5,
mutamicin 6,
gentamicin X₂,
20 gentamicin A,
gentamicin C_{2b},
verdamicin,
gentamicin B, and
gentamicin B₁.

25 Isolate the respective products in the manner described in Example 18A and obtain thereby the following:

1-N-acetylgentamicin C₁,
1-N-acetylgentamicin C_{1a},
1-N-acetylgentamicin C₂,
30 1-N-acetylgentamicin C_{2a},
1-N-acetylgentamicin X₂,
1-N-acetylgentamicin C_{2b},
1-N-acetyl verdamicin,
1-N-acetyl tobramycin,
35 1-N-acetyl Antibiotic G-418,
1-N-acetyl Antibiotic 66-40B,
1-N-acetyl Antibiotic 66-40D,
1-N-acetyl Antibiotic JI-20A,
1-N-acetyl Antibiotic JI-20B,
40 1-N-acetyl Antibiotic G-52,
1-N-acetylmutamicin 1,
1-N-acetylmutamicin 2,
1-N-acetylmutamicin 4,
1-N-acetylmutamicin 5,
45 1-N-acetylmutamicin 6,
1-N-acetylgentamicin A,
1-N-acetylgentamicin B, and
1-N-acetylgentamicin B₁.

50 (C) In the procedure of Example 18A by substituting other acid anhydrides, e.g. *n*-octanoic acid anhydride, phenylacetic acid anhydride, propenoic acid anhydride and *trans*- β -phenylacrylic acid anhydride there is obtained the corresponding 1-N-acyl derivative, e.g. 1-N-(*n*-octanoyl)sisomicin, 1-N-phenylacetylsisomicin, 1-N-propenoylsisomicin and 1-N-(*trans*- β -phenylpropenoyl)sisomicin, respectively.

EXAMPLE 19

1-N-Propionylsisomicin

55 A. Dissolve 1.25 g of sisomicin sulfate in 200 ml of methanol water (2:3, v/v) and chill the solution. Add 1.5 ml of propionic anhydride followed by 0.125 ml of triethylamine in 10 ml of methanol over a 15 minute interval. Allow the reaction mixture to warm to room temperature over a 2 hour interval then evaporate the solvent *in vacuo*.
60 Dissolve the residue in water and convert the product to the free base by passage

of an aqueous solution thereof through Amberlite IRA-401S resin in the hydroxide ion cycle. Lyophilize the column eluate and chromatograph the residue on 50 g of silica gel using the lower phase of (2:1:1) chloroform, methanol, 7% ammonium hydroxide solvent system as eluant. Monitor the fraction via TLC and combine like fractions to obtain thereby 1-N-propionylsomicin. Yield 0.18 g. m.p. 125°-130°C, $[\alpha]_D^{25} = +147^\circ$ (0.3% H₂O) NMR: (D₂O) δ 1.08 (3H, t, J=7.5 Hz, CH₂-CH₃); 1.18 (3H, s, C-CH₃); 2.25 (2H, m, CH₂CH₃); 2.48 (3H, s, NHCH₃); 4.87 (1H, m, =CH-); 5.07 (1H, d, J=4 Hz, H₁''); 5.34 (1H, d, J=2 Hz, H₁'). Mass spectrum M⁺+1 m/e 504, M + m/e=503.

B. In a similar manner, treat an equivalent quantity of acid addition salt of those antibiotics set forth in Example 18B to the process of Example 19A, isolate the respective products in the manner described in Example 19A and obtain thereby the following:

1-N-propionylgentamicin C ₁ ,	1-N-propionylgentamicin C _{1a} ,
1-N-propionylgentamicin C ₂ ,	1-N-propionylgentamicin C _{2a} ,
1-N-propionylgentamicin X ₂ ,	1-N-propionylgentamicin A,
1-N-propionylgentamicin C _{2b} ,	1-N-propionylverdamicin,
1-N-propionyltobramycin,	1-N-propionylantibiotic G-418,
1-N-propionylantibiotic 66-40B,	1-N-propionylantibiotic 66-40D,
1-N-propionylantibiotic JI-20A,	1-N-propionylantibiotic JI-20B,
1-N-propionylantibiotic G-52,	1-N-propionylmutamicin 1,
1-N-propionylmutamicin 2,	1-N-propionylmutamicin 4,
1-N-propionylmutamicin 5,	1-N-propionylmutamicin 6, and
1-N-propionylgentamicin B,	1-N-propionylgentamicin B ₁ .

EXAMPLE 20

1-N-Acetylverdamicin

A. Dissolve 1.25 g. of verdamicin sulfate in a mixture of 10 ml. of methanol and 25 ml. of water. Add 0.125 ml. of triethylamine followed by 1.5 ml. of acetic anhydride. Chill the solution to 0°-5°C and allow to react for a 15 minute interval. Allow the reaction mixture to warm to room temperature over a 2 hour interval then evaporate the solvent *in vacuo*. Dissolve the residue in water and convert the product to the free base by passage of an aqueous solution thereof through a suitable anion exchange resin column in the hydroxide ion cycle e.g. Amberlite IRA-401S. Lyophilize the column eluate and chromatograph the residue in 50 g. of silica gel using the lower phase of (2:1:1) chloroform, methanol, 7% ammonium hydroxide solvent system as eluant. Monitor the fraction via TLC and combine like fractions to obtain 1-N-acetylverdamicin. Yield: 0.241 g.

B. By the process of Example 20, other 1-N-acyl derivatives of verdamicin may be prepared, such as those wherein the acyl group is derived from propionic, butyric, phenylacetic, isobutyric or valeric acids or the like. In like manner, 1-N-acyl derivatives of other 4,6-di-(aminoglycosyl)aminocyclitol antibiotics, such as those set forth in Example 18B, may be prepared.

EXAMPLE 21

1-N-Acetylgentamicin C₁

Dissolve 250 mg. of gentamicin C₁ sulfate in a mixture of 25 ml. of water and 10 ml. of methanol. Add 0.065 ml. of triethylamine. Allow the solution to stand for ten minutes then add 0.5 ml. of acetic anhydride. Let the solution stand for one hour at room temperature then evaporate to a residue *in vacuo*. Dissolve the residue in water and treat with Amberlite IRA 401S in the hydroxyl ion form. Filter the suspension and lyophilize the filtrate. Chromatograph the residue over a silica gel column containing 30 g of adsorbent using the lower phase of a chloroform, methanol, 7% ammonium hydroxide (2:1:1) system and obtain thereby the product of this example. Yield: 50 mg. $[\alpha]_D^{25} = +124^\circ$. Mass Spectrum (M+1)⁺ m/e=520. NMR (D₂O) δ 1.01 (3H, d, J=6.5 Hz, -CHCH₃); 1.15 (3H, s, C-CH₃); 1.95 (3H, s, COCH₃); 2.28 (3H, s, 6' NCH₃); 2.45 (3H, s, 3'' NCH₃); 5.03 (1H, d, J=4.5 Hz, H₁''); 5.09 (1H, d, J=3.5 Hz, H₁').

EXAMPLE 22

A. 1-N-(5-Aminopentanoyl)gentamicin C₁

(1) 1-N-(5-Phthalimidopentanoyl)gentamicin C₁
Dissolve 2.5 g of gentamicin C₁ sulphate in 250 ml of water and add 100 ml.

of methanol. Add 0.65 ml of triethylamine and stir for 10 minutes. Add a solution of 1.2 g. *N*-(5-phthalimidopentanoyloxy)succinimide in 20 ml of dry dimethylformamide dropwise with stirring to the solution of the antibiotic. Stir the mixture at ambient temperature for 16 hours, concentrate the reaction mixture to a residue *in vacuo* and triturate the residue with methanol to yield 3.4 g. of white solids. Chromatograph the residue on 200 g. of silica gel in the lower phase of a chloroform, methanol, 7% ammonium hydroxide (2:1:1) system to give 1-*N*-(5-phthalimidopentanoyl)gentamicin C₁.

(2) 1-*N*-(5-Aminopentanoyl)gentamicin C₁

Heat 0.4 g of 1-*N*-(5-phthalimidopentanoyl)gentamicin C₁ in 5 ml of 5% ethanolic hydrazine hydrate under reflux for 4 hrs. Concentrate the solution and add tetrahydrofuran to precipitate 1-*N*-(5-aminopentanoyl)gentamicin C₁ which is collected by filtration.

B. In a similar manner, treat an equivalent quantity of acid addition salt of the following antibiotics to the process of Example 22A (1) gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin X₂, gentamicin A, gentamicin C_{2b}, verdamicin, tobramycin, Antibiotic G418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamycin 1, mutamycin 2, mutamycin 4, mutamycin 5, mutamycin 6, gentamicin B, gentamicin B₁, and sisomicin.

Isolate the respective products in the manner described in Example 22A (2) and obtain thereby the following:

1-*N*-(5-aminopentanoyl)gentamicin C_{1a},
 1-*N*-(5-aminopentanoyl)gentamicin C₂,
 1-*N*-(5-aminopentanoyl)gentamicin C_{2a},
 1-*N*-(5-aminopentanoyl)gentamicin X₂,
 1-*N*-(5-aminopentanoyl)gentamicin A,
 1-*N*-(5-aminopentanoyl)gentamicin C_{2b},
 1-*N*-(5-aminopentanoyl)verdamicin,
 1-*N*-(5-aminopentanoyl)tobramycin,
 1-*N*-(5-aminopentanoyl)antibiotic G-418,
 1-*N*-(5-aminopentanoyl)antibiotic 66-40B,
 1-*N*-(5-aminopentanoyl)antibiotic 66-40D,
 1-*N*-(5-aminopentanoyl)antibiotic JI-20A,
 1-*N*-(5-aminopentanoyl)antibiotic JI-20B,
 1-*N*-(5-aminopentanoyl)antibiotic G-52,
 1-*N*-(5-aminopentanoyl)mutamycin 1,
 1-*N*-(5-aminopentanoyl)mutamycin 2,
 1-*N*-(5-aminopentanoyl)mutamycin 4,
 1-*N*-(5-aminopentanoyl)mutamycin 5,
 1-*N*-(5-aminopentanoyl)mutamycin 6,
 1-*N*-(5-aminopentanoyl)gentamicin B,
 1-*N*-(5-aminopentanoyl)gentamicin B₁, and
 1-*N*-(5-aminopentanoyl)sisomicin.

EXAMPLE 23

A. 1-*N*-(5-Hydroxypentanoyl)gentamicin C₁

Dissolve 2.5 g. of gentamicin C₁ in 250 ml. of water and add 100 ml. of methanol. Add 0.65 ml. of triethylamine and stir for fifteen minutes. Add a solution of 1.0 g. of *N*-(5-acetoxypentanoyloxy)succinimide with stirring to the solution of the antibiotic, and stir at ambient temperature for 16 hrs. Evaporate the solution *in vacuo* to leave a solid residue. Dissolve the residue in 5 ml. of 5% ethanolic hydrazine hydrate and heat under reflux for fifteen minutes. Concentrate the solution *in vacuo* to leave an oily residue and chromatograph it on 200 g silica gel in the lower phase of a solvent system consisting of chloroform, methanol and 7% ammonium hydroxide (2:1:1) to give 1-*N*-(5-hydroxypentanoyl)gentamicin C₁.

B. In a similar manner, treat an equivalent quantity of acid addition salt of those antibiotics set forth in Example 22B to the process of Example 23A, isolate the respective products in the manner described in Example 23A and obtain thereby the following:

1-*N*-(5-hydroxypentanoyl)gentamicin C_{1a}, 1-*N*-(5-hydroxypentanoyl)gentamicin C₂,
 1-*N*-(5-hydroxypentanoyl)gentamicin C_{2a}, 1-*N*-(5-hydroxypentanoyl)gentamicin X₂,

EXAMPLE 24

A. 1-N-Formylgentamicin C₁
Dissolve 2.5 g. of gentamicin C₁ sulphate in 250 ml. of water and add 100 ml. of methanol. Add 0.65 ml of triethylamine and stir for ten minutes. Add a solution of 2.0 g. of N-formyloxysuccinimide in 20 ml. of dry dimethylformamide dropwise with stirring to the solution of the antibiotic. Stir the mixture at ambient temperature for 16 hours. Concentrate the reaction mixture to a residue *in vacuo* and triturate the residue with methanol to yield the product of this example as a white amorphous solid which may optionally be further purified by the chromatographic procedure set forth in the preceding Example.

the following:

1-*N*-formylgentamicin C_{1a}, 1-*N*-formylgentamicin C₂, 1-*N*-formylgentamicin C_{2a}, 1-*N*-formylgentamicin X₂, 1-*N*-formylgentamicin A, 1-*N*-formylgentamicin C_{2b}, 1-*N*-formylverdamicin, 1-*N*-formyltobramycin, 1-*N*-formylantibiotic G-418, 1-*N*-formylantibiotic 66-40B, 1-*N*-formylantibiotic 66-40D, 1-*N*-formylantibiotic JI-20A, 1-*N*-formylantibiotic JI-20B, 1-*N*-formylantibiotic G-52, 1-*N*-formylmutamicin 1, 1-*N*-formylmutamicin 2, 1-*N*-formylmutamicin 4, 1-*N*-formylmutamicin 5, 1-*N*-formylmutamicin 6, 1-*N*-formylgentamicin B, 1-*N*-formylgentamicin B_i, and 1-*N*-formylsisomicin.

1-N-Acetylgentamicin B

All percentages referred to herein, except those relating to "aqueous" organic solvents are expressed on a weight basis. The percentages relating to "aqueous" organic solvents and the ratios of the components of the chromatography solvents are expressed on a volume basis.

The present invention includes within its scope pharmaceutical compositions comprising at least one of the novel 1-N-substituted derivatives of 4,6-di-(amino-glycosyl)1,3-diaminocyclitols defined below together with a pharmaceutically acceptable carrier or coating. Also included within the invention is a method of eliciting an antibacterial response in a warm-blooded animal having a susceptible bacterial infection which comprises administering to said animals a non-toxic, antibacterially effective

48
 5 tive amount of a 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diamino-
 cyclitol selected from gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁,
 gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂,
 sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic
 66-40D, Antibiotic JI120A, Antibiotic JI120B, Antibiotic G-52, mutamicin 1, muta-
 10 micin 2, mutamicin 4, mutamicin 5 and mutamicin 6 wherein the substituent is
 —CH₂X with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxy-
 alkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl,
 phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and,
 if substituted by amino and hydroxy, bearing the substituents on different carbon
 atoms ; or wherein the substituent is



15 with Z being X defined above and with the proviso that Z together with the carbonyl
 group to which it is attached is other than S-3-amino-2-hydroxypropionyl or S-4-
 amino-2-hydroxybutyryl and in the case of tobramycin also other than S-5-amino-2-
 hydroxyvaleryl; or of a pharmaceutically acceptable acid addition salt of any of the
 1-N-substituted derivatives.

20 The compounds of this invention are broad spectrum antibacterial agents which,
 advantageously, exhibit activity against many organisms, particularly gram-negative
 organisms, which are resistant to their 1-N-unsubstituted precursors. Thus, the com-
 pounds of this invention can be used alone or in combination with other antibiotic
 agents to prevent the growth or reduce the number of bacteria in various environments.
 They may be used, for example, to disinfect laboratory glassware, dental and medical
 25 equipment contaminated with *Staphylococcus aureus* or other bacteria which may
 be inhibited by the compounds of this invention. The activity of the compounds of
 this invention against gram-negative bacteria renders them useful for combating
 infections caused by gram-negative organisms, e.g. species of *Proteus* and *Pseudomonas*.
 The 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, e.g.
 1-N-ethylsisomicin and 1-N-ethylverdamicin have veterinary applications, particularly
 30 in the treatment of mastitis in cattle and *Salmonella* induced diarrhea in domestic
 animals such as the dog and the cat.

The following table sets forth the Minimal Inhibitory Concentration (MIC) of
 some compounds of this invention. The tests were performed in Mueller-Hinto Broth
 (pH 7.2) using standard procedures.

		Minimal Inhibitor Concentration		
		Mg/kg (Mueller-Hinton Broth pH 7.2)		
		1	2	3
40	<i>E. Coli</i>			
	W 677/R55*	0.3	7.5	0.3
	JR 66*	3.0	7.5	3.0
	St. M. 589	7.5	17.5	3.0
	Baker 2	3.0	7.5	3.0
	F-14-BK	3.0	7.5	3.0
	LA 290/R55*	3.0	3.0	3.0
45	ATCC 10536	3.0	3.0	0.75
	<i>Pseudomonas Aeruginosa</i>			
	St. M. 762	17.5	>25	3.0
	1395	>25	>25	7.5
	NRRL 3223	7.5	17.5	3.0
	Stone 20	3.0	3.0	.075
	Stone 39	17.5	17.5	3.0
50	Stone 130*	>25	>25	3.0
	Stone 138*	>25	>25	3.0

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49		1,473,733				49
5	5	<i>Klebsiella pneumoniae</i>				
		Adler 17	3.0	3.0	0.75	
		Adler 18	3.0	3.0	0.75	
		Georgetown 3694*	17.5	17.5	0.3	
		Georgetown 3020*	3.0	7.5	0.30	5
10	10	<i>Staphylococcus aureus</i>				
		209 P	3.0	17.5	0.75	
		Wood	3.0	7.5	0.3	
		<i>Streptococcus pyogenes</i>				
		C	>25	>25	3.0	10
15	15	A. Alvarez	3.0	7.5	7.5	
		<i>Bacillus subtilis</i>				
		6623	.075	0.3	0.03	
		* gentamicin resistant				
		In general, the dosage administered of the compounds of this invention will be dependent upon the age and weight of the animal species being treated, the mode of administration, and the type and severity of bacterial infection being prevented or reduced. In general, the dosage of the derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols employed to combat a given bacterial infection will be similar to the dosage requirements of the corresponding 1-N-unsubstituted-4,6-di-(aminoglycosyl)-1,3-diaminocyclitols.				15
20	20	The compounds of this invention may be administered orally. They may also be applied topically in the form of ointments, both hydrophilic and hydrophobic, in the form of lotions which may be aqueous, non-aqueous or of the emulsion type or in the form of creams. Pharmaceutical carriers useful in the preparation of such formulations will include, for example, such substances as water, oils, greases, polyesters, polyols and the like.				20
		For oral administration the compounds of this invention may be compounded in the form of tablets, capsules, elixirs or the like or may even be admixed with animal feed. It is in these dosage forms that the antibacterials are most effective for treating bacterial infections of the gastro-intestinal tract which infections cause diarrhea.				25
		In general, the topical preparations will contain from about 0.1 to about 3.0 gms. of the compounds of the invention per 100 gms. of ointment, creams or lotion. The topical preparations are usually applied gently to lesions from about 2 to about 5 times a day.				30
		The antibacterials of this invention may be utilized in liquid form such as solutions, suspensions and the like for otic and optic use and may also be administered parenterally via intramuscular injection. The injectable solution or suspension will usually be administered at from about 1 mg. to about 15 mgs, of antibacterial per kilogram of body weight per day divided into about 2 to about 4 doses. The precise dose depends on the stage and severity of the infection, the susceptibility of the infecting organism to the antibacterial and the individual characteristics of the animal species being treated.				35
		The following formulations are to exemplify some of the dosage forms in which the antibacterial agents of this invention may be employed:				40
30	30	Formulation 1				45
		Tablet	10 mg. Tab.	25 mg. Tab.	100 mg. Tab.	
		1-N-ethylsisomicin	10.50* mg.	26.25* mg.	105.00* mg.	
		Lactose, impalpable powder	197.50 mg.	171.25 mg.	126.00 mg.	50
		Corn Starch	25.00 mg.	25.00 mg.	35.00 mg.	
35	35	Polyvinylpyrrolidone	7.50 mg.	7.50 mg.	7.50 mg.	
		Magnesium Stearate	2.50 mg.	2.50 mg.	3.50 mg.	
		* 5% excess				
		Procedure				55
		Prepare a slurry consisting of the 1-N-ethylsisomicin, lactose and polyvinylpyrrolidone. Spray dry the slurry. Add the corn starch and magnesium stearate. Mix and compress into tablets.				

Formulation 2

Ointment	1.0 gm.
1-N-ethylverdamicin	0.5 gm.
Methyl paraben U.S.P.	0.1 gm.
Propyl paraben U.S.P.	to 1000 gm.
Petrolatum	

Procedure

- (1) Melt the petrolatum
- (2) Mix the 1-N-ethylverdamicin, methyl paraben and propyl paraben with about 10% of the molten petrolatum.
- (3) Pass the mixture through a colloid mill.
- (4) Add the remainder of the petrolatum with agitation and cool the mixture until it becomes semi-solid. At this stage the product may be put into suitable containers.

Ointments of other 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols and of the acid addition salts thereof are prepared by substituting an equivalent quantity of another compound of this invention for 1-N-ethylverdamicin in the foregoing example and by following substantially the procedure of the example.

Formulation 3

Injectable Solution	Per 2.0 ml. vial	Per 50 Liters
1-N-ethylsisomicin sulfate	84.0 mgs.*	2100.0 gms.*
Methyl paraben, U.S.P.	3.6 mgs.	90.0 gms.
Propyl paraben, U.S.P.	0.4 mgs.	10.0 gms.
Sodium bisulfite, U.S.P.	6.4 mgs.	160.0 gms.
Disodium ethylenediamine tetraacetate dihydrate, R.G.	0.2 mgs.	5.0 gms.
Water, U.S.P. q.s.	2.0 ml.	50.0 liters

* Includes a 5% manufacturing overcharge.

Procedure: For a 50.0 liter batch

Charge approximately 35 liters of water for injection to a stainless steel jacketed vessel and heat to about 70°C. Charge the methylparaben and propylparaben to the heated water for injection and dissolve with agitation. When the parabens are completely dissolved, cool the contents of the tank to 25—30°C by circulating cold water through the tank jacket. Sparge the solution with nitrogen gas for at least 10 minutes and keep covered with nitrogen gas for at least 10 minutes and keep covered with nitrogen during subsequent processing. Charge and dissolve the disodium EDTA and sodium bisulfite. Charge and dissolve the 1-N-ethylsisomicin sulfate. Bring the batch volume up to 50.0 liters with water for injection and agitate until homogenous.

Under sterile conditions, filter the solution through a suitable bacterial retentive filter collecting the filtrate in a filling tank.

Fill the filtrate aseptically into sterile pyrogen-free multiple dose vials, stopper and seal.

In like manner, injectable solutions of 1-N-propylsisomicin, 1-N-ethylverdamicin and 1-N-propylverdamicin and especially acid addition salts of such antibacterial agents, may be prepared by substituting an equivalent quantity of such compounds for 1-N-ethylsisomicin sulfate and by following the procedure set forth above.

WHAT WE CLAIM IS:—

1. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl, or tolyl, said aliphatic radicals having up to seven carbon



Formulation 2

	Ointment		
	1-N-ethylverdamicin	1.0 gm.	
	Methyl paraben U.S.P.	0.5 gm.	
5	Propyl paraben U.S.P.	0.1 gm.	5
	Petrolatum	to 1000 gm.	

Procedure

- (1) Melt the petrolatum
- (2) Mix the 1-N-ethylverdamicin, methyl paraben and propyl paraben with about 10% of the molten petrolatum.
- (3) Pass the mixture through a colloid mill.
- (4) Add the remainder of the petrolatum with agitation and cool the mixture until it becomes semi-solid. At this stage the product may be put into suitable containers.

Ointments of other 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols and of the acid addition salts thereof are prepared by substituting an equivalent quantity of another compound of this invention for 1-N-ethylverdamicin in the foregoing example and by following substantially the procedure of the example.

Formulation 3

	Injectable Solution	Per 2.0 ml. vial	Per 50 Liters	
20	1-N-ethylsisomicin sulfate	84.0 mgs.*	2100.0 gms.*	20
	Methyl paraben, U.S.P.	3.6 mgs.	90.0 gms.	
	Propyl paraben, U.S.P.	0.4 mgs.	10.0 gms.	
	Sodium bisulfite, U.S.P.	6.4 mgs.	160.0 gms.	
25	Disodium ethylenediamine tetraacetate dihydrate, R.G.	0.2 mgs.	5.0 gms.	25
	Water, U.S.P. q.s.	2.0 ml.	50.0 liters	

* Includes a 5% manufacturing overcharge.

Procedure: For a 50.0 liter batch

Charge approximately 35 liters of water for injection to a stainless steel jacketed vessel and heat to about 70°C. Charge the methylparaben and propylparaben to the heated water for injection and dissolve with agitation. When the parabens are completely dissolved, cool the contents of the tank to 25—30°C by circulating cold water through the tank jacket. Sparge the solution with nitrogen gas for at least 10 minutes and keep covered with nitrogen gas for at least 10 minutes and keep covered with nitrogen during subsequent processing. Charge and dissolve the disodium EDTA and sodium bisulfite. Charge and dissolve the 1-N-ethylsisomicin sulfate. Bring the batch volume up to 50.0 liters with water for injection and agitate until homogenous.

Under sterile conditions, filter the solution through a suitable bacterial retentive filter collecting the filtrate in a filling tank.

Fill the filtrate aseptically into sterile pyrogen-free multiple dose vials, stopper and seal.

In like manner, injectable solutions of 1-N-propylsisomicin, 1-N-ethylverdamicin and 1-N-propylverdamicin and especially acid addition salts of such antibacterial agents, may be prepared by substituting an equivalent quantity of such compounds for 1-N-ethylsisomicin sulfate and by following the procedure set forth above.

WHAT WE CLAIM IS:—

1. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl, or tolyl, said aliphatic radicals having up to seven carbon



* C L M S *

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atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and of the pharmaceutically acceptable acid addition salts thereof, which process comprises treating one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which may have amino-protecting groups at any position other than position 1, with an aldehyde of the formula



with X' being a group as defined for X above wherein any amino or hydroxy group present may be protected, in the presence of a hydride donor-reducing agent, and, if required, removing all protecting groups present in the molecule the last process step being followed by isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

2. Process as claimed in claim 1, wherein the reaction is carried out at a pH in the range of from 1 to 11.

3. Process as claimed in claim 1 or 2, wherein the reaction is carried out at a pH in the range of from 2 to 5.

4. Process as claimed in any of claims 1 to 3, wherein the reaction is carried out at a pH in the range of from 2.5 to 3.5.

5. Process as claimed in any of claims 1 to 4, wherein the reaction is carried out in an inert solvent.

6. Process as claimed in any of claims 1 to 5, wherein the reaction is carried out in an inert, protic solvent.

7. Process as claimed in any of claims 1 to 6, wherein the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol is treated with at least one equivalent of aldehyde in the presence of one equivalent of hydride-donor reducing agent.

8. Process as claimed in any of claims 1 to 7, wherein the hydride-donor reducing agent is a dialkylaminoborane, a tetraalkylammonium cyanoborohydride, an alkali metal cyanoborohydride or an alkali metal borohydride.

9. Process as claimed in any of claims 1 to 8, wherein a hemi-acetal or acetal of the aldehyde is used in acidic medium and the required aldehyde is formed *in situ*.

10. Process as claimed in any of claims 1 to 9, wherein as 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol sisomicin or verdamicin is used, the amino groups of which are in the free form or protected in position 6' or in positions 2' and 6' or in positions 2', 3, and 6'.

11. Process as claimed in any of claims 1 to 10, wherein in the aldehyde of the formula $X'-CHO$, X' represents methyl, ethyl, or propyl.

12. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is $-CH_2X$ with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms, and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and of the pharmaceutically acceptable acid addition salts thereof, which process comprises reducing the NC— double bond in a 1-N=CHX'-substituted derivative of one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, wherein all NH₂ groups are protected and NHCH₃ groups may be protected; with X' being a group as defined for X above, wherein any amino or hydroxy group present may be protected, removing all protecting groups present in the molecule and isolating the desired derivative as such or as a pharmaceutically acceptable acid addition salt.

13. Process as claimed in claim 12, wherein the N,C-double bond is reduced in a 1-N-substituted derivative of Antibiotic 66-40D or of one of the 4-aminoglycosyl-6-garosaminy-1,3-diaminocyclitols gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, Antibiotic G-418, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5, and mutamicin 6 having a 3''-N-4''-O-protecting group of the formula



CHX'

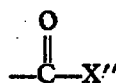
where X' is as defined in claim 12 and is identical with X' in the 1-N=CHX' moiety.

14. Process as claimed in claim 12 or 13, wherein the NH₂ groups of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol starting materials are protected with triphenylmethyl, 2,4-dinitrophenyl, acetyl, trifluoroacetyl, propionyl, benzoyl, methoxycarbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, t-butoxycarbonyl, 2-iodoethoxycarbonyl, carbobenzyloxy or 4-methoxybenzyloxycarbonyl.

15. Process as claimed in any of claims 12 to 14, wherein the reducing agent is a dialkylaminoborane, a tetraalkylammonium cyanoborohydride, an alkali metal cyanoborohydride or an alkali metal borohydride.

16. Process as claimed in any of claims 12 to 15, wherein reduction is effected with sodium borohydride in the presence of sodium methoxide.

17. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is —CH₂X with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms, and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and of the pharmaceutically acceptable acid addition salts thereof, which process comprising treating a 1-N-substituted derivative of one of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols, wherein one or more amino-groups may be protected and the 1-N-substituent is



with X'' being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl, tolyl or hydrocarbyloxy, said aliphatic radicals having up to seven carbon atoms, and if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, and wherein any amino or hydroxy group present may be protected; with an amide-reducing hydride reagent and, if required removing all protecting groups present in the molecule; the last process step being followed by isolating the desired derivative as such or as a pharmaceutically acceptable acid addition salt.

18. Process as claimed in claim 17, wherein the amide-reducing hydride reagent is an aluminum hydride or a borohydride.

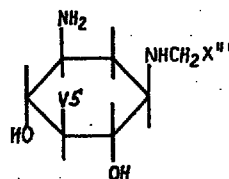
19. Process as claimed in claim 17 or 18, wherein the amide-reducing hydride reagent is lithium aluminum hydride or diborane.

20. Process as claimed in any of claims 17 to 19, wherein the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol is sisomicin or verdamicin, X'' in the 1-N-substituent is methyl, ethyl or propyl and the reducing agent is lithium aluminum hydride.

21. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols sisomicin and mutamicin 2, wherein the substituent is



with X''' being hydrogen, methyl, ethyl or propyl, and of the pharmaceutically acceptable acid addition salts thereof, which process comprises cultivating a mutant strain of the species *Micromonospora inyoensis*, namely *Micromonospora inyoensis* strain 1550F-1G or a strain of the same species corresponding thereto in its capability of producing one of the above defined antibiotics, in a nutrient medium comprising a compound of the formula



wherein V_s is hydroxy or hydrogen, and X''' is as defined above, until substantial antibiotic activity is imparted to the medium and isolating the so-obtained compound as such or in the form of a pharmaceutically acceptable acid addition salt.

22. Process as claimed in claim 21, wherein fermentation is conducted at a pH in the range of from 6.0 to 8.0 and at from 24° to 49°C.

23. Process for the preparation of 1-*N*-substituted derivatives of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5, and mutamicin 6, wherein the substituent is straight chain alkyl having up to five carbon atoms and of the pharmaceutically acceptable acid addition salts thereof which process comprises reacting one of the above named 4,6-di(aminoglycosyl)-1,3-diaminocyclitols, which possesses amino-protecting groups at any position other than position 1, and wherein the 1-amino group may be activated, with an alkylating agent containing the straight chain alkyl group having up to five carbon atoms and a leaving group, removing the protecting groups and, if required, the activating group or groups present in the molecule, and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

24. Process as claimed in claim 23, wherein the free 1-amino group of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitol is alkylated.

25. Process as claimed in claim 23, wherein the 1-amino group of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitol is alkylated in the form of the trifluoromethylsulphonyl derivative.

26. Process as claimed in claim 23, wherein the 1-amino group of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitol is alkylated in the form of the Di-(2-cyanoethyl)-derivative.

27. Process as claimed in any of claims 23 to 26, wherein the alkylating agent is an alkylfluorosulfonate, a dialkylsulfate or an alkyl iodide.

28. Process as claimed in any of claims 23 to 27, wherein the 4,6-di(aminoglycosyl)-1,3-diaminocyclitol, which possesses amino protecting groups at any position other than position 1, is 3''-*N*-4''-*O*-carbonyl-2',3,6'-tri-*N*-*t*-butoxycarbonyl-sisomicin.

29. Process for the preparation of 1-*N*-substituted derivatives of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is methyl, and of the pharmaceutically acceptable acid addition salts thereof which process comprises reacting one of the above named 4,6-di(aminoglycosyl)-1,3-diaminocyclitols which possesses amino-protecting groups at any position other than position 1, with formaldehyde and cyclic imide, treating the so-obtained compound with a hydride-donor reducing agent, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

30. Process as claimed in claim 29, wherein the cyclic imide is succinimide.

31. Process as claimed in claim 29 or 30, wherein the hydride-donor reducing agent is sodium borohydride.

32. Process for the preparation of 1-*N*-substituted derivatives of the 4,6-di(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5, and mutamicin 6, wherein the substituent is methyl, and of the pharmaceutically acceptable acid addition salts thereof which process comprises reacting one of the above named 4,6-di(aminoglycosyl)-1,3-



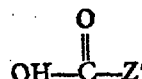
diaminocyclitols which possesses amino protecting groups at any position other than position 1, with formaldehyde in the presence of formic acid, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

5 33. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-
(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁,
gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b},
gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-
10 40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52,
mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the
substituent is 2-hydroxyethyl, and of the pharmaceutically acceptable acid addition
salts thereof which process comprises reacting one of the above named 4,6-di-(amino-
glycosyl)-1,3-diaminocyclitols which possesses amino protecting groups at any position
15 other than position 1, with ethylene oxide, removing all protecting groups present in
the molecule and isolating the derivatives as such or as a pharmaceutically acceptable
acid addition salt.

34. Process for the preparation of 1-N-substituted derivatives of the 4,6-di-
(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁,
gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b},
20 gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-
40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52,
mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the
substituent is



25 with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, amino-
alkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl,
benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if sub-
stituted by amino and hydroxy, bearing the substituents on different carbon atoms,
30 with the proviso that Z together with the carbonyl group to which it is attached is
other than S-3-amino-2-hydroxypropionyl or S-4-amino-2-hydroxybutyryl, and, in case
of tobramycin, also other than S-5-amino-2-hydroxyvaleryl, and of the pharma-
ceutically acceptable acid addition salts thereof; which process comprises treating one
of the above named 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols which may have amino
protecting groups at any position other than position 1, with an acid of the formula



35 with Z' being a group as defined for Z above, wherein any amino or hydroxy group
present may be protected, in the presence of a carbodiimide or with a reactive deriva-
tive of above said acid, and, if required, removing all protecting groups present in the
molecule, the last process step being followed by isolating the derivative as such or as
40 a pharmaceutically acceptable acid addition salt.

35. Process as claimed in claim 34, wherein the 4,6-di-(aminoglycosyl)-1,3-
diaminocyclitol starting compound is used as a free base.

36. Process as claimed in claim 34 or 35, wherein one of the 4,6-di-(amino-
glycosyl)-1,3-diaminocyclitols tobramycin gentamicin B, gentamicin C_{1a}, Antibiotic
45 JI-20A, Antibiotic 66-40B, Antibiotic 66-40D, sisomicin, mutamicin 1, mutamicin 2,
mutamicin 4, mutamicin 5 and mutamicin 6 having a 6'-amino protecting group is
used.

37. Process as claimed in claim 34 or 35, wherein any of the 4,6-di-(amino-
glycosyl)-1,3-diaminocyclitol starting compounds having free amino groups is used.

50 38. Process as claimed in claim 34, wherein 1 to n amino groups of the 4,6-di-
(aminoglycosyl)-1,3-diaminocyclitol starting compound are neutralized by formation
of an acid addition salt and n is the number of amino groups in the molecule.

39. Process as claimed in claim 38, wherein (n-1) amino groups are neutralized
by formation of an acid addition salt.

55 40. Process as claimed in any of claims 34 to 38, wherein a reactive derivative
of the acid is used as acylating agent.



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1,473,733

55

62

41. Process as claimed in any of claims 34 to 40, wherein the reactive derivative of the acid is an ester, an azide, an imidazole derivative or the anhydride.

42. Process as claimed in any of claims 34 to 41, wherein the reactive derivative of the acid is the N-hydroxysuccinimidyl ester.

43. A process as claimed in any of claims 1 to 42, substantially as herein described.

44. A process as claimed in any of claims 1 to 42, substantially as herein described in any of the Examples.

45. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is —CH₂X with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms.

46. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5, and mutamicin 6, wherein the substituent is —CH₂X with X being hydrogen, alkyl, alkenyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.

47. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein said 6-aminoglycosyl is garosaminyl, the 4-aminoglycosyl-6-garosaminyl-1,3-diaminocyclitol being gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, Antibiotic G-418, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 or mutamicin 6, wherein the substituent is —CH₂X and X is as defined in claim 45.

48. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein said 6-aminoglycosyl is garosaminyl and said 1,3-diaminocyclitol is 2-deoxystreptamine, the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamine being gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, or Antibiotic G-418, wherein the substituent is —CH₂X and X is as defined in claim 45.

49. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein said 6-aminoglycosyl is garosaminyl and said 1,3-diaminocyclitol is 2-deoxystreptamine, the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamine being gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin X₂, sisomicin, verdamicin, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52 or Antibiotic G-418, wherein the substituent is —CH₂X and X is as defined in claim 45.

50. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein said 6-aminoglycosyl is garosaminyl and said 1,3-diaminocyclitol is 2-deoxystreptamine, the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamine being gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamicin or Antibiotic G-52, wherein the substituent is —CH₂X and X is as defined in claim 45.

51. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols sisomicin, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is —CH₂X with X being hydrogen or alkyl having up to seven carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.

52. A compound as claimed in claim 51, wherein X is hydrogen or alkyl having up to two carbon atoms.

53. A 1-N-substituted derivative of sisomicin, wherein the substituent is —CH₂X and X is as defined in claim 45.



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56

1,473,733

56

54. A 1-*N*-substituted derivative of verdamicin, wherein the substituent is —CH₂X and X is as defined in claim 45.

55. A 1-*N*-substituted derivative of Antibiotic 66-40D, wherein the substituent is —CH₂X and X is as defined in claim 45.

56. A compound as claimed in any of claims 45 to 50 and 53 to 55, wherein X is hydrogen, alkyl, hydroxyalkyl, aminoalkyl, aminohydroxyalkyl, phenyl or benzyl, said aliphatic radicals having up to seven carbon atoms, and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms.

57. A compound as claimed in any of claims 45 to 50 and 53 to 56, wherein X is hydrogen or alkyl having up to seven carbon atoms.

58. A compound as claimed in any of claims 45 to 50 and 53 to 57, wherein X is hydrogen, methyl, ethyl or propyl.

59. A compound as claimed in any of claims 45 to 50 and 53 to 58, wherein X is methyl or ethyl.

60. A compound as claimed in any of claims 45 to 50 and 53 to 56, wherein X is aminoalkyl having up to seven carbon atoms.

61. A compound as claimed in any of claims 45 to 50 and 53 to 56, wherein X is hydroxyalkyl having up to seven carbon atoms.

62. A compound as claimed in any of claims 45 to 50 and 53 to 56, wherein X is aminohydroxyalkyl having up to three carbon atoms and bearing the substituents on different carbon atoms.

63. A pharmaceutically acceptable acid addition salt of any of the compounds of claims 45, 47 to 50, and 53 to 62.

64. 1-*N*-Ethylsisomicin or a pharmaceutically acceptable acid addition salt thereof.

65. 1-*N*-Ethylverdamicin or a pharmaceutically acceptable acid addition salt thereof.

66. 1-*N*-Ethylgentamicin C_{1a} or a pharmaceutically acceptable acid addition salt thereof.

67. 1-*N*-Ethylgentamicin C₁ or a pharmaceutically acceptable acid addition salt thereof.

68. 1-*N*-Ethyl-Antibiotic G-52 or a pharmaceutically acceptable acid addition salt thereof.

69. 1-*N*-(*n*-Propyl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

70. 1-*N*-(*n*-Propyl)verdamicin or a pharmaceutically acceptable acid addition salt thereof.

71. 1-*N*-(δ -Aminobutyl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

72. 1-*N*-(δ -Aminopropyl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

73. 1-*N*-(*n*-Butyl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

74. 1-*N*-Methylsisomicin or a pharmaceutically acceptable acid addition salt thereof.

75. 1-*N*-Methylverdamicin or a pharmaceutically acceptable acid addition salt thereof.

76. 1-*N*-Ethylmutamicin 6 or a pharmaceutically acceptable acid addition salt thereof.

77. 1-*N*-(*n*-Butyl)verdamicin or a pharmaceutically acceptable acid addition salt thereof.

78. 1-*N*-(*S*-2-Hydroxy-4-aminobutyl)-gentamicin C₁ or a pharmaceutically acceptable acid addition salt thereof.

79. 1-*N*-(*S*-2-Hydroxy-4-aminobutyl)-sisomicin or a pharmaceutically acceptable acid addition salt thereof.

80. 1-*N*-(*S*-2-Hydroxy-4-aminobutyl)-verdamicin or a pharmaceutically acceptable acid addition salt thereof.

81. A compound of any of claims 45 to 80, whenever prepared by the process as claimed in any of claims 1 to 33, 43 and 44.

82. A 1-*N*-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



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1,473,733

57



with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms, and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, with the proviso that Z together with the carbonyl group to which it is attached is other than S-3-amino-2-hydroxypropionyl or S-4-amino-2-hydroxybutyryl, and, in case of tobramycin and gentamicin C₂, also other than S-5-amino-2-hydroxyvaleryl, or a pharmaceutically acceptable acid addition salt thereof.

83. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, aminohydroxyalkyl with at least 5 carbon atoms, N-alkylaminohydroxyalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.

84. A compound of claim 82, wherein Z is hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, N-alkylaminoalkyl, phenyl, benzyl or tolyl, said aliphatic radicals having up to seven carbon atoms.

85. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with Z being as defined in claim 82, or a pharmaceutically acceptable acid addition salt thereof.

86. A 1-N-substituted derivative of one of the 4,6-di-(aminoglycosyl)-1,3-diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin X₂, sisomicin, verdamicin, tobramycin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, mutamicin 1, mutamicin 2, mutamicin 4, mutamicin 5 and mutamicin 6, wherein the substituent is



with Z being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl or aminoalkyl, said aliphatic radicals having up to four carbon atoms, or a pharmaceutically acceptable acid addition salt thereof.

87. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein the 6-aminoglycosyl is garosaminyl and the 1,3-diaminocyclitol is 2-deoxystreptamine, the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines being gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B, or Antibiotic G-52, wherein the substituent is



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58

1,473,733

58



with Z being as defined in any of claims 82 to 84 or a pharmaceutically acceptable acid addition salt thereof.

- 5 88. A 1-N-substituted derivative of a 4,6-di-(aminoglycosyl)-1,3-diaminocyclitol, wherein the 6-aminoglycosyl is garosaminyl and the 1,3-diaminocyclitol is 2-deoxystreptamine, the 4-aminoglycosyl-6-garosaminyl-2-deoxystreptamines being gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamycin or Antibiotic G-52, wherein the substituent is



- 10 with Z being as defined in any of claims 82 to 84 or a pharmaceutically acceptable acid addition salt thereof.

89. A 1-N-substituted derivative of sisomicin, wherein the substituent is



- 15 with Z being as defined in any of claims 82 to 84 or a pharmaceutically acceptable acid addition salt thereof.

90. A 1-N-substituted derivative of verdamycin, wherein the substituent is



- 20 with Z being as defined in any of claims 82 to 84 or a pharmaceutically acceptable acid addition salt thereof.

91. A 1-N-substituted derivative of Antibiotic 66-40D wherein the substituent



- 25 with Z being as defined in any of claims 82 to 84 or a pharmaceutically acceptable acid addition salt thereof.

92. A compound as claimed in any of claims 82 to 91, wherein Z is methyl, ethyl or propyl.

93. 1-N-Acetylsisomicin or a pharmaceutically acceptable acid addition salt thereof.

94. 1-N-Acetylverdamycin or a pharmaceutically acceptable acid addition salts thereof.

30 95. 1-N-Acetylantibiotic G-52 or a pharmaceutically acceptable acid addition salts thereof.

96. 1-N-Acetylgentamicin C_{1a} or a pharmaceutically acceptable acid addition salts thereof.

35 97. A compound of any of claims 82 to 96, whenever prepared by the process as claimed in any of claims 34 to 44.

98. A pharmaceutical composition comprising as active ingredient at least one compound as claimed in claim 45, together with a pharmaceutical carrier or excipient.

99. A composition as claimed in claim 98, in the form of dosage units.

40 100. A composition as claimed in claim 98 or 99, wherein the active ingredient is a compound claimed in any of claims 64 to 80.

101. A pharmaceutical composition comprising at least one compound as claimed in claim 82, together with a pharmaceutical carrier or excipient.

102. A composition as claimed in claim 101, in the form of dosage units.

45 103. A composition as claimed in claim 101 or 102, wherein the active ingredient is a compound claimed in any of claims 93 to 96.



59

1,473,733

59

104. A pharmaceutical composition as claimed in claims 98 or 101, substantially as herein described with specific reference to the Examples.

105. Process for preparing a pharmaceutical composition as claimed in any of claims 98 to 104, wherein the active ingredient is brought into a form suitable for therapeutic administration.

106. Process as claimed in claim 105, wherein the active ingredient is admixed with a pharmaceutical carrier or excipient.

107. Pharmaceutical composition whenever obtained by a process as claimed in claim 105 or 106.

108. A method of eliciting an antibacterial response in a warm-blooded non-human animal having a susceptible bacterial infection, which comprises administering to said animal a non-toxic, antibacterially effective amount of a compound as claimed in any of claims 45 to 80.

109. A method of eliciting an antibacterial response in a warm-blooded non-human animal having a susceptible bacterial infection, which comprises administering to said animal a non-toxic antibacterially effective amount of a compound as claimed in any of claims 82 to 96.

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