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PATENT SPECIFICATION

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367 368 36Y 43X 581 595 597 601 602 627 628 62X 634
63X 640 643 64X 650 652 658 660 662 670 672 67X 695
70Y 790 791 79Y KA KB KH KM KY LH

(54) DERIVATIVES OF DIAMINOCYCLITOLS AND METHODS FOR THEIR PREPARATION

(71) We, SCHERICO LTD., of Töpferstrasse 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 semi-synthetic aminoglycoside aminocyclitol antibacterial agents and to methods for their production.

In particular, this invention relates to antibacterially active 1 - N - substituted derivatives of 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols wherein the 1 - N - substituent is an aminohydroxyacyl group, to the non-toxic acid addition salts of such agents, and to methods for producing such compounds.

In U.S. Patent Specification No. 3,780,018 is described a process whereby 1 - [L - (—) - γ - amino - α - hydroxybutyryl]gentamicin C₁ and 2' [L - (—) - γ - amino - α - hydroxybutyryl]gentamicin C₁ are prepared by reacting gentamicin C₁ with a blocked active ester of L - (—) - γ - amino - α - hydroxybutyric acid followed by deblocking via methods known in the art and separation of the reaction mixture by chromatographic means. In U.S. Patent Specification No. 3,796,698 is described a process for the preparation of 1 - [L - (—) - γ - amino - α - hydroxybutyryl]gentamicin C₂ and in U.S. Patent Specification No. 3,796,699 is described a process for the preparation of 1 - [L - (—) - γ - amino - α - hydroxybutyryl]gentamicin C_{1a}.

The novel antibacterial agents of this invention have surprisingly unexpected properties. Generally, these novel compounds are active against bacterial and/or protozoan strains. Further, they are active against many bacteria which have become substantially insensitive to the underivatized antibiotics.

In one of its product aspects, this 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 C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl with the proviso that in the case of gentamicin C₁ and gentamicin C_{1a} the 1 - N - substituent is S - 3 - amino - 2 - hydroxypropionyl, and the pharmaceutically acceptable acid addition salts thereof.

A preferred group of aminoglycoside compounds from the above defined class consists of 1 - N - substituted derivatives of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl, and the pharmaceutically acceptable acid addition salts thereof.

Another preferred group of compounds consists of 1 - N - substituted derivatives of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin B, gentamicin

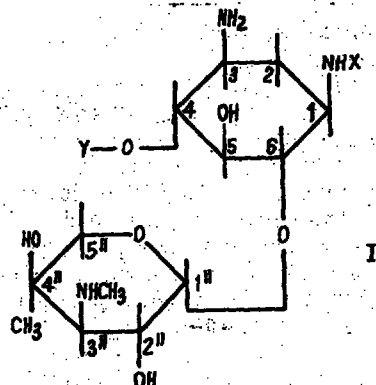
B₁, gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamycin and Antibiotic JI-20B, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl with the proviso that in the case of gentamicin C₁ and gentamicin C_{1a} the 1 - N - substituent is S - 3 - amino - 2 - hydroxypropionyl, and the pharmaceutically acceptable acid addition salts thereof.

An especially preferred group of compounds consists of 1 - N - substituted derivatives of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin B, gentamicin B₁, sisomicin and verdamycin, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl, and the pharmaceutically acceptable acid addition salts thereof.

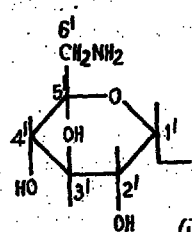
Within this group of compounds the following specifically named compounds 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B₁, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)sisomicin, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)verdamycin and the pharmaceutically acceptable acid addition salts thereof are preferred.

The compounds 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)sisomicin, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)verdamycin and the pharmaceutically acceptable acid addition salts thereof represent an especially preferred group of compounds and the compounds 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B and the pharmaceutically acceptable acid addition salts thereof are most preferred.

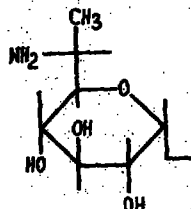
The compounds of this invention are defined by the following structural formulae I, II and III, wherein X is the 1 - N - substituent as hereinbefore defined.



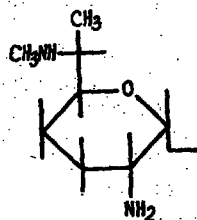
wherein Y is an aminoglycosyl function selected from:



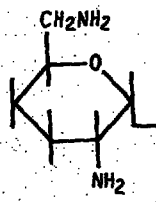
(in 1-N-X-gentamicin B)



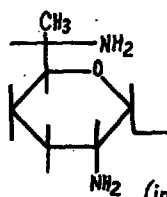
(in 1-N-X-gentamicin B₁)



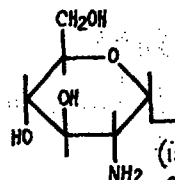
(in 1-N-X-gentamicin C₁)



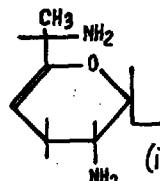
(in 1-N-X-gentamicin C_{1a})



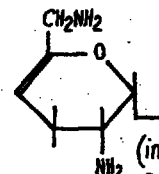
(in 1-N-X-gentamicin C_{2a})



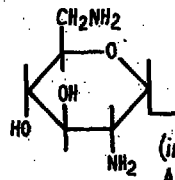
(in 1-N-X-gentamicin X₂)



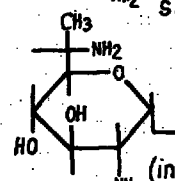
(in 1-N-X-verdamicin)



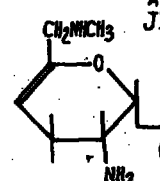
(in 1-N-X-sisomicin)



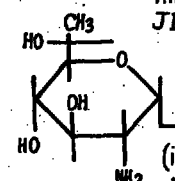
(in 1-N-X-Antibiotic JI-20A)



(in 1-N-X-Antibiotic JI-20B)



(in 1-N-X-Antibiotic G-52) and,

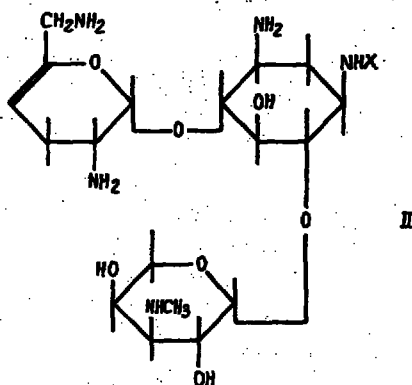


(in 1-N-X-Antibiotic G-419)

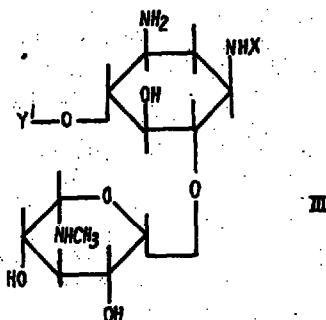
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1 - N - X - Antibiotic 66-40D of the following formula II

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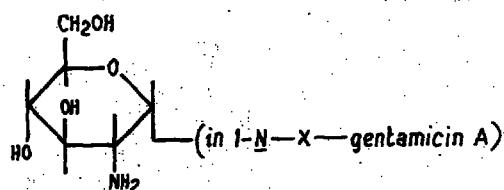
and 1 - N - X - gentamicin A and 1 - N - X - Antibiotic 66-40B of the following formula III



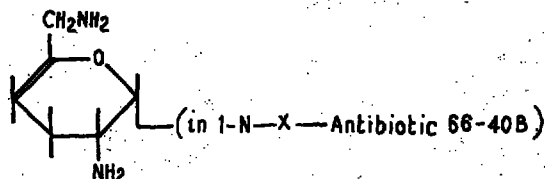
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wherein Y' is

10



and Y' is



The underivatized (parent) antibiotics (defined by above structural formulae I, II and III, wherein X is hydrogen) are all known in the art except gentamicin C_{2a}. Gentamicin C_{2a} may be prepared in the following manner:

Dissolve 96 gms. of gentamicin base (prepared from the sulfate salt obtained by the procedure of Example 4 of U.S. Patent Specification No. 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 the tubes 290—360 with fresh tubes containing 40 ml. of upper phase and 40 ml. of lower phase. Re-set the apparatus for an additional 2800 transfers and repeat the chromatographic procedure performed above. Combine the tubes 1—16 and concentrate *in vacuo* to obtain 1.3 gms. of gentamicin C_{2a} having the following properties:

(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°C 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=6.5Hz, CH—CH₃); 1.17 (3H, s, C—CH₃); 2.47 (3H, s, N—CH₃); 2.51 (1H, d, J=10.5Hz, H-3''); 3.75 (1H, q, J=10.5, 4Hz, H-2''); 4.00 (1H, d, J=12Hz, H-5''eq); 5.04 (1H, d, J=4Hz, H-1''); 5.13 (1H, d, J=3.5Hz, H-1').

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

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

The compound referred to herein as gentamicin X₂ is also known in the art as gentamicin X.

The compounds of the invention may be conveniently produced from novel intermediates which include 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols selected from gentamicin B, Antibiotic JI-20A, Antibiotic 66-40B, Antibiotic 66-40D, and sisomicin, wherein the amino group in position 6' is protected, and acid addition salts thereof. Typical amino-protecting groups are trifluoroacetyl and t-butoxycarbonyl. Exemplary of such intermediates are 6' - N - trifluoroacetyl gentamicin B, 6' - N -

trifluoroacetyl Antibiotic JI-20A, 6' - N - trifluoroacetyl Antibiotic 66-40B, 6' - N - trifluoroacetyl Antibiotic 66-40D, and 6' - N - t - butoxycarbonyl sisomicin.

Other valuable intermediates are 6' - N - trifluoroacetyl gentamicin C₁, and, furthermore, gentamicin C₁ having an amino-protecting group in position 2' or in positions 2' and 3, and the acid addition salts of such compounds. Exemplary are 2' - N - trifluoroacetyl gentamicin C₁ and 2',3 - di - N - trifluoroacetyl gentamicin C₁.

In one of its process aspects, this invention comprises 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 C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B and Antibiotic G-52, wherein the 1 - N - substituent is designated X, which represents S - 3 - amino - 2 - hydroxypropionyl or S - 4 - amino - 2 - hydroxybutyryl with the proviso that, in the case of gentamicin C₁ and gentamicin C_{1a} X is S - 3 - amino - 2 - hydroxypropionyl; 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 acid of the formula



wherein X' is a group as defined for X above, wherein the amino group and/or the hydroxy group may be protected, in the presence of a carbodiimide, such as dicyclohexylcarbodiimide, or with a reactive derivative of above said acid, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

Generally, it is preferred to use those starting compounds having a 6'-CH₂-NH₂ group in the form of their 6' - N - protected derivatives. Exemplary of preferred protecting groups are trifluoroacetyl and t - butoxycarbonyl. Gentamicin C₁ may advantageously be used as the 2',3 - di - N - protected derivative. Gentamicin A, gentamicin B₁, gentamicin C_{2a}, gentamicin C_{2a3}, gentamicin X₂, verdamicin, Antibiotic G-418, Antibiotic JI-20B and Antibiotic G-52 may be used in their protected form but also can advantageously be used in their free form.

In carrying out the process of this invention specific acylation at the amino group in position 1 rather than random acylation may be achieved by using a 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol which is partially neutralized by formation of an acid addition salt.

Protonation of an amino group provides the same effect as the presence of a "usual chemical" blocking group. It surprisingly has been found that the amino group in position 1 of a 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol is the last one which is protonated upon addition of acid or, if a per acid addition salt is treated with base, the first one which is de-protonated. Thus "blocking groups" (in the form of protons) can be facily introduced into the molecule by merely adding a desired amount of acid to the aminoglycoside or by adding a desired amount of base to a per acid addition salt thereof. The inventive process takes advantage of these findings and thus provides a convenient method for the preparation of the 1 - N - acyl derivatives of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols by using partially neutralized and therefore partially blocked starting compounds.

As used herein, the expression "partially neutralized by formation of an acid addition salt" means that each mole of 4,6 - di(aminoglycosyl) - 1,3 - diaminocyclitol has associated therewith less than the stoichiometric number of moles of acid that is required to form the per acid addition salt. Furthermore, this expression means that each mole of 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol has at least one mole of acid associated therewith.

For example, one equivalent of gentamicin C₁ having five amino groups would require five equivalents of acid to form the per acid addition salt. The inventive process is effected on an acid addition salt of gentamicin C₁ having less than five equivalents and at least one equivalent of acid (e.g. 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5 or 1.0 equivalents of acid).

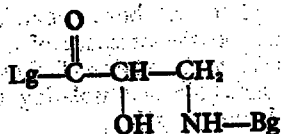
In the preferred feature of this process the starting compound is neutralized with (n-1) equivalents of acid, n being the number of amino groups in the molecule. Thus, (n-1) amino groups are neutralized by formation of an acid addition salt. It will, however, be understood that the process also can advantageously be carried out on partially neutralized starting compounds, wherein more or less than (n-1) equivalents of acid give rise to formation of the acid addition salt within the limits given above.

In terms of pH-ranges the process is carried out in a range of from 5.0 to 9.0, preferably from 5.0 to 8.0. The most preferred range of the reaction medium is a pH of from 6.5 to 7.5, especially 6.8 to 7.2.

The term "acid addition salt" embraces such salts as may be formed between the basic 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol 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 or trifluoroacetic. 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.

It is also possible to use partially neutralized starting compounds which have chemical blocking groups. This, however, is generally not necessary.

The hydroxyamino acylating agents employed in the processes of this invention are preferentially used in the form of reactive derivatives, for example in the form of esters, anhydrides, azides or acid imidazolidine derivatives. Further, it is preferred that the amino group in the hydroxyamino acylating agent is blocked. Thus, when the hydroxyaminoacyl product desired is the iso-serinyl-derivative, the preferred acylating agent would take the form of



wherein the carboxyl function is preferably activated in the form of an active ester (leaving group Lg) and the amino group is preferably blocked (blocking group Bg) by a benzyloxy carbonyl group which is readily removed by hydrogenolysis or a *t*-butoxycarbonyl group or a trifluoroacetyl group, the former being conveniently removed with acid and the latter with base.

If pharmaceutically acceptable acid addition salts of the compounds of this invention are desired, they may be prepared by adjusting an aqueous solution of said agent to pH 4.0, followed by lyophilization. The salts produced thereby are the functional equivalents of the free nitrogen base. Exemplary of acids which may be used for salt formation are sulfuric, hydrochloric, phosphoric, nitric, acetic, propionic, succinic, oxalic or maleic acid.

As used herein, the term "blocking group" or "protecting group" refers to groups which render the blocked or protected amino groups inert to subsequent desired chemical manipulation, but which can easily be removed at the end of the synthetic sequence without cleaving the desired *N* - aminohydroxyacyl group.

Amino-protecting groups are generally known in the art. However, for this invention trifluoroacetyl, 2,2,2 - trichloroethoxycarbonyl, *t* - butoxycarbonyl, benzyloxy-carbonyl and 4 - methoxy - benzyloxycarbonyl groups are among those preferred. Particularly preferred from the foregoing are trifluoroacetyl, *t* - butoxycarbonyl and benzyloxycarbonyl groups.

In the blocking process, the protecting group is usually employed in the form of an acid imidazole derivative, an acid azide or as active esters such as ethyl thiolo-trifluoroacetate, *t* - butoxycarbonyl azide, *N* - benzyloxycarbonyloxy 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 *N* - imidazolyl. Thus the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin B, sisomicin, Antibiotic 66-40B, Antibiotic 66-40D, and Antibiotic JI-20A having a 6' - amino - protecting group and the acid addition salts thereof may be prepared by treating one of the above named 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols with a compound of the formula BgLg, wherein Bg becomes an amino-protecting group and Lg is a leaving group, and isolating the so-obtained compound as such or as an acid addition salt. Preferably about equimolar quantities of reactants are used and Bg represents trifluoroacetyl or *t*-butoxycarbonyl.

For example, 6' - *N* - trifluoroacetyl - gentamicin C_{1a}, and the acid addition salts thereof may be prepared by reacting gentamicin C_{1a}, with a compound of the formula CF₃CO—Lg, wherein Lg is a leaving group, such as —SC₂H₅, and isolating

the so-obtained compound as such or as an acid addition salt. Preferably about equimolar quantities of reactants are used.

Similarly, 2' - N - protected or 2',3 - di - N - protected gentamicin C₁ and the acid addition salts thereof may be prepared by reacting gentamicin C₁ with either about one or about two molar equivalents of a compound of the formula BgLg defined above, and isolating the so-obtained compound as such or as an acid addition salt. In the compound BgLg, part Bg is preferably trifluoroacetyl.

The following Examples illustrate the invention.

Example 1

1-N-(S-4-Amino-2-hydroxybutyryl)sisomicin

A. S-4-Trifluoroacetamido-2-Hydroxybutyric Acid

Dissolve 5 g. of S - 4 - benzyloxycarbonylamine - 2 - hydroxybutyric acid in a mixture of 80 ml. of dioxane and 20 ml. of water. Add 200 mg. of 30% palladium-on-carbon and hydrogenate at ambient temperature and 3.4 atmospheres for 3 hours. Remove the catalyst by filtration and concentrate the filtrate to a residue *in vacuo*. Dry the residue in high vacuum for 72 hours. Dissolve the dried residue in 30 ml. of cold trifluoroacetic anhydride with agitation. Stir the solution for 3 hours at ambient temperature, then concentrate *in vacuo* to a residue. Triturate the residue with benzene and obtain a grey solid which is isolated by filtration, washed with benzene and dried to give the product of this step.

B. N-(S-4-Trifluoroacetamido-2-hydroxybutyryloxy)succinimide

Dissolve 20 mmoles of the product of step A in 50 ml. of ethyl acetate, add 2.31 g. of N - hydroxysuccinimide with agitation and cool the resulting solution in an ice bath. Add 4.3 g. of dicyclohexylcarbodiimide to the solution and stir the reaction mixture for 3 hours at room temperature. Filter to remove the precipitate and concentrate the filtrate to dryness thereby yielding the title product which is dried in high vacuum and used in step D.

C. 6'-N-Trifluoroacetylsisomicin

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 (60MHz, D₂O) δ 5.37 doublet, J=2Hz, H-1'; 5.12 doublet, J=4Hz, H-1''; 4.96 broad singlet, H-4'; 2.57, singlet, N-CH₃; 1.26 singlet, C-CH₃.

D. 1-N-(S-4-amino-2-hydroxybutyryl)sisomicin

Dissolve 814 mg. (1.5 mmoles) of 6' - N - trifluoroacetyl sisomicin in 12 ml. of 50% aqueous methanol and add dropwise with stirring a solution of 1.5 mmoles of the product of Step B dissolved in 3 ml. of dimethylformamide. Stir the resulting mixture at room temperature for 18 hours and concentrate *in vacuo* to a residue. Dissolve the residue in 10 ml. of concentrated ammonium hydroxide and allow to stand for 2 hours (to remove the trifluoroacetyl groups). Evaporate the solvent to obtain the title product as a residue. Chromatograph the residue on a silica gel column using the lower phase of a chloroform:methanol:ammonium hydroxide (2:1:1) solvent system as eluent. Fractions containing the title product are pooled and concentrated to a residue which is dissolved in water and lyophilized to yield an amorphous white solid.

In a manner similar to that described above treat each of the following antibiotics with ethyl thioltrifluoroacetate in methanol: gentamicin B, Antibiotic JI-20A, Antibiotic 66-40B and Antibiotic 66-40D.

Isolate the resultant products in a manner similar to that described in step C to obtain, respectively: 6' - N - trifluoroacetyl gentamicin B, 6' - N - trifluoroacetyl Antibiotic JI-20A, 6' - N - trifluoroacetyl Antibiotic 66-40B, and 6' - N - trifluoroacetyl Antibiotic 66-40D.

Subject the foregoing 6' - N - trifluoroacetyl intermediates to the procedure of step D and obtain thereby the following:

- 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B,
- 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)antibiotic 66-40B, and
- 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)antibiotic 66-40D.

In an analogous manner, by substituting an equivalent quantity of *S*-3-benzyl-oxycarbonylamino-2-hydroxypropionic acid for *S*-4-benzyl-oxycarbonylamino-2-hydroxybutyric acid, and by following the procedure of this Example, the following compounds may be prepared:

1-*N*-(*S*-3-amino-2-hydroxypropionyl)sisomicin, $[\alpha]_D^{25} +139^\circ$ (C, 0.3 in water) Anal. Calcd. for $C_{22}H_{42}O_8N_6 \cdot 1/2H_2CO_3$; C=47.74; H=7.66; N=14.85; Found C=47.39; H=7.14; N=15.16 Pmr. 100 MHz D_2O δ 1.22 (S), C—Me, δ 2.60 (S) *N*—Me 4.22 (Q), J=4Hz, 7.5Hz; δ 5.09 (d), $H_{1''}$, $J_{1'',2''}=4.0$ Hz; δ 5.34 (d), $H_{1'}$, $J_{1',2'}=2$ Hz,

1-*N*-(*S*-3-amino-2-hydroxypropionyl)gentamicin C_{120} Pmr 100 MHz, D_2O : δ 1.22 C—Me; 2.59 *N*—Me; $[\alpha]_D^{25} +115.4^\circ$ (C, 0.3 in water); 5.09 $H_{1''}$, $J_{1'',2''}=4$ Hz, 5.17 $H_{1'}$, $J_{1',2'}=3.5$ Hz Anal. Calcd. for $C_{22}H_{44}O_8N_6 \cdot H_2O \cdot CO_2$; C=45.01; H=7.39; N=13.70; Found C=44.98; H=7.81; N=13.68%,

1-*N*-(*S*-3-amino-2-hydroxypropionyl)gentamicin B, Pmr 100 MHz, D_2O+DCI ; δ 1.18 C—Me (S); 2.77 *N*—Me (S); δ 4.35 (Q), J=4.5, 8.0; $H_{1''}$, δ 5.01 (d), $J_{1'',2''}=4.0$ Hz; $H_{1'}$, 5.43 (d), $J_{1',2'}=3.5$ Hz,

1-*N*-(*S*-3-amino-2-hydroxypropionyl)antibiotic JI-20A,

1-*N*-(*S*-3-amino-2-hydroxypropionyl)antibiotic 66-40B, and

1-*N*-(*S*-3-amino-2-hydroxypropionyl)antibiotic 66-40D.

Example 2

1-*N*-(*S*-4-amino-2-hydroxybutyryl)sisomicin

Dissolve 1.6 g. of 6'-*N*-trifluoroacetylsisomicin (prepared as described in Example 1, step C) in a mixture consisting of water (10 ml.) and methanol (5 ml.). Add a solution of *N*-(*S*-4-phthalimido-2-hydroxybutyryloxy)succinimide (3 mmoles) in dimethylformamide (3 ml.). Stir the mixture at room temperature for 3 hours. Evaporate the solvent *in vacuo*, dissolve the residue in ethanol (15 ml.) and add hydrazine hydrate (0.3 g.). Heat the solution under reflux for 3 hours and then evaporate the solution *in vacuo* to a residue. Chromatograph the residue on silica gel (50 g.) using the lower phase of a chloroform:methanol:concentrated ammonium hydroxide (2:1:1) solvent system as eluant. Monitor the fractions by TLC and pool fractions containing the pure major component with mobility less than sisomicin. Yield 250 mg.

Example 3

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

A. 6'-*N*-*t*-butoxycarbonylgentamicin B

Dissolve 1 g. of gentamicin B in 30 ml. of 50% aqueous methanol and cool to $5^\circ C$. 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 (60MHz, D_2O) 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=4.5Hz, $H_{1''}$) 5.23 (1H, d, J=3.0 Hz, $H_{1'}$) PPM, Calcd. for $C_{24}H_{46}N_6O_{12} \cdot CO_2 \cdot H_2O$; C=46.57; H=7.51; N, 8.69%; Found C=46.80; H=7.82; N=8.54% $[\alpha]_D^{25} +124^\circ$ (C, 1 in methanol).

B. 1-*N*-(*S*-4-amino-2-hydroxybutyryl)-6'-*N*-*t*-butoxycarbonyl gentamicin B

Dissolve 0.582 g. of 6'-*N*-*t*-butoxycarbonyl gentamicin B in 25 ml. of methanol:water (1:3). Add a solution of 0.334 g. of *N*-(*S*-4-carbobenzyloxy-amino-2-hydroxybutyryloxy)succinimide in 5 ml. of dimethylformamide dropwise with agitation. Stir the reaction mixture at $5^\circ C$ for 5 hours, then evaporate to dryness *in vacuo*. Chromatograph the residue on 60 g. silica gel using the lower phase of a 2:1:1 chloroform:methanol:ammonium hydroxide solvent system as eluant. Collect 3 ml. fractions and monitor the content thereof by TLC. Combine fractions 160—235 and evaporate to a residue of 0.280 g., migrating as a single spot on TLC. Dissolve the residue in 80 ml. of methanol and 10 ml. of water and hydrogenate at 3.7 atmospheres over 60 mg. of 5% palladium-on-charcoal catalyst. Remove the catalyst by filtration, evaporate the filtrate to a residue and chromatograph on 20 g. of silica gel using the lower phase of a 1:1:1 chloroform:methanol:ammonium hydroxide solvent system as eluant. Combine fractions 79—127 (2 ml. each) consisting of 1-*N*-

(*S* - 4 - amino - 2 - hydroxybutyryl) - 6' - *N* - *t* - butoxycarbonyl gentamicin B. Yield 86 mg. Pmr δ 1.21 (3H, s, C-CH₃), 1.43 (9H, s, C-(CH₃)₃), 2.51 (3H, s, N-CH₃), 5.08 (1H, d, *J*=4Hz, H-1''), 5.25 (1H, d, *J*=3.5Hz, H-1') PPM.

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

Dissolve the product of step B in 0.3 ml. of trifluoroacetic acid and after 5 minutes add 30 ml. of ethyl ether. A precipitate of the compound of this Example as its trifluoroacetate salt forms and is recovered by filtration. Dissolve the precipitate in water and pass the solution through a basic ion exchange resin column in the hydroxide ion form to convert the acid addition salt to the free base. Lyophilize the column effluent to yield the compound of this Example as a white amorphous solid. Yield 72 mg. Pmr D₂O 100 MHz: δ 1.14 C-methyl (*S*), 2.45 (*S*) *N*-methyl, 5.04 (d), H-1'', *J*_{1'',2''}=4.0Hz; 5.30 (d), H-1', *J*_{1',2'}=3.5Hz.

In a similar manner, by substituting an equivalent quantity of other aminoglycoside aminocyclitol antibiotics, such as gentamicin C_{1a} or Antibiotic JI-20A, and by following the procedure of step A, the following compounds may be prepared: 6' - *N* - *t* - butoxycarbonylgentamicin C_{1a} and 6' - *N* - *t* - butoxycarbonyl Antibiotic JI-20A.

Similarly, by subjecting the latter of the foregoing compounds to the procedures of steps B and C, 1 - *N* - (*S* - 4 - amino - 2 - hydroxybutyryl)antibiotic JI-20A may be prepared.

In an analogous manner, by substituting an equivalent quantity of *N* - (*S* - 3 - carbobenzyloxyamino - 2 - hydroxypropionyloxy)succinimide for *N* - (*S* - 4 - carbobenzyloxyamino - 2 - hydroxybutyryloxy)succinimide in step B, by reacting the 6' - *N* - *t* - butoxycarbonyl antibiotics therewith and by subjecting the products obtained thereby to the procedure of step C, the following compounds may be prepared: 1 - *N* - (*S* - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 1 - *N* - (*S* - 3 - amino - 2 - hydroxypropionyl)gentamicin C_{1a} and 1 - *N* - (*S* - 3 - amino - 2 - hydroxypropionyl)antibiotic JI-20A.

Example 4

A. 1-*N*-(*S*-3-Amino-2-hydroxypropionyl)gentamicin C₁
1-*N*-(*S*-Carbobenzyloxyamino-2-hydroxypropionyl)-2'-3-di-*N*-trifluoroacetylgentamicin C₁

Dissolve 0.84 g. of 2',3 - di - *N* - trifluoroacetyl gentamicin C₁ in 40 ml. of tetrahydrofuran and add a solution of 1.19 g. of *N* - (*S* - 3 - carbobenzyloxyamino - 2 - hydroxypropionyloxy)succinimide in 24 ml. of tetrahydrofuran dropwise with stirring. Stir the reaction mixture for 24 hours, then evaporate to a residue. Chromatograph the residue on silica gel using the lower phase of a chloroform:methanol:concentrated ammonium hydroxide:water (2:1:0.2:0.8 v/v) solvent system as eluant. Monitor the chromatogram by TLC and combine fractions containing like product to obtain the major product of the reaction having the following constants: MP 125°-131°C; $[\alpha]_D^{25} = +93^\circ$ (CH₃OH) Anal. Calc. (for dihydrate) C=47.47; H=6.20; N=9.23%; Found: C=47.85; H=6.67; N=9.08%.

B. 1-*N*-(*S*-3-Carbobenzyloxyamino-2-hydroxypropionyl)gentamicin C₁

Dissolve 0.55 g. of the product of step A in 55 ml. of methanol and add 25 ml. of concentrated ammonium hydroxide with stirring. Stir for 3 days, at which time TLC on silica gel plates using the lower phase of a 1:1:1 mixture of chloroform:methanol:concentrated ammonium hydroxide solvent system as developer indicates substantially complete removal of the trifluoroacetyl blocking groups. Evaporate the solution to dryness *in vacuo*. Yield 0.2 g.; M.P. 109°-112°C; $[\alpha]_D^{25} = +73^\circ$ (H₂O).

C. 1-*N*-(*S*-Amino-2-hydroxypropionyl)gentamicin C₁

Dissolve 0.153 g. of the residue from step B in 8 ml. of acetic acid and add 0.05 g. of 10% palladium-on-carbon catalyst. Hydrogenate the solution at 4.0 atmospheres and at 25°C until TLC (using the solvent system used in step B) indicates the complete conversion to the title product (i.e. 16 hours→3 days). Remove the catalyst by filtration and evaporate the solution to a residue *in vacuo*. Chromatograph the residue over 7 g. of silica gel using the lower phase of a 2:1:1 mixture of chloroform:methanol:ammonium hydroxide as eluant. Monitor the column using TLC, combine like materials and obtain thereby the product of this Example. Yield 112 mg. M.P. 109°-119°C, $[\alpha]_D^{25} = +98^\circ$ (H₂O). Anal. Calc. (monohydrate) C=49.47; H=8.65; N=14.42%; Found: C=49.24; H=8.53; N=14.10%.

Example 5

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

A. 1-N-(S-4-Phthalimido-2-hydroxybutyryl)verdamicin

Dissolve 4.61 g. of verdamicin in 50 ml. of a methanol:water (1:3) solution and cool to 0°—5°C. Add a solution of 3.81 g. of N-(S-4-phthalimido-2-hydroxybutyryloxy)succinimide in 20 ml. dimethylformamide dropwise with stirring to the antibiotic solution. Stir for an additional 16 hours then concentrate to a residue *in vacuo*. Chromatograph the residue on a 250 g. silica gel column using the lower phase of a 2:1:1 mixture of chloroform:methanol:concentrated ammonium hydroxide as the eluant. Monitor the eluate by TLC and combine fractions containing like materials. Evaporate the combined fractions containing the major product to obtain thereby 1-N-(S-4-phthalimido-2-hydroxybutyryl)verdamicin and an isomer thereof.

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

Dissolve 4.0 g. of the product of the foregoing step in 35 ml. of ethanol and add 1.0 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 with the lower phase of a 1:1:1 (v/v) mixture of chloroform:methanol:concentrated ammonium hydroxide. Combine and evaporate the fractions which contain the major component of the reaction as demonstrated by TLC to obtain the compound of this Example as a white amorphous solid.

In a similar manner, subject to the process described in this Example the following antibiotics: gentamicin A, gentamicin B₁, gentamicin C_{2a}, gentamicin X₂, Antibiotic G-418, Antibiotic JI-20B, and Antibiotic G-52.

Isolate the resultant products in the manner described to obtain respectively: 1-N-(S-4-amino-2-hydroxybutyryl)gentamicin A, 1-N-(S-4-amino-2-hydroxybutyryl)gentamicin B₁, 1-N-(S-4-amino-2-hydroxybutyryl)gentamicin C_{2a}, 1-N-(S-4-amino-2-hydroxybutyryl)gentamicin X₂, 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic G-418, 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic JI-20B, and 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic G-52.

In an analogous manner, by substituting an equivalent quantity of N-(S-phthalimido-2-hydroxypropionyloxy)succinimide for the N-(S-phthalimido-2-hydroxybutyryloxy)succinimide and by following the process described in this Example, the 1-N-(S-3-amino-2-hydroxypropionyl)derivatives of the foregoing antibiotics may be prepared. Accordingly, using N-(S-3-phthalimido-2-hydroxypropionyloxy)succinimide in step A, subject the following antibiotics to the process of this Example: verdamicin, gentamicin A, gentamicin B₁, gentamicin C_{2a}, gentamicin X₂, Antibiotic G-418, Antibiotic JI-20B, and Antibiotic G-52.

Isolate the resultant products in the manner described in this Example to obtain, respectively: 1-N-(S-3-amino-2-hydroxypropionyl)verdamicin, 1-N-(S-3-amino-2-hydroxypropionyl)gentamicin A, 1-N-(S-3-amino-2-hydroxypropionyl)gentamicin B₁, 1-N-(S-3-amino-2-hydroxypropionyl)gentamicin C_{2a}, 1-N-(S-3-amino-2-hydroxypropionyl)gentamicin X₂, 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic G-418, 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic JI-20B, and 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic G-52.

Example 6

1. N-(S-3-*t*-Butoxycarbonylamino-2-hydroxypropionyloxy)succinimideA. S-3-*t*-Butoxycarbonylamino-2-hydroxypropionic acid

Dissolve 8.0 g. of S-3-carbobenzyloxyamino-2-hydroxypropionic acid in 110 ml. of dioxane-water (4:1). Add 200 mg. of palladium-on-carbon catalyst and hydrogenate at 3.4 atmospheres for 3 hours. Filter off the catalyst and evaporate the filtrate to a residue comprising S-3-amino-2-hydroxypropionic acid. Dissolve the acid in methanol (40 ml.) and triethylamine (8.5 ml.), cool the solution in an ice-bath and add *t*-butoxycarbonyl azide (4.76 g.). Allow the mixture to stand overnight with gradual warming to room temperature. Evaporate most of the methanol, dilute with water and acidify with dilute hydrochloric acid to pH 3. Extract the aqueous mixture several times with ethyl acetate, combine and dry the ethyl acetate extracts and evaporate to a residue *in vacuo*. Recrystallize the residue from ethyl acetate-hexane to give the title compound of this Example. Yield 3.5 g. M.P. 92—94°, $[\alpha]_D^{25}$; +14.9°, (c=0.68, H₂O), calculation for C₈H₁₅NO₅ requires: C=46.82; H=7.37; N=6.83%. Found: C=46.81; H=7.61; N=6.63%.

B. *N*-(*S*-3-*t*-Butoxycarbonylamino-2-hydroxypropionyloxy)succinimide

Dissolve 2.3 g. of *S*-3-*t*-butoxycarbonylamino-2-hydroxypropionic acid in a mixture of tetrahydrofuran (25 ml.) and ethyl acetate (60 ml.). With stirring add 1.42 g. of *N*-hydroxysuccinimide, followed by a solution of dicyclohexylcarbodiimide (2.51 g.) in ethyl acetate (10 ml.). Allow the mixture to stir for 16 hours, then filter off the precipitated solids. Evaporate the filtrate to obtain the product of this Example. Yield 3.56 g.

In a similar manner substitute an equivalent quantity of *S*-4-carbobenzyloxy-amino-2-hydroxybutyric acid for the *S*-3-carbobenzyloxyamino-2-hydroxypropionic acid in step A. Subject this acid to the procedure of this Example and obtain *N*-(*S*-4-*t*-butoxycarbonylamino-2-hydroxybutyryloxy)succinimide.

2. 2',3-Di-*N*-Trifluoroacetyl Gentamicin C₁

A. 2'-*N*-Trifluoroacetyl Gentamicin C₁

Dissolve 1.7 g. of gentamicin C₁ in 20 ml. of methanol, cool the mixture to 4°C and add 0.46 ml. (0.563 g.) of ethyl thiolotrifluoroacetate 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. 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, $[\alpha]_D^{25} = +128^\circ$ (*c*=0.3%, H₂O). Analysis for C₂₃H₄₂N₆O₈F₃ · H₂O 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%.

B. 2',3-Di-*N*-Trifluoroacetyl Gentamicin C₁

Dissolve 0.66 g. of the product of step A in 10 ml. of methanol, cool the mixture to 4°C and add 0.148 ml. (0.182 g.) of ethyl thiolotrifluoroacetate, 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 A. 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, $[\alpha]_D^{25} = 121^\circ$ (*c*=0.3%; H₂O). Analysis for C₂₅H₄₁N₆O₈F₆ requires C=44.84%; H=6.17%; N=10.46%. Found: C=44.94%; H=6.35%; N=10.17%.

C. Alternatively, steps 2A and 2B of this Example may be combined as follows: Dissolve 4.95 g. of Gentamicin C₁ in 100 ml. of methanol at about 25°C and add dropwise 4.05 g. of ethyl thiolotrifluoroacetate to the solution with stirring. Continue stirring overnight, concentrate the mixture to a residue *in vacuo* and chromatograph as described in step 2B to obtain the title compound of step 2B.

Example 7

1-*N*-(*S*-4-Amino-2-Hydroxybutyryl)Gentamicin C_{1a}

A. 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}. Yield 70 mg. 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).

B. 1-*N*-(*S*-4-Amino-2-Hydroxybutyryl)Gentamicin C_{1a}

Dissolve the product of step A 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 4.0 atmospheres and 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=9.5Hz, 4.0Hz, side chain CHOH); 5.04 (2H, m, H-1', and H-1''). Mass Spectral data: (M—H₂O) m/e 532.

Example 8

1-N-(S-4-Amino-2-Hydroxybutyryl)Gentamicin B

A. 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=4Hz); 5.25 (1H, d, J=3.5Hz).

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

Dissolve the product of step A 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 3.4 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 washing 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. Yield 12 mg.

In a similar manner, treat an equivalent quantity of the following 4,6 - di - (aminoglycosyl)aminocyclitol antibiotics to this process: gentamicin C_{2a}, gentamicin C_{2ab}, gentamicin A, gentamicin X₂, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B, and gentamicin B₁.

Isolate the respective products in the manner described in this Example and obtain thereby the following: 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin C₁, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin C_{2a}, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin A, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin X₂, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)antibiotic G-418, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)antibiotic JI-20A, 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)antibiotic JI-20B, and 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B₁.

In an analogous manner, by substituting an equivalent quantity of N - (S - 3 - benzoyloxycarbonylamino - 2 - hydroxypropionyl)succinimide for N - (S - 4 - benzoyloxycarbonylamino - 2 - hydroxybutyryl)succinimide, and by subjecting the above-named antibiotics to this process, the following products may be obtained: 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin C₁, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin C_{2a}, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin A, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin X₂, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)antibiotic G-418, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)antibiotic JI-20A, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)antibiotic JI-20B, and 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B₁.

Example 9

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

A. 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.

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

Dissolve the product of step A 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 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.

In a similar manner, subject to this process an equivalent quantity of the following antibiotics: sisomicin, Antibiotic G-52, Antibiotic 66-40B, and antibiotic 66-40D.

Isolate the respective products in the manner described and obtain thereby the following:

- 1-N-(S-4-amino-2-hydroxybutyryl)sisomicin,
- 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic G-52,
- 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic 66-40B, and
- 1-N-(S-4-amino-2-hydroxybutyryl)antibiotic 66-40D.

In an analogous manner, by substituting an equivalent quantity of N - (S - 3 - phthalimido - 2 - hydroxypropionyloxy)succinimide for N - (S - 4 - phthalimido - 2 - hydroxybutyryloxy)succinimide, and by subjecting the above named antibiotics to this process the following products may be obtained:

- 1-N-(S-3-amino-2-hydroxypropionyl)sisomicin,
- 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic G-52,
- 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic 66-40B, and
- 1-N-(S-3-amino-2-hydroxypropionyl)antibiotic 66-40D.

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, excipient or coating and processes for producing pharmaceutical compositions wherein the active ingredients are brought into a form suitable for therapeutic administration by mixing with a suitable pharmaceutical carrier or excipient or by applying a coating. Also included within the invention is 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 1 - N - substituted derivative of a 4,6 - di - (amino-glycosyl) - 1,3 - diaminocyclitol selected from gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B and Antibiotic G-52, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl with the proviso that in the case of gentamicin C₁ and gentamicin C_{1a}, the 1 - N - substituent is S - 3 - amino - 2 - hydroxypropionyl, or of a pharmaceutically acceptable acid addition salt thereof.

The compounds of this invention are broad spectrum antibacterial agents which, advantageously, exhibit activity against many organisms, which are resistant to their 1 - N - unsubstituted precursors. Thus, the compounds 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. The compounds of this invention are more active against many organisms which inactivate the parent antibiotics by acetylation of the 3-amino group and/or by adenylation of the 2''-hydroxyl group. Thus, the compounds of this invention have the potential of becoming antibacterial agents and may be employed for the same uses as their underivatized (parent) antibiotics, e.g. they may be used as a bacteriostatic rinse for hospital glassware, surgical

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instruments, bath tubs or for cleansing areas wherein laboratory animals are housed, or the like.

In addition to their utility as antibacterial agents, the compounds of this invention are useful as intermediates in the preparation of a novel class of compounds which also possess unexpectedly enhanced antibacterial activity. Evidence of this utility may be found in Belgian Patent Specification No. 818,431. Embraced by that patent are 1 - N - substituted 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols wherein the 1 - N - substituent is, amongst others, S - 4 - amino - 2 - hydroxybutyl or S - 3 - amino - 2 hydroxypropyl. These compounds may be obtained by reducing the compounds of the present invention according to standard procedures. Preferred reducing agents are aluminium hydrides and borohydrides.

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.

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 and polyols.

For oral administration the compounds of this invention may be compounded in the form of tablets, capsules or elixirs or may even be admixed with animal feeds. 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.

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 5 times a day.

The antibacterials of this invention may be utilized in liquid form such as solutions or suspensions 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.

The following formulations are to exemplify some of the dosage forms in which the antibacterial agents of this invention may be employed:

Formulation 1

Tablet	10 mg. Tab.
1-N-(S-3-amino-2-hydroxy propionyl)gentamicin B	10.50* mg.
Lactose, impalpable powder	197.50 mg.
Corn Starch	25.00 mg.
Polyvinylpyrrolidone	7.50 mg.
Magnesium Stearate	2.50 mg.

* 5% excess

Procedure

Prepare a slurry consisting of the 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)-gentamicin B, lactose and polyvinylpyrrolidone. Spray-dry the slurry. Add the corn starch and magnesium stearate. Mix and compress into tablets.

Formulation 2

Ointment	
1-N-(S-3-amino-2-hydroxy-propionyl)gentamicin B	1.0 gm.
Methyl paraben U.S.P.	0.5 gm.
Propyl paraben U.S.P.	0.1 gm.
Petrolatum	to 1000 gm.

Procedure

- (1) Melt the petrolatum
- (2) Mix the 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 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.

Formulation 3

Injectable Solution	Per 2.0ml. vial
1-N-(S-4-amino-2-hydroxy-butyl)gentamicin B sulfate	84.0 mgs.*
Methyl paraben, U.S.P	3.6 mgs.
Propyl paraben, U.S.P.	0.4 mgs.
Sodium bisulfite, U.S.P.	6.4 mgs.
Disodium Ethylenediamine tetra-acetate dihydrate, R.G.	0.2 mgs.
Water, U.S.P. q.s.	2.0 ml.

*Includes a 5 % manufacturing overcharge

Procedure: For a 50.0 liter batch

Charge approximately 35 liters of water for injection into a suitable stainless steel jacketed vessel and heat to about 70°C. Charge the methylparaben and propylparaben into 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 during subsequent processing. Charge and dissolve the disodium EDTA and sodium bisulfite. Charge and dissolve the active ingredient. Bring the batch volume up to 50.0 liters with water for injection and agitate until homogeneous.

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

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

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 C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is designated X, which represents S - 3 - amino - 2 - hydroxypropionyl or S - 4 - amino - 2 - hydroxybutyryl with the proviso that, in case of gentamicin C₁ and gentamicin C_{1a}, X is S - 3 - amino - 2 - hydroxypropionyl; and of the pharmaceutically acceptable acid addition salts thereof; which 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



wherein X' is a group as defined for X above, wherein the amino group and/or the hydroxy group may be protected, in the presence of a carbodiimide, or with a reactive derivative of above said acid, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

2. Process as claimed in claim 1, wherein one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol starting compounds gentamicin C_{1a}, gentamicin B, Antibiotic JI-20A, Antibiotic 66-40B, Antibiotic 66-40D and sisomicin having a 6' - amino - protecting group is used.

3. Process as claimed in claim 2, wherein the amino protecting group is trifluoroacetyl or t-butoxycarbonyl.



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Procedure

- (1) Melt the petrolatum
- (2) Mix the 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 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.

Formulation 3

Injectable Solution	Per 2.0ml. vial
1-N-(S-4-amino-2-hydroxybutyryl)gentamicin B sulfate	84.0 mgs.*
Methyl paraben, U.S.P.	3.6 mgs.
Propyl paraben, U.S.P.	0.4 mgs.
Sodium bisulfite, U.S.P.	6.4 mgs.
Disodium Ethylenediamine tetraacetate dihydrate, R.G.	0.2 mgs.
Water, U.S.P. q.s.	2.0 ml.

*Includes a 5% manufacturing overcharge

Procedure: For a 50.0 liter batch

Charge approximately 35 liters of water for injection into a suitable stainless steel jacketed vessel and heat to about 70°C. Charge the methylparaben and propylparaben into 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 during subsequent processing. Charge and dissolve the disodium EDTA and sodium bisulfite. Charge and dissolve the active ingredient. Bring the batch volume up to 50.0 liters with water for injection and agitate until homogeneous.

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

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

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 C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is designated X, which represents S - 3 - amino - 2 - hydroxypropionyl or S - 4 - amino - 2 - hydroxybutyryl with the proviso that, in case of gentamicin C₁ and gentamicin C_{1a}, X is S - 3 - amino - 2 - hydroxypropionyl; and of the pharmaceutically acceptable acid addition salts thereof; which 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



wherein X' is a group as defined for X above; wherein the amino group and/or the hydroxy group may be protected, in the presence of a carbodiimide, or with a reactive derivative of above said acid, removing all protecting groups present in the molecule and isolating the derivative as such or as a pharmaceutically acceptable acid addition salt.

2. Process as claimed in claim 1, wherein one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol starting compounds gentamicin C_{1a}, gentamicin B, Antibiotic JI-20A, Antibiotic 66-40B, Antibiotic 66-40D and sisomicin having a 6' - amino - protecting group is used.

3. Process as claimed in claim 2, wherein the amino protecting group is trifluoroacetyl or t-butoxycarbonyl.

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4. Process as claimed in claim 1, wherein gentamicin C₁ having amino-protecting groups in positions 2' and 3 is used as the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol starting compound.

5. Process as claimed in claim 4, wherein the amino-protecting groups are trifluoroacetyl.

6. Process as claimed in claim 1, wherein one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol starting compounds gentamicin A, gentamicin B₁, gentamicin C_{2a}, gentamicin C_{2as}, gentamicin X₂, verdamicin, Antibiotic G-418, Antibiotic JI-20B and Antibiotic G-52 having free amino groups is used.

7. Process as claimed in any of claims 1 to 6, wherein a reactive derivative of the acid HO—X' is used as acylating agent.

8. Process as claimed in any of claims 1 to 7, wherein the reactive derivative of the acid HO—X' is an ester, an azide, an acid imidazolidine derivative or the anhydride.

9. Process as claimed in any of claims 1 to 8, wherein the reactive derivative of the acid HO—X' is the N-hydroxysuccinimide ester.

10. Process as claimed in any one of claims 1 to 9, wherein one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52 is used as starting material.

11. Process as claimed in claim 1, wherein the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol is partially neutralized by formation of an acid addition salt.

12. Process as claimed in claim 11, wherein the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitol starting compound is neutralized with (n-1) equivalents of acid, n being the number of amino groups in the molecule.

13. Process as claimed in any of claims 1 to 12, wherein X' is S - 3 - amino - 2 - hydroxypropionyl or S - 4 - amino - 2 - hydroxybutyryl having an amino-protecting group.

14. A process as claimed in any of claims 1 to 13, substantially as herein described with specific reference to the Examples.

15. 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 C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl with the proviso that in case of gentamicin C₁ and gentamicin C_{1a}, the 1-N-substituent is S-3-amino-2-hydroxypropionyl, or a pharmaceutically acceptable acid addition salt thereof.

16. A 1 - N - substituted derivative of one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin C_{2a}, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl, and the pharmaceutically acceptable acid addition salts thereof.

17. A 1 - N - substituted derivative of one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin A, gentamicin B, gentamicin B₁, gentamicin X₂, sisomicin, verdamicin, Antibiotic G-418, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, and Antibiotic G-52, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl, and the pharmaceutically acceptable acid addition salts thereof.

18. A 1 - N - Substituted derivative of one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, sisomicin, verdamicin and Antibiotic JI-20B, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl with the proviso that in the case of gentamicin C₁ and gentamicin C_{1a} the 1 - N - substituent is S - 3 - amino - 2 - hydroxypropionyl, and the pharmaceutically acceptable acid addition salts thereof.

19. A 1 - N - Substituted derivative of one of the 4,6 - di - (aminoglycosyl) - 1,3 - diaminocyclitols gentamicin B, gentamicin B₁, sisomicin and verdamicin, wherein the 1 - N - substituent is a member selected from S - 3 - amino - 2 - hydroxypropionyl and S - 4 - amino - 2 - hydroxybutyryl, and the pharmaceutically acceptable acid addition salts thereof.

20. A compound selected from 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 1 - N - (S -



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4 - amino - 2 - hydroxybutyryl)gentamicin B₁, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)sisomicin, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)verdamicin and the pharmaceutically acceptable acid addition salts thereof.

21. A compound selected from 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)sisomicin, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)verdamicin and the pharmaceutically acceptable acid addition salts thereof.

22. A compound selected from 1 - N - (S - 4 - amino - 2 - hydroxybutyryl)gentamicin B, 1 - N - (S - 3 - amino - 2 - hydroxypropionyl)gentamicin B and the pharmaceutically acceptable acid addition salts thereof.

23. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

24. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic 66-40B or a pharmaceutically acceptable acid addition salt thereof.

25. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic 66-40D or a pharmaceutically acceptable acid addition salt thereof.

26. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic G-52 or a pharmaceutically acceptable acid addition salt thereof.

27. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)verdamicin or a pharmaceutically acceptable acid addition salt thereof.

28. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin C₁ or a pharmaceutically acceptable acid addition salt thereof.

29. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin C_{1a} or a pharmaceutically acceptable acid addition salt thereof.

30. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin C_{2a} or a pharmaceutically acceptable acid addition salt thereof.

31. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin X₂ or a pharmaceutically acceptable acid addition salt thereof.

32. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin A or a pharmaceutically acceptable acid addition salt thereof.

33. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin B or a pharmaceutically acceptable acid addition salt thereof.

34. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)gentamicin B₁ or a pharmaceutically acceptable acid addition salt thereof.

35. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic JI-20A or a pharmaceutically acceptable acid addition salt thereof.

36. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic JI-20B or a pharmaceutically acceptable acid addition salt thereof.

37. 1 - N - (S - 3 - Amino - 2 - hydroxypropionyl)antibiotic G-418 or a pharmaceutically acceptable acid addition salt thereof.

38. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)sisomicin or a pharmaceutically acceptable acid addition salt thereof.

39. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic 66-40B or a pharmaceutically acceptable acid addition salt thereof.

40. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic 66-40D or a pharmaceutically acceptable acid addition salt thereof.

41. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic G-52 or a pharmaceutically acceptable acid addition salt thereof.

42. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)verdamicin or a pharmaceutically acceptable acid addition salt thereof.

43. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)gentamicin C_{2a} or a pharmaceutically acceptable acid addition salt thereof.

44. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)gentamicin X₂ or a pharmaceutically acceptable acid addition salt thereof.

45. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)gentamicin A or a pharmaceutically acceptable acid addition salt thereof.

46. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)gentamicin B or a pharmaceutically acceptable acid addition salt thereof.

47. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)gentamicin B₁ or a pharmaceutically acceptable acid addition salt thereof.

48. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic JI-20A or a pharmaceutically acceptable acid addition salt thereof.

49. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic JI-20B or a pharmaceutically acceptable acid addition salt thereof.

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50. 1 - N - (S - 4 - Amino - 2 - hydroxybutyryl)antibiotic G-418 or a pharmaceutically acceptable acid addition salt thereof.

51. A compound of any of claims 15 to 50, whenever prepared by a process as claimed in any one of claims 1 to 14.

52. Pharmaceutical composition comprising as active ingredient at least one compound as claimed in any one of claims 15 to 50 together with a pharmaceutical carrier, excipient or coating.

53. A composition as claimed in claim 52, in the form of a dosage unit.

54. A composition as claimed in claim 52 or 53, substantially as herein described with specific reference to the Formulations.

55. Process for preparing a pharmaceutical composition comprising as active ingredient at least one compound as claimed in any one of claims 15 to 50, wherein the active ingredient is brought into a form suitable for therapeutical administration by mixing with a suitable pharmaceutical carrier, excipient or by applying a coating.

56. A pharmaceutical composition whenever prepared by a process as claimed in claim 55.

57. 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 one of claims 15 to 50.

MATHYS & SQUIRE,
Chartered Patent Agents,
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London E.C.4.
Agents for the Applicants.

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