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54 **Selective N-protection of aminoglycosides and novel, selectively protected aminoglycoside intermediates.**

57 This invention relates to the selective N-protection of aminoglycosides and to novel, selectively protected aminoglycoside intermediates. Modifications in aminoglycoside molecules are usually accomplished by multi-stage reactions whereby in most cases amino groups need to be protected in order to avoid side reactions. To obtain 1-N-alkyl- and 1-N-acyl- substituted- 4,6-di-O- (aminoglycosyl) -1,3-diaminocyclitols which are very potent antibiotics, ideally all other amino groups are protected before introduction of the 1-N-substituent into the 4,6-di-O- (aminoglycosyl) -1,3-diaminocyclitol. A process is provided for the selective protection of the 3"-amino function in a 1,3"-di-N- unprotected-poly-N- protected -4,6-di-O- (aminoglycosyl) -1,3-diaminocyclitol, wherein the 6-O-aminoglycosyl has an amino function at the 3"-position and hydroxyl functions at the 2" and 4" positions. Also, the respective 1-N-unprotected-poly-N- protected -4,6-di-O- (aminoglycosyl) -1,3-diaminocyclitol intermediates are described.

Selective protection of the 3"-amino function is accomplished by reaction with no more than one molar equivalent of available 1-Z-imidazole wherein Z is an amino protecting group.

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SELECTIVE N-PROTECTION OF AMINOGLYCOSIDES
AND NOVEL, SELECTIVELY PROTECTED AMINOGLY-
COSIDE INTERMEDIATES

This invention relates to a process whereby 1,3"-di-N-un-
5 protected-poly-N-protected-4,6-di-O- (aminoglycosyl)-1,3-
diaminocyclitols are selectively protected at the 3"-amino
function and to novel 1-N-unprotected-poly-N-protected-
4,6-di-O- (aminoglycosyl)-1,3-diaminocyclitols. The compounds
are useful as intermediates in the preparation of 1-N-sub-
10 stituted-4,6-di-O- (aminoglycosyl)-1,3-diaminocyclitols, very
potent antibacterial agents such as netilmicin (1-N-ethyl-
sisomicin).

U.S. Patent No. 4,002,742 describes a multistep
15 method whereby 4,6-di-O- (aminoglycosyl)-1,3-diaminocyclitols
are converted to certain 1-N-unprotected-poly-N-protected-
4,6-di-O- (aminoglycosyl)-1,3-diaminocyclitols which are
disclosed as intermediates in the preparation of 1-N-
alkylated derivatives.

French Patent No. 74-23811 describes the per-N-formylation of an aminoglycoside which is further reacted to produce a 1-N-unprotected-poly-N-formylated aminoglycoside which can be further reacted to produce a 1-N-substituted amino-
5 glycoside.

By this invention, a novel chemical process was developed whereby a 4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol, also known herein as an aminoglycoside, is converted to a 1-N-unprotected-poly-N-protected aminoglycoside, many of
10 which were heretofore unknown and could not be prepared by prior art processes.

The process of this invention resides in the concept of the selective protection of a 3"-amino function in a 1,3"-di-N-unprotected-poly-N-protected-4,6-di-O-(aminoglycosyl)-
15 1,3-diaminocyclitol, wherein the 6-O-aminoglycosyl has an amino function at the 3" position and hydroxyl functions at the 2" and 4" positions, and is characterised in that the 1,3"-di-N-unprotected-poly-N-protected-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol is reacted in a solvent with
20 no more than one molar equivalent of available 1-Z-imidazole, wherein Z is an amino protecting group selected from lower alkanoyl, lower alkoxycarbonyl, aralkoxycarbonyl, trichloroethoxycarbonyl and N-carbonylimidazole. Thereby are produced 1-N-unprotected-poly-N-protected-4,6-di-O-

(aminoglycosyl)-1,3-diaminocyclitols, said compounds being useful as intermediates in the preparation of 1-N-substituted aminoglycosides.

As used herein, the term "aminoglycoside" refers to a 4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol, "per-N-protection" refers to protection on all the amino functions of an aminoglycoside, and "poly-N-protection" refers to protection on all amino functions unless otherwise excluded as, for instance, the terminology 1,3"-di-N-unprotected-poly-N-protected.

The novel process for the selective 3"-amino protection of 1,3"-di-N-unprotected-poly-N-protected aminoglycosides to produce 1-N-unprotected-poly-N-protected aminoglycosides was found in an effort to provide another process for the production of 1-N-substituted aminoglycosides, especially 1-N-ethylsisomicin, whereby side products/reactions are minimized.

Chemical transformation on an aminoglycoside wherein the site of reaction is to be one of the amino functions should ideally be carried out on intermediates wherein every other amino function is selectively protected by a blocking group; otherwise, mixtures of various mono-N- and poly-N-derivatives are formed which require tedious separation

techniques (usually one to several column chromatographies) to isolate the desired transformation product.

However, by the prior art methods, it is not always possible to prepare the ideal selectively blocked intermediate, so that with any of the amino functions unprotected, multiple products are produced, and the desired transformation product is therefore obtained in low yields.

The present invention provides such ideally protected intermediates for the production of 1-N-substituted aminoglycosides with increased yields.

In U.S. Patent 4,002,742, which relates to the production of 1-N-substituted aminoglycosides, there is described a multistep method for preparing a 1-N-unprotected-poly-N-protected aminoglycoside, the sequence of steps being as follows:

Sisomicin is converted to:

- a) Penta-N-carbobenzyloxysisomicin $\longrightarrow$
- b) 1,3',2',6'-tetra-N-carbobenzyloxy-3"-N-4"-O-carbonylsisomicin $\longrightarrow$
- c) 3"-N-4"-O-carbonylsisomicin $\longrightarrow$
- d) 2',3',6'-tri-N-t-butoxycarbonyl-3"-N-4"-O-carbonylsisomicin.

The aforementioned process produces the product in inherently low yields. Furthermore, this process cannot produce the N-alkanoyl protected compounds claimed in this invention.

5 The French Patent No. 74-23811 describes a method whereby, for example, kanamycin B is formylated to 1,3,2',6',3"-penta-N-formylated kanamycin B, thence treated with base to produce 3,2',6',3"-tetra-N-formylated kanamycin B. The
10 aforementioned process is, however, restricted to the preparation of formyl derivatives, and is such that the product is produced in low yields (15 - 25%).

In view of the heretofore inherent problems in blocking all the amino groups, regardless of configuration, other than the 1-N-position in an aminoglycoside, it has been the
15 practice to block some of the amino functions (keeping 1 unblocked), then introduce the 1-N-substituent. Of course, inherent in this is the possibility of getting a multiplicity of compounds wherein substitution has taken place at every unblocked amino function.

20 The process of this invention utilizes as starting compounds 1,3"-di-N-unprotected-poly-N-protected aminoglycosides which are prepared by using a transition-metal (e.g. copper) complexing process, said process being described in German

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Heretofore, if the aforementioned 1,3"-di-N-unprotected-poly-N-protected-aminoglycosides were further acylated by the prior art methods, it would be expected that the 1-
5 amino function would be preferentially acylated, not the 3"-amino function since the 1 amino function is primary and less hindered than the 3"-amino function, the latter having neighboring 2" and 4"-hydroxyl functions, and in the case of aminoglycosides such as sisomicin and gentamicin, being a secondary amine. Indeed this is illustrated
10 in Example 3 wherein 1,3"-di-N-unprotected-3,2',6'-tri-N-(trichloroethoxycarbonyl) sisomicin is reacted with N-acetoxysuccinimide, deblocked at the 3,2',6'-amino functions to produce 1-N-acetylsisomicin.

15 By this invention it was found that in a 1,3"-di-N-unprotected-poly-N-protected aminoglycoside, the 3"-N-position can preferentially be protected by means of 1-Z-imidazole, this reaction taking place cleanly and in good yields. The 1-Z-imidazole is a compound wherein Z is an amino protecting group which is lower alkanoyl (acetyl, propionyl,
20 butyryl, trifluoroacetyl), lower alkoxycarbonyl (ethoxycarbonyl, t-butoxycarbonyl, trichloroethoxycarbonyl), aralkoxycarbonyl (benzyloxycarbonyl) and N-carbonylimidazole. In this invention, 1-Z-imidazole is preferentially

1-lower alkanoyl-imidazole, 1-acetylimidazole being the most preferred.

The 1-Z imidazoles are a known class of compounds. Those which are not readily available are conveniently prepared by reaction of imidazole with the appropriate acyl chloride or chloroformate.

By this process the 1,3"-di-N-unprotected-poly-N-protected aminoglycoside is allowed to react with a 1-Z-imidazole in an inert organic solvent, water and/or lower alkanol at ambient temperatures for a period of from about 0.5 - 24 hours. Using separation techniques described hereinafter, the 1-N-unprotected-poly-N-protected aminoglycoside is obtained. For example, 3,2',6'-tri-N-acetylsisomicin is reacted with acetylimidazole in a water/tetrahydrofuran mixture at room temperature for about 2 hours, then isolated utilizing known techniques to obtain 3,2',6',3"-tetra-N-acetylsisomicin.

The inert organic solvents employed in this process are usually water miscible and will not take part in the reaction, e.g. tetrahydrofuran, dioxane, dimethoxyethane, dimethylformamide and acetonitrile. In the instances where inert organic solvents are utilized alone, the ratio of the imidazole to the aminoglycoside is about equimolar.

When the reaction is carried out in a solvent containing water and/or lower alkanol (wherein the lower alkanol has up to 4 carbon atoms), the molar ratio of the imidazole to the aminoglycoside should be up to 2:1, as the water/
5 lower alkanol solvents will partially react with the imidazole compounds.

The ambient temperature utilized in this reaction can be from 5°C to about 40°C. The reaction is generally a rapid one, from about 0.5 - 2 hours but can be run over a longer
10 period of time, up to about 24 hours.

When, in this process 1-Z-imidazole is utilized wherein Z is lower alkanyl, the lower alkanoyl protects only the 3"-N-position. However, when Z is other than lower alkanoyl, the protecting group will bridge the 3"-N and 4"-O-positions to become a 3",4"-N,O-carbonyl moiety. When gentamicin A, Antibiotic 66-40B, kanamycin A, kanamycin B,
15 3',4'-dideoxykanamycin B and tobramycin are utilized, a mixture will be obtained wherein bridging will occur not only at the 3"-N and 4"-O-positions, but also at the 3"-
20 N and 2"-O-positions.

A preferred group of starting materials are 1,3"-di-N-unprotected-poly-N-protected gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂,

gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, Antibiotic G-52, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B, kanamycin A, kanamycin B, 3',4'-dideoxykanamycin B, verdamicin, sisomicin, tobramycin and the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the foregoing. 3,2',6'-tri-N-acetylsisomicin and 3,6'-di-N-acetylkanamycin A are specifically useful compounds.

The 5-epi-, 5-deoxy-, 5-epi-fluoro-5-deoxy- analogs are best prepared prior to the introduction of amino protecting groups. The preparation of 5-epi-aminoglycosides is described in U.S. Patent 4,000,261 and the preparation of 5-deoxy-aminoglycosides is described in U.S. Patent 4,053,591.

The 5-epi-fluoro-5-deoxy aminoglycosides (e.g. 5-epi-fluoro-5-deoxysisomicin) are prepared by reacting an aminoglycoside having a 5-hydroxyl function (e.g. sisomicin) and having all its amino and hydroxyl functions protected, other than the 5-hydroxyl function, with a dialkylamino-sulfur trifluoride (e.g. diethylaminosulfur trifluoride) in an inert organic solvent in the temperature range of from -100°C to about -50°C, followed by removal of the protecting groups (c.f. Example 4).

The preparation of 1-N-substituted aminoglycosides, for which the compounds of this invention are useful as intermediates, are described in U.S. Patent 4,002,742.

It has been found, however, that when utilizing a 1-N-unprotected-poly-N-acylamino-
 5 glycoside (e.g. 3,2',6',3"-tetra-N-acetylsisomicin) for conversion to a 1-N-ethyl-aminoglycoside (e.g. 1-N-ethylsisomicin), a convenient hydride donor reducing agent to be employed is sodium borohydride.

10 In its product aspect, this invention relates to novel 1-N-unprotected-poly-N-protected aminoglycosides. Particularly, this invention relates to novel 1-N-unprotected-poly-N-R-3"-N-Z'-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols, selected from

- 15 3,2'-di-N-R-3"-N-Z'-gentamicin A,
 3,5'-di-N-R-3"-N-Z'-gentamicin B,
 3,6'-di-N-R-3"-N-Z'-gentamicin B₁,
 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C₁,
 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{1a},
 20 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C₂,
 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{2a},
 3,2',5'-tri-N-R-3"-N-Z'-gentamicin C_{2b},
 3,2'-di-N-R-3"-N-Z'-gentamicin X₂,
 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic G-52,
 25 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic 66-40E.

3,2',6'-tri-N-R-3"-N-Z'-Antibiotic 6G-40D,
3,2'-di-N-R-3"-N-Z'-Antibiotic G-418,
3,2',6'-tri-N-R-3"-N-Z'-Antibiotic JI-20A,
3,2',6'-tri-N-R-3"-N-Z'-Antibiotic JI-20B,
5 3,6'-di-N-R-3"-N-Z'-kanamycin A,
3,2',6'-tri-N-R-3"-N-Z'-kanamycin B,
3,2',6'-tri-N-R-3"-N-Z'-3',4'-dideoxykanamycin B,
3,2',6'-tri-N-R-3"-N-Z'-verdamicin,
3,2',6'-tri-N-R-3"-N-Z'-sisomicin,
10 3,2',6'-tri-N-R-3"-N-Z'-tobramycin, and
the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the
foregoing, wherein R is lower alkanoyl, aroyl, lower alkoxy-
carbonyl, trichloroethoxycarbonyl, aralkoxycarbonyl, and
Z' is lower alkanoyl with at least two carbon atoms.

15 Of these the derivatives of gentamicins B, B₁ and C_{1a},
kanamycin A and sisomicin represent a preferred range of
compounds.

The definitions of the protecting groups have been des-
cribed hereinabove in more detail. Specifically, preferred
20 is that both R and Z' be lower alkanoyl, preferentially
acetyl.

Particularly valuable compounds of this invention are 3,2',
6',3"-tetra-N-acetylsisomicin, which compound is used to

obtain 1-N-ethylisomycin and 3,6',3"-tri-N-acetylkana-
mycin A.

The term "lower" as used herein for defining protecting
groups R, Z and Z' comprises such radicals having up to
six carbon atoms.

The following Examples illustrate the invention.

EXAMPLE 1

1-N-Unsubstituted-poly-N-protected-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols

5 A. 3,2',6',3"-tetra-N-acetylsisomicin

To a solution of 3 g (5.2 mmol) 3,2',6'-tri-N-acetylsisomicin in 150 ml of water, add 572 mg (5.2 mmol) of 1-acetylimidazole in 8 ml of tetrahydrofuran. Stir the solution at room temperature for 30 minutes and then add 10 572 mg (5.2 mmol) of acetylimidazole. Stir the solution a further 2 hours and then evaporate off the solvent and chromatograph the residue on 150 g of silica gel, eluting with the lower phase of a chloroform-methanol-15% ammonium hydroxide (2:1:1) solvent mixture to obtain 3,2',6',3"-tetra-N-acetylsisomicin (1.96 g, 61% yield) $[\alpha]_D^{26} + 204^\circ$ (H₂O); δ (D₂O) 1.05 (rotamers, C-CH₃), 2.25, 2.05, 1.95 (12H, COCH₃), 3.1, 3.0 (3H, rotamers, N-CH₃), 4.85 (1H, m, H-4'), 5.3 (1H, m, H-1'', J = 4 Hz) and 5.5 ppm (1H, d, J = 2 Hz, H-1').

20 B. 3,6',3'-Tri-N-acetylkanamycin A

(1) Dissolve 300 mg of kanamycin A and 1.9 g of cupric acetate hydrate in 13.5 ml of dimethylformamide and 4 ml of water and stir the mixture at 25°C for 30 minutes. Then

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add 0.17 g of acetic anhydride in 2 ml of dimethylformamide and stir for 1 hour at 25°C. Bubble hydrogen sulfide through the solution, filter off the solids and wash with dimethylformamide. Evaporate the filtrate to dryness and chromatograph the residue on a silica gel column (110 x 2.5 cm) using chloroform-methanol-3.5 % ammonium hydroxide (1:2:1) as the eluant to obtain 3,6'-di-N-acetylkanamycin A (130 mg, 37% yield); $[\alpha]_D^{26} + 94.6^\circ$ (H₂O); $[\theta]_{290} + 1002$ (TaCu); δ (D₂O) 2.05 (3H, s, NAc), 2.08 (3H, s, NAc), 5.13 (1H, m, H_{1''}) and 5.41 ppm (1H, m, H_{1'}).

(2) To a solution of 100 mg of 3,6'-di-N-acetylkanamycin A in 10 ml of (1:1) tetrahydrofuran-water, add 29.1 mg of acetylimidazole and stir the mixture at 25°C for 23 hours. Evaporate the solution to dryness and chromatograph the residue on a silica gel column (110 x 1.5 cm) using chloroform-methanol-3.5% ammonium hydroxide (1:2:1) as the eluant to obtain 3,6',3"-tri-N-acetylkanamycin A (41 mg, 38% yield); $[\alpha]_D^{26} + 104.7^\circ$ (H₂O); δ (D₂O) 2.02 (3H, s, NAc), 2.07 (3H, s, NAc), 2.09 (3H, s, NAc), 5.17 (1H, d, J_{1'',2''} 3.5 Hz, H_{1''}), and 5.41 ppm (1H, d, J_{1',2'} 3.0 Hz, H_{1'}).

1-N-unsubstituted-poly-N-acetyl-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols

In a manner similar to Example 1A, by utilizing as starting compounds

- a) 3,2'-di-N-acetylgentamicin A,
- b) 3,6'-di-N-acetylgentamicin B,
- c) 3,6'-di-N-acetylgentamicin B₁,
- d) 3,2',6'-tri-N-acetylgentamicin C₁,
- 5 e) 3,2',6'-tri-N-acetylgentamicin C_{1a},
- f) 3,2',6'-tri-N-acetylgentamicin C₂,
- g) 3,2',6'-tri-N-acetylgentamicin C_{2a},
- h) 3,2',6'-tri-N-acetylgentamicin C_{2b},
- i) 3,2'-di-N-acetylgentamicin X₂,
- 10 j) 3,2',6'-tri-N-acetyl Antibiotic G-52,
- k) 3,2',6'-tri-N-acetyl Antibiotic 66-40B,
- l) 3,2',6'-tri-N-acetyl Antibiotic 66-40D,
- m) 3,2'-di-N-acetyl Antibiotic G-418,
- n) 3,2',6'-tri-N-acetyl Antibiotic JI-20A,
- 15 o) 3,2',6'-tri-N-acetyl Antibiotic JI-20B,
- p) 3,2',6'-tri-N-acetylkanamycin B,
- q) 3,2',6'-tri-N-acetyl-3',4'-dideoxykanamycin B,
- r) 3,2',6'-tri-N-acetylverdamicin,
- s) 3,2',6'-tri-N-acetyltobramycin, and
- 20 t) the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of
the foregoing and of 3,2',6'-tri-N-acetylsisomicin and
3,6'-di-N-acetylkanamycin A there are obtained respectively:
- a) 3,2',3"-tri-N-acetylgentamicin A,
- b) 3,6',3"-tri-N-acetylgentamicin B,
- 25 c) 3,6',3"-tri-N-acetylgentamicin B₁,
- d) 3,2',6',3"-tetra-N-acetylgentamicin C₁,

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- e) 3,2',6',3"-tetra-N-acetylgentamicin C_{1a},
- f) 3,2',6',3"-tetra-N-acetylgentamicin C₂,
- g) 3,2',6',3"-tetra-N-acetylgentamicin C_{2a},
- h) 3,2',6',3"-tetra-N-acetylgentamicin C_{2b},
- i) 3,2',3"-tri-N-acetylgentamicin X₂,
- j) 3,2',6',3"-tetra-N-acetyl Antibiotic G-52,
- k) 3,2',6',3"-tetra-N-acetyl Antibiotic 66-40B,
- l) 3,2',6',3"-tetra-N-acetyl Antibiotic 66-40D,
- m) 3,2',3"-tri-N-acetyl Antibiotic G-418,
- n) 3,2',6',3"-tetra-N-acetyl Antibiotic JI-20A,
- o) 3,2',6',3"-tetra-N-acetyl Antibiotic JI-20B,
- p) 3,2',6',3"-tetra-N-acetylkanamycin B,
- q) 3,2',6',3"-tetra-N-acetyl-3',4'-dideoxykanamycin B,
- r) 3,2',6',3"-tetra-N-acetylverdamicin,
- s) 3,2',6',3"-tetra-N-acetyltobramycin, and
- t) the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the foregoing and of 3,2',6',3"-tetra-N-acetylsisomicin, and of 3,6',3"-tri-N-acetylkanamycin A.

D. 3,2',6'-Tri-N-(2,2,2-trichloroethoxycarbonyl)-3"-N-acetylgentamicin C_{1a}

Dissolve 500 mg of 3,2',6'-tri-N-(2,2,2-trichloroethoxycarbonyl)-gentamicin C_{1a} in 20 ml (1:1) tetrahydrofuran and water. Add 84.6 mg of acetyl imidazole dissolved in 4 ml tetrahydrofuran. Stir the solution at room temperature for 2 hours. Evaporate the tetrahydrofuran and decant off

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the remaining water. Dissolve the resultant residue in a small amount of chloroform and wash with water. Evaporate the chloroform and azeotrope the resultant residue with benzene. Chromatograph the azeotroped resultant residue on a silica gel column (30 x 3 cm) eluting with 10% methanol/
 5 chloroform. Combine the fractions to obtain 3,2',6'-tri-N-(2,2,2-trichloroethoxycarbonyl)-3"-N-acetyl gentamicin C_{1a} (428 mg, 82% yield); $[\alpha]_D^{26} + 69.9^\circ$ (CHCl₃); ν max. (KBr) 1720, 1620 cm.⁻¹; δ (CDCl₃) 1.08 (3H, s, 4"-CH₃), 2.16 (3H, s, NAc), 3.12 (3H, s, 3"-N-CH₃) and
 10 4.76 ppm (6H, s, CO₂CH₂CCl₃).

E. 3,2',6'-Tri-N-(2,2,2-trichloroethoxycarbonyl)-3"-N, 4"-O-carbonylgentamicin C_{1a}

(1) Dissolve 20 g of imidazole in 180 ml of tetrahydro-
 15 furan; cool the resultant solution to 0°C. To this solution, add 31 g of 2,2,2-trichloroethylchloroformate in 180 ml of tetrahydrofuran dropwise over a period of 1 hour. Then stir the mixture at 25°C for 1 hour, filter the mixture and concentrate the filtrate to dryness. Wash the resultant
 20 solid with water and dry to obtain 1-N-(2,2,2-trichloroethoxycarbonyl)-imidazole: m.p. = 80°C;

ν max. (CHCl₃) 3000, 1780, 1400, 1310, 1280, 1235, 1165, 1015 cm.⁻¹; δ (CDCl₃) 5.03 (2H, s, -CH₂CCl₃), 7.13 (1H, m, H₄), 7.48 (1H, m, H₅) and 8.20 ppm (1H, s, H₂).

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(2) To a solution of 500 mg of 3,2',6'-tri-N-(2,2,2-trichloroethoxycarbonyl)-gentamicin C_{1a} in 10 ml of anhydrous tetrahydrofuran, add 624 mg of 1-N-(2,2,2-trichloroethoxycarbonyl)-imidazole and then allow the solution to remain at 25°C for 24 hours. Evaporate the solution, dissolve the residue in ethyl acetate and wash with water, then dry the ethyl acetate layer over MgSO₄. Filter the ethyl acetate layer, evaporate to dryness and chromatograph the residue on a silica gel column (110 x 2.5 cm) using 4% methanol in chloroform as the eluant to obtain 3,2',6'-tri-N-(2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin C_{1a} (290 mg, 56% yield) $[\alpha]_D^{26} + 78.7^\circ$ (CHCl₃), ν max. (KBr) 3375, 2940, 1730, 1520 cm⁻¹; δ (CDCl₃) 1.38 (3H, s, 4"-CH₃), 2.98 (3H, s, 3"-NCH₃), 4.77 (6H, broad s, -CH₂CCl₃), 5.08 (1H, m, H_{1"}), and 5.43 ppm (1H, m, H₁).

F. 1-N-unsubstituted-3"-N-4"-O-carbonyl-poly-N-(2,2,2-trichloroethoxycarbonyl)-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols

20 In a manner similar to Example 1E there are obtained the following compounds, respectively:

- a) 3,6'-di-N-(2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin B,
- b) 3,6'-di-N-(2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin B₁,

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- c) 3,2',6'-tri-H (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin C_1 ,
- d) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin C_2 ,
- 5 e) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin C_{2a} ,
- f) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin C_{2b} ,
- g) 3,2'-di-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylgentamicin X_2 ,
- 10 h) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonyl Antibiotic G-52,
- i) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonyl Antibiotic 66-40D,
- 15 j) 3,2'-di-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonyl Antibiotic G-418,
- k) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonyl Antibiotic JI-20A,
- l) 3,2',6'-tri-H (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonyl Antibiotic JI-20B,
- 20 m) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylsisomicin,
- n) 3,2',6'-tri-N (2,2,2-trichloroethoxycarbonyl)-3"-N-4"-O-carbonylverdamycin, and

the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the foregoing and of 3,2',6'-tri-N-(2,2,2-trichloroethoxy-carbonyl)-3"-N,4"-O-carbonylgentamicin C_{1a}.

EXAMPLE 2

5 1-N-Substituted-4,6-di-O-(aminoglycosyl)-1,3-diaminocycli-
 tols

A. 1-N-Ethylsisomicin

To a stirred solution of 40.5 g of 3,2',6',3"-tetra-N-acetylsisomicin in 500 ml of isopropanol, add 6.5 ml of
10 acetaldehyde and 2.3 g of sodium borohydride in 100 ml of isopropanol, continue stirring for 3 hours at room temperature. After 3 hours, add water and then remove the isopropanol by evaporation. Add 150 ml of 50% sodium hydroxide to the residue and heat at 100°C for 18 hours in an argon
15 atmosphere. Cool the solution, adjust the pH to 6 and treat the solution with 2.15 liter of IRC-50 (NH₄⁺) ion exchange resin, wash the resin with water and elute with 2N aqueous ammonium hydroxide. Concentrate the ammoniacal eluant to dryness and dissolve the residue in the lower phase of a
20 chloroform-isopropanol-14% ammonium hydroxide (2:1:1) solvent mixture and chromatograph on a 300 g silica gel column utilizing the same solvent to obtain 1-N-ethylsisomicin (25 gm, 80% yield).

like eluates and evaporate and pass the residue through a column of IRA-401S (OH^-) ion exchange resin, eluting with water. The eluate was lyophilized to give 1-N-acetylsisomicin identical with authentic material. (0.9 gm - 60% yield).

5

EXAMPLE 4

5-Epi-fluoro-5-deoxysisomicin

A. To a stirred solution of 4 g of 1,3,2',6'-tetra-N-benzyloxycarbonyl-2"-O-benzoyl-3",4"-N,O-carbonylsisomicin in 60 ml of dry methylene chloride at -78°C under an atmosphere of argon, add 3 ml (6 eq.) of diethylamino-sulfur trifluoride (DAST). Stir the reaction mixture at -78°C for two hours and then allow to warm to 0°C . Add 50 ml of 5% sodium bicarbonate and separate the organic phase. Wash this organic phase with 50 ml water and then dry over 25 gm sodium sulfate. Evaporate the methylene chloride under vacuum. Triturate the residual yellow foam with a small volume of ether to obtain 3.6 g of 5-epi-fluoro-5-deoxy-1,3,2',6'-tetra-N-benzyloxycarbonyl-2"-O-benzoyl-3",4"-N,O-carbonylsisomicin as a white solid; m.p. = 225°C .

D. 1-N-Ethyl-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols

1. In a manner similar to Example 2A, by utilizing the products of Example 1A, B and C there are obtained the corresponding 1-N-ethyl-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols.
2. In a manner similar to Example 2A, by utilizing the products of Example 1E and F there are obtained the corresponding 1-N-ethyl-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitols.

EXAMPLE 3

1-N-Acetylsisomicin

To a stirred solution of 3.0 g of 3,2',6'-tri-N-(tri-chloroethoxycarbonyl) sisomicin in 75 ml of methanol and 15 ml of water, add 0.55 g of N-acetoxysuccinimide. Stir the reactants for 20 hours at room temperature, then add 7 ml of acetic acid and 6 g of zinc dust and heat the mixture for 2.5 hours. Cool and filter the reaction, washing with methanol. Evaporate the filtrate and dissolve the residue in 30 ml of water, add a solution of 3 g sodium carbonate in 600 ml of water, and heat to boiling. Filter the formed salts and wash the salts with water. Evaporate the filtrate and reflux the residue with 150 ml of isopropanol. Filter, evaporate the filtrate and chromatograph the residue on 70 g of silica gel, eluting with chloroform, methanol, concentrated ammonia (3:1:0.15). Combine

B. To a solution of 4 g of 5-epi-fluoro-5-deoxy-1,3,2', 6'-tetra-N-benzyloxycarbonyl-2"-O-benzoyl-3",4"-N,O-carbo-nylsisomicin in 5 ml of tetrahydrofuran and 60 ml liquid ammonia slowly add 2 g of sodium with stirring. Continue to stir for two hours, then add 15 ml of methanol dropwise and allow the ammonia to evaporate by warming to room temperature overnight. Dissolve the resultant residue in 10 ml 5% sodium hydroxide, and heat at 100°C for two hours under an atmosphere of argon. Cool and pass the solution through IRC-50 (H⁺) resin. Wash the resin with water and elute the product with 100 ml of 1N ammonium hydroxide. Concentrate the ammonium hydroxide eluates to a residue comprising 5-epi-fluoro-5-deoxysisomicin. Purify the product by chromatographing on a silica gel column eluting with the lower phase of a chloroform-methanol-15% ammonium hydroxide (2:1:1) solvent system. Combine the like eluates as determined by thin layer chromatography and lyophilize to a residue to obtain 750 mg 5-epi-fluoro-5-deoxysisomicin as a white solid; m.p. 100°C - 102°C; $[\alpha]_D^{26} + 200^\circ$ (MeOH); PMR (100 MHz) (D₂O), 5.1 (1H, d, J=57.0 Hz, 5-H), 5.13 (1H, d, J=2.5 Hz, 1'-H), 5.03 (1H, d, J=4.0 Hz, 1"-H), 4.88 (1H, br, s, 4'-H), 3.85 (1H, d, J=12.5 Hz, 5_e"-H), 3.36 (1H, d, J=12.5 Hz, 5_a"-H), 2.56 (1H, d, J=10.5 Hz, 3"-H), 2.47 (3H, s, 3"-N-CH₃), 1.17 (3H, s, 4'-C-CH₃).

C L A I M S

1. Process for the selective protection of a 3"-amino function in a 1,3"-di-N-unprotected-poly-N-protected-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol, wherein the 6-O-aminoglycosyl has an amino function at the 3"-position and hydroxyl functions at the 2"- and 4"-positions, characterised in that the 1,3"-di-N-unprotected-poly-N-protected-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol is reacted in a solvent with no more than one molar equivalent of available 1-Z-imidazole, wherein Z is an amino protecting group selected from lower alkanoyl, lower alkoxy-carbonyl, aralkoxycarbonyl, trichloroethoxycarbonyl and N-carbonylimidazole.
2. Process according to claim 1, characterised in that the reaction is carried out in an inert organic solvent and about equimolar amounts of reactants are used.
3. Process according to claim 1, characterised in that the reaction is carried out in a solvent containing water and/or a lower alkanol and up to two molar equivalents of 1-Z-imidazole are used.
4. Process according to any one of claims 1 to 3, characterised in that as 1-Z-imidazole is used 1-lower alkanoylimidazole.

5. Process according to any one of claims 1 to 4, characterised in that as 1-Z-imidazole is used 1-acetyl-imidazole.

6. Process according to any one of claims 1 to 5, characterised in that the starting compound is selected from 1,3"-di-N-unprotected-poly-N-protected gentamicin A, gentamicin B, gentamicin B₁, gentamicin C₁ gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, gentamicin X₂, Antibiotic G-52, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic G-418, Antibiotic JI-20A, Antibiotic JI-20B, kanamycin A, kanamycin B, 3',4'-dideoxykanamycin B, verdamycin, sisomicin, tobramycin and the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the foregoing.

7. Process according to any one of claims 1 to 6, characterised in that two molar equivalents of 1-acetyl-imidazole are reacted with 3,2',6'-tri-N-acetylsisomicin in water and tetrahydrofuran.

8. Process according to any one of claims 1 to 6, characterised in that up to two molar equivalents of 1-acetyl-imidazole are reacted with 3,6'-di-N-acetylkanamycin A in water and tetrahydrofuran.

9. A 1-N-unprotected-poly-N-protected-4,6-di-O-aminoglycosyl-1,3-diaminocyclitol when obtained by the process of any one of claims 1 to 8.

10. A 1-N-unprotected-poly-N-R-3"-N-Z'-4,6-di-O-(aminoglycosyl)-1,3-diaminocyclitol, selected from

- 3,2'-di-N-R-3"-N-Z'-gentamicin A,
- 3,6'-di-N-R-3"-N-Z'-gentamicin B,
- 3,6',di-N-R-3"-N-Z'-gentamicin B₁,
- 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C₁,
- 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{1a},
- 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C₂,
- 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{2a},
- 3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{2b},
- 3,2'-di-N-R-3"-N-Z'-gentamicin X₂,
- 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic G-52,
- 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic 66-40B,
- 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic 66-40D,
- 3,2'-di-N-R-3"-N-Z'-Antibiotic G-418,
- 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic JI-20A,
- 3,2',6'-tri-N-R-3"-N-Z'-Antibiotic JI-20B,
- 3,6'-di-N-R-3"-N-Z'-kanamycin A,
- 3,2',6'-tri-N-R-3"-N-Z'-kanamycin B,
- 3,2',6'-tri-N-R-3"-N-Z'-3',4'-dideoxykanamycin B,
- 3,2',6'-tri-N-R-3"-N-Z'-verdamicin,
- 3,2',6'-tri-N-R-3"-N-Z'-sisomicin,

3,2',6'-tri-N-R-3"-N-Z'-tobramycin, and the 5-epi, 5-deoxy, 5-epi-fluoro-5-deoxy analogs of the foregoing wherein R is lower alkanoyl, aroyl, lower alkoxy-carbonyl, trichloroethoxycarbonyl or aralkoxycarbonyl, and Z' is lower alkanoyl with at least two carbon atoms.

11. A compound of claim 10, selected from

3,6'-di-N-R-3"-N-Z'-gentamicin B,

3,6'-di-N-R-3"-N-Z'-gentamicin B₁,

3,2',6'-tri-N-R-3"-N-Z'-gentamicin C_{1a},

3,6'-di-N-R-3"-N-Z'-kanamycin A, and

3,2',6'-tri-N-R-3"-N-Z'-sisomicin

wherein R and Z' are as defined in claim 10.

12. A compound of claim 10 or 11, wherein R is lower alkanoyl.

13. A compound of claims 10 to 12, wherein R and Z' are acetyl.

14. 3,2',6',3"-tetra-N-acetylsisomicin and 3,6',3"-tri-N-acetylkanamycin A.



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	THE JOURNAL OF ANTIBIOTICS, 29 (7) 1976, 714-719 * Page 714, paragraph 2; page 715, paragraph 3 *	1,2,4, 5	C 07 H 15/22
	FR - A - 2 264 556 (ZAIDAN) * Page 5, lines 32-35 *	1	
D	FR - A - 2 236 873 (TAKEDA) * Page 20 *	1,10	TECHNICAL FIELDS SEARCHED (Int.Cl.)
D	FR - A - 2 240 015 (SCHERICO) & US 4 002 742 * Pages 116-120 *	1	C 07 H 15/22
			CATEGORY OF CITED DOCUMENTS
			X: particularly relevant A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: conflicting application D: document cited in the application L: citation for other reasons
			&: member of the same patent family, corresponding document
☒ The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 13-09-1978	Examiner 00000000