

PATENT SPECIFICATION

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(54) PROCESS OF SELECTIVELY BLOCKING AMINO GROUPS

(71) We, SCHERICO LTD., of Topferstrasse 5, Lucerne, Switzerland, a Swiss body corporate, 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 a process of selectively blocking certain amino groups in a polyamino-organic compound and to novel, selectively blocked-amine derivatives produced thereby.

When carrying out certain chemical transformations of a polyamino-organic compound, particularly those in the carbohydrate and aminocyclitol-aminoglycoside art, in order to obtain good yields of a desired product, it is necessary to first prepare derivatives of amine functions in the molecule with "blocking" or "protecting" groups to prevent the amino functions from entering into competing reactions with the reagents being employed. Commonly employed blocking groups are acyl blocking groups which are easily attached to amino groups and can be removed after the desired transformation has been completed but which are stable under the reaction conditions to be employed.

Chemical transformations in a polyamino-organic compound wherein the site of reaction is to be one of the amino functions, are ideally 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 chromatography or several chromatographies) to isolate the desired transformation product. However, it is not always possible to prepare the ideal selectively blocked intermediate by prior art methods so that, with amino functions unprotected, the desired transformation product is produced only in low yields.

When a polyamino-organic compound contains amino functions of various degrees of reactivity due either to steric factors and/or to the primary and/or secondary and/or tertiary nature of the amine groups, it is sometimes possible, although in moderate to low yields, to selectively protect some amino functions while leaving other amino functions unprotected at which a desired reaction may take place. Such procedures usually involve multistep transformations requiring the isolation and purification of each intermediate. Frequently, it is impossible to protect all amino functions other than the one of which a transformation is desired, such as, for example, when two amino functions are of equal reactivity, so that at best only some amino functions are blocked which minimizes the number of products formed in a given reaction but which still will give rise to a mixture of products resulting in poor yields of desired products isolatable and purifiable only with difficulty.

By this invention, it is now possible to easily prepare, in good yields, selectively blocked amino derivatives of polyamino-organic compounds in which at least one amino function has an available neighboring hydroxyl group. The invention finds its greatest use in the carbohydrate and aminocyclitol-aminoglycoside art whereby it is now possible to easily prepare in good yields *N*-acylated derivatives heretofore impossible to make or heretofore produced only in small quantities. Additionally,

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the selectively *N*-acylated polyaminoorganic compound prepared is of sufficient purity and predictable yield to allow further reactions to be carried out without isolation of the *N*-acylated polyamino intermediate.

Prior to this invention, divalent transition metal salt complexes of neighboring amino and hydroxyl group pairs were unknown. The prior art postulates (Bull. Chem. Soc. Japan, Vol. 39, No. 6 1235—1248 (1966)) the existence of cupraammonium complexes between amines on a chiral center and adjacent vicinal hydroxyl groups on a chiral center *in situ* in dilute aqueous ammonia solutions, said cupra-ammonium complexes being used for optical rotatory studies as an analytical tool in determining the absolute spatial relationship between vicinal amino/hydroxyl group pairs.

It has now been discovered that divalent transition metal salt complexes of neighboring amino/hydroxyl group pairs (wherein the amino and hydroxyl groups may or may not be vicinal and may or may not be on chiral centers) can be formed and, moreover, can be formed in an organic solvent with a minimal quantity of divalent transition metal ion without having to use ammonium hydroxide to form a transition metal ammonium ion (e.g. cupraammonium ion). It has also been discovered that the polyamino-organic compound-transition metal salt complexes of this invention will survive under acylating reaction conditions which transform a free amino group to an *N*-acylated derivative thereof and, furthermore, that the transition metal salt complexes of this invention will prevent the complexed amino/hydroxyl groups from entering into the reaction.

Accordingly, the invention relates to a process for producing an organic compound, having at least one amino group having an available neighboring hydroxyl group and at least one amino group blocked with an acyl blocking group Y; and at least one of said amino groups being either devoid of, or sterically less available to, an available neighboring hydroxyl group; which comprises the reaction of said polyamino-organic compound in an inert organic solvent, with a salt of a divalent transition metal cation selected from copper (II), nickel (II), cobalt (II), cadmium (II) or with mixtures thereof, whereby is formed a complex of said polyamino-organic compound between said transition metal salt and at least one pair of groups consisting of said amino group having an available neighboring hydroxyl group and the said neighboring hydroxyl group; followed by the reaction of the resulting polyamino-organic compound-transition metal salt complex with an amine blocking reagent having an acyl blocking group, Y; then reaction of the resulting polyamino-organic compound-transition metal salt complex having acyl blocking groups: Y, on non-complexed amino groups, with a transition metal precipitating reagent or with ammonium hydroxide, whereby the transition metal cation is removed.

As used herein the phrases "one of said amino groups having an available neighboring hydroxyl group" and "available neighboring amino and hydroxyl group pairs" include amino and hydroxyl functions which are positioned on adjacent carbon atoms in a *cis*-vicinal or diequatorial *trans*-vicinal manner and also include amino and hydroxyl groups which are not positioned on vicinal carbons but which are spatially adjacent (i.e. they are close enough to be hydrogen bonded) and available to each other. Further, by the term "available" is meant that, in addition, to being available to each other, to be useful in the process of this invention, the neighboring amino and hydroxyl group pairs must be so situated in the polyamino-organic compound molecule so as to be sterically available to an approaching reagent such as a cation of a divalent transition metal. Thus, for example, in a 4,6-di-*O*-(aminoglycosyl)-2-deoxystreptamine having a 2'-amino group such as in sisomicin, tobramycin, verdamicin and gentamicins C₁, C₁₂ and C₂, although the 5-hydroxyl group of the 2-deoxystreptamine moiety is neighboring and available to the 2'-amino group, the 5-hydroxyl group is so sterically hindered that a salt of a divalent transition metal such as a cupric acetate, cannot get into sufficiently close proximity to the 5-hydroxyl-2'-amino pair to form a transition metal salt complex thereof.

Transition metal salts useful as complexing agents in this process include any divalent salt of copper (II), nickel (II), cobalt (II) and cadmium (II). Among those which have strongest complexing activity are divalent transition metal salts of weak acids, preferably weak organic acids such as benzoic, propionic, and acetic acid. Preferred divalent transition metal salts include the acetate salt of copper (II), nickel (II), cobalt (II), and cadmium (II) and mixtures thereof. Of particular use are nickel (II) acetate, copper (II) acetate and cobalt (II) acetate.

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When monovalent transition metal salts are used, e.g. cuprous acetate, the cuprous acetate complex of available neighboring hydroxyl and amino group pairs is formed in poor yields and is weakly bound so that when the *N*-acylation step is carried out, *N*-acylation occurs at the sites of weakly complexed amino groups (albeit at a somewhat slower rate) as well as at the non-complexed amino group sites resulting in mixtures of products and poorer yields of desired product.

The process of this invention is carried out in an inert organic solvent which includes any organic solvent in which the polyamino-organic compound and transition metal salt as well as the resulting polyamino-organic compound-transition metal salt complex intermediate are reasonably soluble, and which will not react to any great extent with the reagents of this process. Preferred solvents are polar, aprotic organic solvents, particularly dialkylamides (e.g. dimethylformamide) and dialkylsulfoxides (e.g. dimethylsulfoxide). Polar protic solvents such as lower alkanols, particularly methanol and ethanol, may also be used in the process when such solvents are required for solubility reasons. When using protic solvents, it is usually desirable to employ a greater quantity of divalent transition metal salt than when using aprotic solvents since protic solvents weaken the complexed functions in the polyamino-organic compound-transition metal salt complex.

It is preferable to utilize substantially anhydrous solvents in order to obtain maximum yields. However, water may be present (and is frequently desirable for solubility reasons) even in amounts up to about 25% (by volume) and higher without affecting the yields of selectively blocked *N*-acylated polyamino product, provided additional divalent transition metal salt is employed since water, being a protic solvent, also weakens the complexed functions in the polyamino-organic compound transition metal salt complex intermediates.

In this process an equilibrium appears to exist between the polyamino-organic compound having at least one available neighboring amino/hydroxy group pair [A], the transition metal salt (e.g. cupric acetate, Cu(OAc)₂) and the solvent which may be indicated as follows:

$$\begin{array}{ccc} \text{[A]} + \text{Cu(OAc)}_2 + \text{solvent} & \xrightleftharpoons{\text{aprotic solvent}} & \text{A} \cdot \text{Cu(OAc)}_2 \cdot \text{solvent} + \text{Cu(OAc)}_2 \cdot \text{solvent} + \text{solvent} \\ & & \text{solvent} + \text{solvent} \\ \text{[A]} + \text{Cu(OAc)}_2 + \text{solvent} & \xrightleftharpoons{\text{protic solvent}} & \text{A} \cdot \text{Cu(OAc)}_2 \cdot \text{solvent} + \text{Cu(OAc)}_2 \cdot \text{solvent} + \text{solvent} \end{array}$$

With an aprotic solvent, the equilibrium favors the formation of a transition metal salt complex of a polyamino-organic compound having available, neighboring amino/hydroxyl groups, while an organic protic solvent or the presence of excess water in an organic aprotic solvent favors decomplexing of the polyamino-organic compound transition metal salt complex. Thus, in the latter case, in order to obtain high yields of the polyamino-organic compound-salt complex in solution, greater quantities of transition metal salt are required to force the equilibrium in the desired direction than is necessary when an essentially anhydrous aprotic solvent is used.

Usually, when carrying out the process of this invention, the molar quantity of transition metal salt used in the first step of the process is at least equal to the molar quantity of the polyamino-organic compound times the number of available, neighboring amino- and hydroxyl group pairs therein, and the molar quantity of acylating agent used in the second step of the process, which is preferably carried out *in situ*, is equal to the molar quantity of the polyamino-organic compound times the number of non-complexed amino functions in the molecule which are to be protected.

However, when there exists a difference in the reactivity of the non-complexed amines, one may use less *N*-acylating reagent if one desires to *N*-acylate only the more reactive amines.

After the polyamino-organic compound-transition metal salt complex has been reacted with an acylating agent to *N*-acylate the non-complexed amino groups, the transition metal cation is then conveniently removed from the *N*-

acylated polyamino-organic compound-transition metal salt complex by means of a transition metal precipitating reagent or by means of ammonium hydroxide. In the latter case, the transition metal cation is removed by the preferential formation of a complex with ammonium hydroxide which is soluble in ammonium hydroxide and water. This method of removing the transition metal cation is convenient when the selectively blocked *N*-acyl polyaminoorganic derivative is soluble in organic solvents since it may then be extracted from the aqueous organic solvent mixture which contains the ammonium hydroxide transition metal salt complex.

It is usually more convenient to remove the transition metal cation by means of precipitating reagents known in the art. Useful precipitating reagents include dioxime of dimethylglyoxal, 1,3-dicarbonylalkanes such as acetyl-acetone and heptane-3,5-dione as well as sulfide precipitating reagents such as ammonium sulfide, alkali metal sulfides (e.g. sodium sulfide), alkaline earth metal sulfides (e.g. calcium sulfide), alkaline earth metal hydrosulfides (e.g. sodium hydrosulfide) and hydrogen sulfide. Of the foregoing, particularly useful precipitating reagents are dioxime of dimethylglyoxal, acetylacetone and hydrogen sulfide.

When removing the transition metal cation, hydrogen sulfide is a precipitating reagent of choice since it is a simple procedure to merely bubble hydrogen sulfide through the reaction mixture; also the resulting transition metal sulfide is completely precipitated in a short period of time and is easily removed by filtration.

The acyl blocking groups, Y, and the corresponding acylating reagents whereby they are formed are well known in the art as well as methods for their removal after a desired chemical transformation has been carried out at some other side in the molecule. As used herein, acyl blocking groups, Y, which may be selectively introduced on to non-complexed amino functions in a polyamino-organic compound transition metal salt complex intermediate are contemplated as including benzyloxycarbonyl and substituted benzyloxycarbonyl groups such as *p*-nitrobenzyloxy-carbonyl and *p*-methoxybenzyloxycarbonyl (which are easily removable by catalytic reduction); aryloxycarbonyl groups such as phenoxycarbonyl; and alkoxycarbonyl groups such as methoxycarbonyl, ethoxycarbonyl and the like (which are preferentially removed by basic hydrolysis); trichloroethoxycarbonyl groups (removable by zinc in acetic acid); tertiaryalkoxycarbonyl groups such as *tert*-butoxycarbonyl and *tert*-amyloxycarbonyl groups (removable by mild acid hydrolysis); halogenalkylcarbonyl groups such as chloroacetyl (removable with base or with thiourea or a similar reagent) and trifluoroacetyl (easily removed under mild basic conditions); succinimido and phthalimido groups (easily removed by means of hydrazine) and alkanoyl groups such as acetyl, propionyl, and the like as well as aroyl groups such as benzoyl (which are removed by basic hydrolysis). The reaction conditions necessary to introduce any of the foregoing *N*-acyl derivatives on a free amino function are well known in the art.

Polyamino-organic compounds useful as starting compounds in the process include any polyamino-organic compound which has at least one amino function which has an available neighboring hydroxyl group and which also has at least one amino function which is either devoid of an available neighboring hydroxyl group and therefore cannot form a complex with a transition metal salt or which is sterically less available to a neighboring hydroxyl so that any salt complex formed at that site would be less stable than other strongly complexed amino/hydroxyl pairs, thus rendering the amino function available to *N*-acylation in the second step of the process.

The polyamino-organic starting compounds may be substituted by groups other than hydroxyl groups and may contain hereto atoms provided they do not react with transition metal salts or with the acylating reagents. Thus, the polyamino-organic starting compounds of this process are preferably devoid of sulfhydryl and mercaptyl groups.

Contemplated as included among the polyamino-organic starting compounds of the process are monocyclic-, bicyclic- and tricyclic-polyaminoaryl hydroxides such as 2,4 - diaminophenol, 2,4 - diamino - 1 - naphthol, 2,6 - diamino - 1 - naphthol, 1,4 - diamino - 2 - naphthol and 5,8 - diamino - 1 - naphthol; as well as polyaminocycloalkanols such as (*cis, cis, cis*) - 2,4 - diaminocyclohexanol and (*trans, cis, trans*) - 2,4 - diaminocyclohexanol and acyclic polyamino compounds such as 2,6 - diaminohexanol.

In acyclic polyamino-organic starting compounds of the process, amino and hydroxyl groups are neighboring and available to each other which are positioned so that the carbon chain (or hetero carbon chain) containing the amino/hydroxyl

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group pairs can form a five, six or seven membered ring together with the transition metal cation.

The process is particularly useful in the carbohydrate art when carrying out chemical transformations of pseudodisaccharides such as 4 - O - (6' - amino - 6' - deoxyglucosyl)deoxystreptamine, neamine, garamine, kanamine, paromamine, and the gentamines (e.g. C₁, C_{1a}, C₂, C_{2b}, etc.).

The process finds its greatest use, however, in the aminocyclitol-aminoglycoside art where chemical transformations of aminoglycoside antibiotics are continually being made to synthesize new derivatives having enhanced antibacterial activity and/or a more favorable antibacterial spectrum. Most of these transformations are multistep, low yield processes in which several initial steps are directed merely to protecting amino and/or hydroxyl functions with blocking groups prior to carrying out the main chemical transformation step, in an attempt to improve the yield of desired transformation product. By this process, the amino groups which require protection are selectively blocked in excellent yield in a sample two-step-one-vessel procedure.

4,5-Linked aminoglycoside antibiotics containing deoxystreptamine which are useful starting compounds of the process include ribostamycin (in which the 1-amino-6-hydroxyl group pair is converted *in situ* to a transition metal salt complex, then all other amino functions are *N*-acylated followed by removal of the transition metal salt, then methylation of the resulting 1 - *N* - unsubstituted - poly - *N* - acylribostamycin utilizing known methylation techniques to produce 1 - *N* - methylribostamycin having antibacterial activity); xylostamycin, 3' - deoxyribostamycin, and 3', 4', 5' - trideoxyribostamycin. Other useful starting compounds include 4 - O - linked - streptamine containing aminocyclitol - aminoglycosides such as 1,3 - di - de - *N* - aminodihydrostreptomycin.

The preferred mode of the process is that wherein amino groups are selectively blocked in a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol antibacterial agent. By the novel process of this invention, it is now possible to easily prepare in good yields selectively blocked amino-glycoside antibiotic derivatives such as 2',6' - di - *N* - acyl, 3,6' - di - *N* - acyl, 3,2',6' - tri - *N* - acyl, and 1,3,2',6' - tetra - *N* - acyl derivatives of 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol derivatives by a two - step - one - vessel process, all of which were heretofore unknown and which are valuable as intermediates in preparing antibacterially active transformation products of the parent aminoglycoside.

A particularly valuable mode is that whereby is prepared a 3,2',6' - tri - *N* - acyl derivative of a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol such as sisomicin, tobramycin, verdamicin, gentamicin C₁, gentamicin C_{1a}, and gentamicin C₂, which are valuable intermediates in preparing the corresponding 1 - *N* - alkylaminoglycosides (after removal of the *N*-acyl groups at the 3,2' and 6' positions) which are valuable antibacterial agents described in U.S. Patent No. 4,002,742. Of the foregoing 3,2',6' - tri - *N* - acylsisomicins, particularly, 3,2',6' - tri - *N* - acetylsisomicin, are of great value as intermediates in preparing 1 - *N* - ethylsisomicin, a potent antibacterial agent.

In carrying out the process of this invention, it has been found that mixtures of transition metal salt complexes are sometimes advantageously employed to produce good yields of selectively *N*-acylated polyamino compounds. It is believed this is due to the fact that some transition metal salts form more strongly bound transition metal salt complexes with certain available, neighboring amino/hydroxyl group pairs than do other transition metal salts. Thus, when preparing 3,2',6' - tri - *N* - acetylsisomicin, improved yields are obtained when the process is carried out utilizing as divalent transition metal salt an equimolar mixture of nickel (II) acetate and cupric acetate (the total molar equivalents of transition metal salts being four times that of sisomicin). The yield thereby obtained is at least 90% theory as compared with a 76% theoretical yield when cupric acetate was used alone. A particularly valuable mode is that utilizing cobalt (II) acetate as transition metal whereby is obtained almost theoretical yields of essentially pure selectively blocked *N*-acylated derivatives.

By modifying the quantity of transition metal salt and acylating reagent and (incidentally) changing the solvent, one can obtain good yields of 1,3,2',6' - tetra - *N* - acetylsisomicin instead of 3,2',6' - tri - *N* - acetylsisomicin. Thus, by adding from about a half to an equimolar equivalent amount of cupric acetate hydrate per mole of sisomicin in an aqueous methanolic solution, there is obtained *in situ* a sisomicin-cupric acetate complex involving the 3'' - amino - 4'' - hydroxyl group pair. Reaction of the foregoing *in situ* with about 4 molar equivalents of

acetic anhydride followed by removal of the cupric cation by precipitation as the sulfide salt and then isolation and purification utilizing known techniques yields 1,3,2',6' - tetra - *N* - acetylsisomicin in yields of almost 80% theory, an intermediate useful when transformation at the 3"-amino group is desired.

The process of this invention also provides the possibility to prepare stepwise *in situ* mixed *N*-acyl derivatives of the remaining non-complexed amino groups when said amines have different degrees of reactivity. Depending upon the types of *N*-acyl groups introduced into the molecule and their relative ease of removal, it is possible to prepare any desired combination of selectively *N*-acylated polyamino-organic compound. Thus, for example, upon reaction of sisomicin in 95% aqueous methanol with about an equimolar amount of cupric acetate, there is obtained *in situ* a sisomicin-cupric acetate complex essentially involving the 3"-amino and 4"-hydroxyl groups. Reaction of the foregoing sisomicin-cupric acetate complex with about a molar equivalent of ethylthioltrifluoro acetate produces a monoacyl derivative of the most reactive non-complexed amino function therein; namely, the 6'-amino, to produce *in situ* 6' - *N* - trifluoroacetylsisomicin - cupric acetate complex. By subsequently adding about three molar equivalents of acetic anhydride to the foregoing derivative *in situ*, the remaining three non-complexed amino groups are *N*-acetylated to produce 1,3,2' - tri - *N* - acetyl - 6' - *N* - trifluoroacetylsisomicin - cupric acetate complex. After destroying the cupric acetate complex followed by treatment of the residue containing 1,3,2' - tri - *N* - acetyl - 6' - *N* - trifluoroacetylsisomicin with ammonium hydroxide which causes hydrolysis of the 6' - *N* - trifluoroacetyl group without disturbing the *N* - acetyl groups, 1,3,2' - tri - *N* - acetylsisomicin is isolated by known techniques. The 1,3,2' - tri - *N* - acylaminoglycosides are valuable intermediates in preparing 6' - *N* - alkylaminoglycosides which are useful antibacterial agents.

Additionally, by forming *in situ* 6' - *N* - trifluoroacetylsisomicin - cupric acetate nickel (II) acetate complex in dimethylsulfoxide and adding two equivalents of acetic anhydride, there is formed a di - *N* - acetyl derivative involving the two more reactive non-complexed amino functions at the 3- and 2'-positions to produce *in situ* 3,2' - di - *N* - acetyl - 6' - *N* - trifluoroacetylsisomicincupric acetate complex, which, after removal of the complex with hydrogen sulfide and hydrolysis of the 6' - *N* - trifluoroacetyl group by means of mild basic hydrolysis, yields 3,2' - di - *N* - acetylsisomicin. The 3,2' - di - *N* - acetylaminocyclitol - aminoglycosides are valuable intermediates in the preparation of 1,6' - di - *N* - alkylaminoglycosides, valuable antibacterial agents described in Belgium Patent No. 847,900.

Also, mixed *N*-acyl derivatives of a polyamino-organic compound may be prepared by introducing, *in situ*, some *N*-acyl derivatives on the polyamino-organic compound-transition metal salt complex, then destroying the complex followed by *N*-acylation of the amino functions which formerly had been rendered inactive due to the presence of the complexes. By utilizing this procedure, it is possible to obtain a 1,3,3" - tri - *N* - acetylsisomicin derivative, a useful intermediate for the preparation of 2',6' - di - *N* - substituted derivatives such as 2',6' - di - *N* - alkylsisomicin having antibacterial activity. Thus, for example, treatment of sisomicin with about 2 to 3 molar equivalents of nickel (II) acetate in methanol yields *in situ* a sisomicin-nickel (II) acetate complex involving the 3" - amino - 4" - hydroxyl group pair and the 1 - amino - 2" - hydroxy group pair. Reaction of the sisomicin-nickel (II) acetate complex *in situ* with about two moles of *N* - *tert* - butoxycarbonyloxyphthalimide produces a di - *N* - acyl derivative involving the more reactive non-complexed 2' and 6'-amino groups as compared with the non-complexed 3-amino group to produce 2',6' - di - *N* - *tert* - butoxycarbonylsisomicin - nickel (II) acetate complex. Removal of the nickel (II) ion as nickel sulfide followed by reaction *in situ* of the resulting filtrate containing 2',6' - di - *N* - *tert* - butoxycarbonylsisomicin with acetic anhydride yields 1,3,3" - tri - *N* - acetyl - 2',6' - di - *N* - *tert* - butoxycarbonylsisomicin which, upon controlled acid hydrolysis *in situ* such as by 5% trifluoroacetic acid in tetrahydrofuran yields 1,3,3" - tri - *N* - acetylsisomicin.

Alternatively, the 2',6' - di - *N* - *tert* - butoxycarbonylsisomicin intermediate prepared by the above procedure may be isolated *per se*, then reduced with lithium aluminum hydride in tetrahydrofuran to produce 2',6' - di - *N* - methylsisomicin useful as antibacterial agent.

The above discussion describing the preparation of selectively *N*-acylated sisomicin derivatives, particularly valuable intermediates prepared by this process,

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illustrates the flexibility and versatility of the process and the valuable intermediates thereby obtained.

Accordingly, this invention also relates to the novel selectively blocked aminocyclitol-aminoglycoside derivatives which are prepared by the above process and may be defined as 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols being selectively blocked in positions 3,2' and 6' which are selected from:

3,2',6' - tri - N - Y - sisomicin, 3,2',6' - tri - N - Y - verdamycin, 3,2',6' - tri - N - Y - tobramycin, 3,2',6' - tri - N - Y - gentamicin C₁, 3,2',6' - tri - N - Y - gentamicin C_{1a}, 3,2',6' - tri - N - Y - gentamicin C₂, 3,2',6' - tri - N - Y - gentamicin C_{2a}, 3,2',6' - tri - N - Y - gentamicin C_{2b}, 3,2',6' - tri - N - Y - Antibiotic 66-40B, 3,2',6' - tri - N - Y - Antibiotic 66-40D, 3,2',6' - tri - N - Y - Antibiotic JI-20A, 3,2',6' - tri - N - Y - Antibiotic JI-20B, 3,2',6' - tri - N - Y - Antibiotic G-52 the 5-epi- and 5-epi- azido - 5 - deoxy analogs of the foregoing;

3,2',6' - tri - N - Y - kanamycin B, 3,2',6' - tri - N - Y - 3',4' - dideoxykanamycin B, 3,2',6' - tri - N - Y - nebramycin factor 4, 3,2',6' - tri - N - Y - nebramycin factor 5', 3,2',6' - tri - N - Y - 3',4' - dideoxy - 3',4' - dehydrokanamycin B, 3,2',6' - tri - N - Y - 3',4' - dideoxy - 6' - N - methylkanamycin B, and 3,2',6' - tri - N - Y - 5 - deoxysisomicin; wherein Y is an acyl group, including acetyl, 2,2,2 - trichloroethoxycarbonyl and benzyloxycarbonyl.

Of particular value as intermediates are those compounds, wherein Y is lower alkanoyl, preferably acetyl. Of these, a particularly preferred species is 3,2',6' - tri - N - acetylsisomicin.

Other valuable 3,2',6' - tri - N - acylaminocyclitol - aminoglycosides are those wherein Y is 2,2,2 - trichloroethoxycarbonyl, a particularly preferred species being 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin, a valuable intermediate in the preparation of 1 - N - acyl - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols, particularly 1 - N - acyl derivatives of sisomicin, e.g. 1 - N - acetylsisomicin, valuable as antibacterial agents and as intermediates in preparing the corresponding 1 - N - alkyl derivatives.

Other novel intermediates are 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols being selectively blocked in positions 2',6', which are selected from

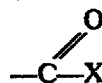
2',6' - di - N - Y - sisomicin, 2',6' - di - N - Y - verdamycin, 2',6' - di - N - Y - tobramycin, 2',6' - di - N - Y - gentamicin C₁, 2',6' - di - N - Y - gentamicin C_{1a}, 2',6' - di - N - Y - gentamicin C₂, 2',6' - di - N - Y - gentamicin C_{2a}, 2',6' - di - N - Y - Antibiotic 66-40B, 2',6' - di - N - Y - Antibiotic 66-40D, 2',6' - di - N - Y - Antibiotic JI-20A, 2',6' - di - N - Y - Antibiotic JI-20B, 2',6' - di - N - Y - Antibiotic G-52, the 5-epi- and 5-epi- azido - 5 - deoxy analogs of the foregoing and of 2',6' - di - N - Y - gentamicin C_{2b};

2',6' - di - N - Y - kanamycin B, 2',6' - di - N - Y - 3',4' - dideoxykanamycin B, 2',6' - di - N - Y - nebramycin factor 4, 2',6' - di - N - Y - nebramycin factor 5', 2',6' - di - N - Y - 3',4' - dideoxy - 3',4' - dehydrokanamycin B, 2',6' - di - N - Y - 3',4' - dideoxy - 6' - N - methylkanamycin B, and 2',6' - di - N - Y - 5 - deoxysisomicin; wherein Y is an acyl group.

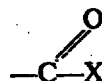
Particularly valuable derivatives of the foregoing are those wherein Y is 2,2,2 - trichloroethoxycarbonyl or *tert.* - butoxycarbonyl, particularly 2',6' - di - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin and 2',6' - di - N - (*tert.* - butoxycarbonyl)sisomicin, valuable intermediates in preparing 2',6' - di - N - alkyl - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols having antibacterial activity, e.g. 2',6' - di - N - ethylsisomicin.

Still other novel intermediates are 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols being selectively blocked in positions 3,6' which are selected from 3,6' - di - N - Y - kanamycin A, 3,6' - di - N - Y - gentamicin B, 3,6' - di - N - Y - gentamicin B₁, 3,6' - di - N - Y - gentamicin A₃, 3,6' - di - N - Y - 6' - N - methylkanamycin A wherein Y is an acyl group, preferably acetyl, *tert.* - butoxycarbonyl, benzyloxycarbonyl and 2,2,2 - trichloroethoxycarbonyl. When Y is acetyl, the foregoing derivatives are valuable mainly as intermediates in the preparation of the corresponding 1 - N - alkyl derivatives. When Y is such as *tert.* - butoxycarbonyl, benzyloxycarbonyl and 2,2,2 - trichloroethoxycarbonyl, the foregoing compounds are of particular value in the preparation of the corresponding 1 - N - (γ - amino - α - hydroxybutyryl), 1 - N - (β - amino - α - hydroxypropionyl) and 1 - N - (δ - amino - α - hydroxyvaleryl) derivatives which, when the 3,6' - di - N - acyl groups are removed, are potent antibacterial agents known in the art.

The above described derivatives, as obtained in the inventive process, are useful for preparing 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols having substituents at amino groups which were not protected during the process for their preparation. Substitution in position 1 of the molecule yields particularly preferred compounds. Accordingly, this invention also relates to a process for the preparation of 1 - N - substituted derivatives of the 4,5 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols sisomicin, verdamicin, tobramycin, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, of the 5-epi- and 5 - epi - azido - 5 - deoxy analogs of the foregoing; kanamycin B, 3',4' - dideoxykanamycin B, nebramycin factor 4, nebramycin factor 5',3',4' - dideoxy - 3',4' - dehydrokanamycin B, 3',4' - dideoxy - 6' - N - methylkanamycin B, 5 - deoxysisomicin, gentamicin B, gentamicin B₁, gentamicin A₃, kanamycin A, 6' - N - methylkanamycin A, kanamycin C, Antibiotic G-418, gentamicin A, gentamicin A₁ and gentamicin X₂; wherein the 1 - N - substituent is —CH₂X or



with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, mono- or di - N - alkylaminoalkyl, aminohydroxyalkyl, mono- or di - N - alkylaminohydroxyalkyl, phenyl, benzyl, or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms and of the pharmaceutically acceptable acid addition salts thereof, which comprises the reaction of any of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols in an inert organic solvent with a salt of a divalent transition metal cation selected from copper (II), nickel (II), cobalt (II) and cadmium (II) or with mixtures thereof, whereby is formed a complex of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol between said transition metal salt and pairs of amino and hydroxy groups; followed by the reaction *in situ* of the resulting complex with an amine blocking reagent having an acyl blocking group Y; then reaction of the resulting complex having acyl blocking groups, Y, on non-complexed amino groups, with a transition metal precipitating reagent or with ammonium hydroxide, whereby said transition metal cation is removed and a 3,2',6' - tri - N - Y - or a 3,6' - di - N - Y - or a 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is obtained; followed by the introduction of the 1 - N - substituent —CH₂X or

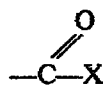


wherein X is as defined above; and then removal of all amino blocking groups present in the molecule and isolating the 1 - N - substituted derivative as such or as a pharmaceutically acceptable acid addition salt.

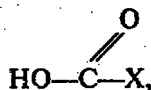
Introduction of the 1 - N - substituent —CH₂X can be effected according to methods known in the art for alkylating primary amino groups. For example, such methods are described in Belgium Patent No. 818,431 and a specifically preferred method comprises the reaction of an acid addition salt of the 3,2',6' - tri - N - Y - or the 3,6' - di - N - Y - or the 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with about one equivalent of a hydride-donor reducing reagent selected from the group consisting of dialkylaminoborane, tetraalkylammonium cyanoborohydride, alkali metal cyanoborohydride and alkali metal borohydride in an inert solvent and with at least one equivalent of an aldehyde having the formula XCHO wherein X is as defined above. After removal of the N - acyl blocking groups, the 1 - N - alkyl derivative is obtained in much greater overall yields (e.g. usually above 50% theory) of purer product than by presently known methods of preparing 1 - N - alkylaminoglycosides (usually less than 20% theory).

For example, when preparing 1 - N - ethylsisomicin by first converting sisomicin to 3,2',6' - tri - N - acetylsisomicin via the transition metal salt complex *in situ* process of this invention followed by reaction of a sulfuric acid addition salt of the resulting 3,2',6' - tri - N - acetylsisomicin with acetaldehyde followed by reduction with sodium cyanoborohydride, then removal of 3,2',6' - tri - N - acetyl

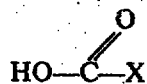
functions via basic hydrolysis, there is obtained 1 - *N* - ethylsisomicin of high purity in yields of about 60% theory; whereas conversion of sisomicin to 1 - *N* - ethylsisomicin by the same process but without acetylating at the 3,2' and 6' positions produces 1 - *N* - ethylsisomicin in yields of about 11% theory. Introduction of the 1 - *N* - substituent



comprises the reaction of the 3,2',6' - tri - *N* - Y - or the 3,6' - di - *N* - Y - or the 3,2' - di - *N* - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol, wherein Y is a blocking group removable without affecting the 1 - *N* - acyl substituent, with an acid of the formula



wherein X is as defined above with any primary or secondary amino group being protected, in the presence of a carbodiimide, or with a reactive derivative of said acid. In a preferred mode of this process, 3,6' - di - *N* - Y derivatives of kanamycin A, gentamicin B and gentamicin B₁, wherein Y is *tert.* - butyloxycarbonyl or benzyloxycarbonyl are used and the acyl moiety of the acid



is β - amino - α - hydroxypropionyl, γ - amino - α - hydroxybutyryl or δ - amino - α - hydroxyvaleryl the amino group therein being protected.

It is to be noted that the 1 - *N* - (aminohydroxyalkanyoyl) - substituent in the so obtained 1 - *N* - (aminohydroxyalkanoyl)aminocyclitol - aminoglycosides may be in the R,S- form or in the R- form or in the S- form. In accordance with this invention all three forms are included.

By means of the novel 3,6' - di - *N* - acyl derivatives of gentamicins B and B₁, and of kanamycin A, prepared via the transition metal complexes, each of gentamicins B and B₁ and kanamycin A can now be converted to the corresponding 1 - *N* - aminohydroxyalkanoyl derivatives in high yields of pure product. Thus, for example, kanamycin A is convertible to crystalline, 3,6' - di - *N* - benzyloxycarbonylkanamycin A without requiring chromatographic purification via the di-nickel (II) acetate complex in dimethylsulfoxide in over 80% theoretical yield. Reaction of the foregoing 3,6' - di - *N* - acyl derivative with *N* - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide followed by treatment of the resulting 1 - *N* - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - *N* - benzyloxycarbonylkanamycin A with hydrogen and a catalyst to remove the benzyloxycarbonyl protecting groups produces 1 - *N* - (S - γ - amino - α - hydroxybutyryl)kanamycin A (also known as amikacin and as Antibiotic BB-K-8) of high purity in excellent overall yields of over 50%.

This invention thus represents a great improvement over the prior art methods of converting kanamycin A to amikacin such as that disclosed in J. Antibiotics 25, 695-708 (1972) whereby kanamycin A is first converted to the 6' - *N* - benzyloxycarbonyl derivative in 45% yields which, when taken through essentially the same conversions described hereinabove produces purified amikacin in overall yields of less than 10%. Similarly, by the method of this invention, kanamycin A via the 3,6' - di - *N* - benzyloxycarbonyl intermediate is convertible to 1 - *N* - (β - amino - α - hydroxypropionyl)kanamycin A (also known as 1 - *N* - isoserylkanamycin A) in overall yields greater than 50% theory as compared with overall yields of less than 8% via prior art methods utilizing the 6' - *N* - *tert.* - butyloxycarbonyl intermediate such as described in U.S. Patent No. 3,939,143.

Similarly, by reacting gentamicin B in dimethylsulfoxide with about 3 molar equivalents of cupric acetate there is obtained *in situ* the corresponding gentamicin B-cupric acetate complex involving the 3" - amino - 4" - hydroxyl and the 1 - amino - 2" - hydroxyl group pairs. Reaction of the foregoing *in situ* with about 1 molar equivalent of *N* - *tert.*-butyloxycarbonyloxypthalimide yields the

corresponding 6' - *N* - *tert* - butoxycarbonylgentamicin B - di - cupric acetate complex, while reaction with at least two moles of the *N* - *tert* - butoxycarbonyloxyphthalimide yields the corresponding 3,6' - di - *N* - *tert* - butoxycarbonylgentamicin B - di - cupric acetate complex. Reaction of each of the foregoing with hydrogen sulfide followed by separation of the resulting cupric sulfide by filtration yields a solution comprising 6' - *N* - *tert* - butoxycarbonylgentamicin B or 3,6' - di - *N* - *tert* - butoxycarbonylgentamicin B, respectively. Each of the foregoing may be isolated from their respective reaction solutions and purified in known manner and then reacted with *N* - (*S* - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide or with *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide or with *N* - (*S* - δ - amino - α - hydroxyvaleryl)succinimide to obtain 1 - *N* - (*S* - γ - amino - α - hydroxybutyryl)gentamicin B, or 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B, or 1 - *N* - (*S* - δ - amino - α - hydroxyvaleryl)gentamicin B, respectively, all of which are valuable antibacterial agents.

Alternatively, one may carry out the chemical conversion at C-1 *in situ* without isolating the 3,6' - di - *N* - *tert* - butoxycarbonylgentamicin B to obtain the corresponding 1 - *N* - (*S* - γ - amino - α - hydroxybutyryl)gentamicin B or 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B or 1 - *N* - (*S* - δ - amino - α - hydroxyvaleryl)gentamicin B in excellent overall yields of about 50% theory. The foregoing also represents a significant improvement over prior art methods for preparing these compounds such as described by P. J. L. Daniels et al. in *J. Antibiotics* 27, 889 (1974) which produce 1 - *N* - aminohydroxyalkanoylgentamicin C₁ derivatives in less than 15% yields.

It is to be noted, also, that the yield of 6' - *N* - *tert* - butoxycarbonylgentamicin B is nearly quantitative and requires no chromatographic purification in contrast to prior art procedures which give only about 46% yields and requires chromatographic isolation.

When 3,6' - di - *N* - acyl - gentamicin B intermediates, e.g. 3,6' - di - *N* - benzyloxycarbonylgentamicin B, are used in the conversion of gentamicin B to a 1 - *N* - (aminohydroxyalkanoyl) derivative thereof, e.g. 1 - *N* - (β - amino - α - hydroxypropionyl)gentamicin B, reaction of the 3,6' - di - *N* - acyl derivative with a racemic reagent, e.g. *N* - (β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide will yield a 3,6' - di - *N* - acyl - 1 - *N* - (*R,S* - aminohydroxyalkanoyl)gentamicin B diastereoisomeric mixture (e.g. 3,6' - di - *N* - (benzyloxycarbonyl) - 1 - *N* - (*R,S* - β - benzyloxycarbonylamino - α - hydroxypropionyl)gentamicin B). Surprisingly, this mixture can easily be separated via chromatographic techniques into each diastereoisomer, e.g. into 3,6' - di - *N* - benzyloxycarbonyl - 1 - *N* - (*R* - β - benzyloxycarbonylamino - α - hydroxypropionyl)gentamicin B and 3,6' - di - *N* - (benzyloxycarbonyl) - 1 - *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyl)gentamicin B which, upon removal of the 3,6' - di - *N* - blocking groups yield the respective diastereoisomeric antibacterial agent, e.g. 1 - *N* - (*R* - β - amino - α - hydroxypropionyl)gentamicin B and 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B, respectively. Alternatively, removal of the protecting groups from 3,6' - di - *N* - (benzyloxycarbonyl) - 1 - *N* - (*R,S* - β - benzyloxycarbonylamino - α - hydroxypropionyl)gentamicin B yields 1 - *N* - (*R,S* - β - amino - α - hydroxypropionyl)gentamicin B which can be obtained pure from impurities by chromatography on silica gel but which is not thereby separated into the respective diastereoisomers.

The polyamino-organic compound-transition metal salt complexes are conveniently used as intermediates *in situ* in the solvent medium in which they are prepared, and therefore, there is usually no need to isolate the transition metal salt complexes of this invention. Thus, an important aspect of this invention resides in a composition comprising a polyamino-organic compound transition metal salt complex in an inert organic solvent. The transition metal salt complexes can be isolated, however, either by evaporating the solvent medium *in vacuo* or by precipitating the complex by pouring the solution thereof into ether, whereby is produced a polyamino-organic compound-transition metal salt complex as an amorphous powder which is characterized by colors different from the transition metal salt and of the polyamino-organic compound precursor, and which are also characterized by infrared spectrum taken in the solid state.

Preferred transition metal salt complexes of this invention are those derived from cupric acetate, nickel (II) acetate, cobalt (II) acetate or mixtures thereof.

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particularly those wherein the polyamino-organic compound is an aminocyclitol-aminoglycoside, preferably a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

Included among the preferred polyamino-organic compound-transition metal salt complexes of this invention are sisomicin-dicupric acetate complex, sisomicin-dicobalt (II) acetate complex, sisomicin-dinickel (II) acetate complex and the mixed sisomicin-cupric acetate-nickel (II) acetate complex, all of which are valuable intermediates in preparing 3,2',6' - tri - N - acylsisomicin selectively blocked compounds.

Other particularly valuable polyamino-organic compound-transition metal salt complexes of this invention are gentamicin B-dicupric acetate complex, gentamicin B-dicobalt (II) acetate complex and kanamycin A-dinickel (II) acetate complex, valuable intermediates in preparing the 3,6' - di - N - acyl derivatives of gentamicin B and kanamycin A, e.g. 3,6' - di - N - tert - butoxycarbonylgentamicin B and 3,6' - di - N - benzyloxycarbonylkanamycin A.

The following Examples illustrate the invention.

EXAMPLE 1

3,2',6'-Tri-N-acetylsisomicin

A. Via Cupric Acetate Complex

Add cupric acetate hydrate (9 gms., 45 mmol) to a stirred solution of sisomicin (1.3 gms., 2.9 mmol) in water (16 ml) and dimethylformamide (54 ml). Stir at room temperature for 35 minutes, then to the cupric salt complex thereby formed add dropwise at a rate of about 25 drops per minute 9.3 ml of a 1 molar solution of acetic anhydride in dimethylformamide (9.3 mmol). Stir the reaction mixture for an additional 30 minutes, then add 30 ml of water and bubble hydrogen sulfide through the solution for about 10 minutes, stir the mixture for an additional 30 minutes, then filter the solution through a pad of Celite (Registered Trade Mark) and wash the cupric sulfide residue with three 20 ml portions of water. Concentrate the combined filtrate and water washings and chromatograph the resultant residue on silica gel (150 gms., 60-200 mesh) eluting with chloroform:methanol:ammonium hydroxide (30:10:1). Combine like fractions as determined by thin layer chromatography on silica gel using a solvent system consisting of chloroform:methanol:ammonium hydroxide (2:1:0.34) and evaporate the fractions containing the major product *in vacuo* and lyophilize the resultant aqueous mixture to a residue comprising 3,2',6' - tri - N - acetylsisomicin (1.29 gms., 76% yield); $[\alpha]_D^{25} + 186.7^\circ$ (c, 4.4 in water); pmr (ppm) (D_2O): δ 1.22 (4" - CH_2); 1.94, 1.98, 2.0 (N-acetyls); 2.51 (3" - $N-CH_2$); 2.59 (H-3", $J_{2,3} = 9.5$ Hz); 5.10 (H-1", $J_{1,2} = 4.0$ Hz); 5.51 (H-1', $J_{1',2'} = 2.5$ Hz; mass spectrum: (M^+) m/e 573, also m/e 392, 374, 364, 346, 233, 215, 205, 187, 160, 443, 425, 415, 397, 169. Analysis calculated for $C_{25}H_{43}O_{10}N_5 \cdot H_2CO_3$; C, 49.13; H, 7.14; N, 11.02%. Found: C, 49.10; H, 7.02; N, 11.38%.

B. Via Mixture of Cupric Acetate and Nickel (II) Acetate Complexes

(1) Add cupric acetate hydrate (8 gm., 40 mmol) and nickel (II) acetate tetrahydrate (10 gm, 40 mmol) to a stirred solution of sisomicin (8.94 gm 20 mmol) in dimethylsulfoxide (400 ml). After 30 minutes at room temperature add dropwise a solution of acetic anhydride (5.4 ml in 50 ml tetrahydrofuran, 60 mmol). After the addition is complete, stir the reaction mixture for an additional 30 minutes. Pour the reaction mixture into 1.5 l. of ether and thoroughly mix the contents. After letting the mixture settle, decant the ether layer. Repeat the above procedure three times using 500 ml. ether each time. Dissolve the semi-solid residue in 400 ml. methanol and bubble hydrogen sulfide through the solution to remove cupric and nickel (II) ions. Remove the solids by filtration through Celite and wash the residue with methanol.

Concentrate the combined filtrates *in vacuo* and dissolve the residue in water (150 ml). Treat the aqueous solution with amberlite (Registered Trade Mark) IRA-401S ion exchange resin in the hydroxide cycle until the pH is about 9. Remove the resin by filtration, wash thoroughly and either lyophilize the aqueous solution to give 3,2',6' - tri - N - acetylsisomicin in 90% yield (10.3 gms) or concentrate the aqueous solution to a thick syrup, dissolve the residue in minimum amount of isopropanol and precipitate the product with excess ether.

(2) Alternatively, after removal of the cupric and nickel (II) ions by treatment with hydrogen sulfide and filtration, concentrate the filtrate and dissolve the residue in a minimum amount of isopropanol and precipitate the product with

excess ether to obtain 3,2',6' - tri - *N* - acetylsisomicin acetic acid salt in 90% yield.

C. Via Cobalt (II) Acetate Complex

(1) Stir sisomicin (0.447 gms, 1 mmol) in dimethylformamide (20 ml) and add cobalt (II) acetate tetrahydrate (0.516 gms, 2.07 mmol). Stir for 20 minutes at room temperature, then to the cobalt (II) acetate complex thereby formed add dropwise a 1 molar solution of acetic anhydride in tetrahydrofuran (3 ml). Continue stirring for 1 hour, then add water (10 ml) and bubble hydrogen sulfide through the solution until all the cobalt (II) is precipitated. Remove the cobalt (II) sulfide by filtration through a pad of Celite and wash the residue with water. Evaporate the filtrate *in vacuo* and dissolve the resultant residue in a minimum quantity of chloroform:methanol:ammonium hydroxide (2:1:0.35). Pass the solution through a column of silica gel (50 gms, 60–200 mesh), washing the column with the same solvent. Collect the homogeneous fractions containing the 3,2',6' - tri - *N* - acetylsisomicin as determined by thin layer chromatography, evaporate the combined fractions *in vacuo* and lyophilize the resultant residue to obtain 3,2',6' - tri - *N* - acetylsisomicin (yield=0.498 gms, 88% theory).

(2) In the above procedure, by substituting dimethylsulfoxide for dimethylformamide and by isolating and purifying the resultant product in a manner similar to that described in Example 1B, there is obtained 3,2',6' - tri - *N* - acetylsisomicin in good yields.

D. Utilizing Acetyl-Acetone as Transition Metal Precipitating Reagent

In the procedures of Examples 1A, 1B and 1C in place of hydrogen sulfide utilize acetyl-acetone as the transition metal precipitating reagent and separate by filtration the resultant precipitate of the corresponding transition metal acetyl-acetonate then isolate and purify each of the resultant product in a manner similar to that described to obtain 3,2',6' - tri - *N* - acetylsisomicin.

EXAMPLE 2

3,2',6'-Tri-*N*-acetylverdamicin via Cupric Acetate Complex

To a stirred solution of verdamicin (4.61 gms, 10 mmol) in dimethylformamide (300 ml) and water (90 ml) add cupric acetate monohydrate (14 gms, 70 mmol), stir the reaction mixture at room temperature for about 30 minutes, then to the cupric salt complex thereby formed add dropwise 33 ml of a 1 molar solution of acetic anhydride in dimethylformamide (3.3 equivalents). Continue stirring at room temperature for about 18–20 hours, then add water and bubble hydrogen sulfide gas through the solution. Filter off the copper sulfide thereby formed through filter aid on a sintered glass funnel rinsing with water (50–200 ml). Evaporate the filtrate *in vacuo*, dissolve the resultant residue in water and adjust the pH of the aqueous solution to about 9 using Amberlite IRA-401S ion exchange resin in the hydroxide form. Freeze dry the solution to a residue comprising 3,2',6' - tri - *N* - acetylverdamicin (5.5 gms). Purify by chromatographing on 300 gm silica gel (60–200 mesh) eluting with chloroform:methanol:ammonium hydroxide (28%) (30:10:1). Combine like fractions as determined by thin layer chromatography and evaporate the combined fractions *in vacuo* to a residue comprising 3,2',6' - tri - *N* - acetylverdamicin, yield 1.68 gm (2.8 mmol, 28% theory); pmr (ppm) (D_2O): δ 1.25 ($4''-C-CH_3$); 1.36 ($6'-C-CH_3$, $J_{1,2}'=7$ Hz); 1.97, 2.04 (*N* - Acetyls); 2.56 ($3''-N-CH_3$); 4.91 ($H-4''$); 5.11 ($H-1''$, $J_{1,2}''=4$ Hz); 5.60 (d, $H-1''$, $J_{1,2}''=2.5$ Hz); mass spectrum: m/e 587 $[M]^+$; 392, 374, 364, 346; 233, 215, 205, 187; 457, 439, 429, 411; 183.

EXAMPLE 3

3,2',6'-Tri-*N*-(2,2,2-Trichloroethoxycarbonyl) Derivatives of Sisomicin and Gentamicin C_{1a} via Cupric Acetate Complex

A. 3,2',6' - Tri - *N* - (2,2,2 - Trichloroethoxycarbonyl)sisomicin

Add cupric acetate hydrate (14 gms, 70 mmol) to a stirred solution of sisomicin (5.0 gms, 11.1 mmol) in dimethylsulfoxide (300 ml) stirring for 45 minutes, then to the cupric salt complex thereby formed add in portions *N* - (2,2,2 - trichloroethoxycarbonyloxy)succinimide (9.25 gms, 32 mmol) (over a 2 to 3 minute period). Stir the resulting green solution for one hour, then dilute the reaction mixture with 2 *N* ammonium hydroxide (3 liters) and extract with ethyl acetate (two

12	13	1,575,982	13
0%		600 ml portions). Combine the organic extracts, wash with 2 N ammonium hydroxide (600 ml) containing sodium chloride (60 gms), dry over sodium sulfate and evaporate <i>in vacuo</i> . Chromatograph the resultant residue on silica gel (300—350 gms) eluting with a chloroform:methanol:concentrated ammonium hydroxide solvent mixture (7:2:0.1 by volume).	
add om vise ring tion tion e in of gh a ame N - the ,6' -	5	Combine the like eluates containing the desired product as determined by thin layer chromatography and evaporate the combined eluates <i>in vacuo</i> , then dry the resultant residue at 60°C and 0.1 mm to give 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin, yield 7.52 gms. (77% theory); m.p. 124—127°C [α] _D ²⁵ +91.0° (c, 0.5 in chloroform); pmr (ppm) (CDCl ₃): δ 1.17 (4''—C—CH ₃) 2.58 (3''—N—CH ₃) and 4.77 (—CH ₂ CCl ₃).	5
	10		10
	15	B. 3,2',6' - Tri - N - (2,2,2 - Trichloroethoxycarbonyl)gentamicin C _{1a} Add cupric acetate hydrate (2.8 gms; 14 mmol) to a stirred solution of gentamicin C _{1a} (1.0 gm, 2.22 mmol) in dimethylsulfoxide (56 ml) at 25°C. Continue stirring for 1 hour, then to the cupric salt complex thereby formed add portionwise N - (2,2,2 - trichloroethoxycarbonyloxy) succinimide (1.8 gms. 62 mmol) over a 15 minute period. Continue stirring for 2 hours, then dilute the reaction mixture with 2 N ammonium hydroxide (800 ml) and extract with ethyl acetate (3x75 ml). Evaporate the combined extracts <i>in vacuo</i> and chromatograph the resultant residue on a silica gel column (110x2.5 cm) eluting first with chloroform (250 ml) and then eluting with chloroform:methanol:concentrated ammonium hydroxide (7:2:0.1 by volume). Combine the like eluates containing the desired product as determined by thin layer chromatography and evaporate the combined eluates to a residue of 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl)gentamicin C _{1a} , yield 1.51 gms (78% theory); [α] _D ²⁵ +80.0° (c, 0.3 in chloroform) $\nu_{\text{max}}^{\text{KBr}}$ 3330, 1730, 1520, 1040, 1025 cm ⁻¹ ; pmr (ppm) (CDCl ₃): δ 1.14 (4''—C—CH ₃), 2.55 (3''—N—CH ₃) and 4.64 (—CH ₂ CCl ₃).	15
for in a - N -	20		20
lfide te by cetyl- milar	25		25
	30	EXAMPLE 4 2',6'-Di-N-Substituted Sisomicin A. 2',6' - di - N - (2,2,2 - Trichloroethoxycarbonyl)sisomicin Via Nickel (II) Acetate Complex Add sisomicin (5 gms, 11.1 mmol) and nickel (II) acetate hydrate (15 gms, 75 mmol) to methanol (350 ml) and stir until dissolved. To the solution of the nickel (II) salt complex thereby formed cooled in an ice bath add N - (2,2,2 - trichloroethoxycarbonyloxy)succinimide (6.40 gms, 22 mmol) in portions over a 2 to 3 minute period and stir the reaction mixture at room temperature for an hour. Add concentrated ammonium hydroxide (5 ml) to the reaction mixture, evaporate <i>in vacuo</i> to a volume of about 100 ml, add 2 N ammonium hydroxide (500 ml) together with sodium chloride (50 gms) and extract with chloroform (three 300 ml portions). Combine the organic phases, dry over sodium sulfate and evaporate. Chromatograph the resultant residue on 300 gms of silica gel eluting with a chloroform:methanol:concentrated ammonium hydroxide solvent mixture (3:1:0.1 by volume). Combine like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined fractions <i>in vacuo</i> . Dry the precipitate at 60°C at 0.1 mm pressure to a residue of 2',6' - di - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin, yield=5.80 gms (72% theory); m.p. 115—119°C; [α] _D ²⁵ +94° (c, 0.4 in chloroform); pmr (ppm) (CDCl ₃): δ 1.16 (4''—C—CH ₃); 1.33 (H—2, J _{ax,2eq} =J _{1,2ax} =J _{2,3ax} =10 Hz); 2.60 (3''—N—CH ₃); 4.73 (—CH ₂ CCl ₃); 4.95 (H—1'' and H—4'); 5.50 (H—1', J _{1',2'} =3 Hz); 6.28 (2'—NH, J=8 Hz) and 7.45 (6'—NH).	30
amide l), stir cupric hydride ire for gh the on a ate in queous roxide - N - l (60— 10:1). porate - N - δ 1.25 6 (3''— .5 Hz); 19, 429,	35		35
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omicin then to (2,2,2 - minute reaction ate (two	55	B. 2',6' - Di - N - Tert. - Butoxycarbonylsisomicin Via Cupric Acetate Complex Add cupric acetate hydrate (0.6 gm, 3 mmol) to a solution of sisomicin (447 mg 1 mmol) in dimethylsulfoxide (10 ml), and stir for 10 minutes at room temperature. To the cupric salt complex thereby formed add dropwise a solution of N - tert. - butoxycarbonyloxyphthalimide (576 mg., 2.2 mmol) in dimethylsulfoxide (3 ml). Stir for 18 hours at room temperature, then add the reaction solution dropwise into stirred diethyl ether (75 ml). Allow the resultant precipitate to settle, decant the diethyl ether solution and triturate the precipitate twice with 75 ml portions of diethyl ether. Dissolve the precipitate in methanol, and bubble hydrogen sulfide through the methanol solution, separate the resultant cupric sulfide precipitate by filtration, deionize the methanolic solution with Amberlite IRA-401S (OH ⁻) ion	55
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exchange resin (20 gm), filter, concentrate the filtrate *in vacuo* and chromatograph the resultant residue on alumina (75 gms) in a column (2.4x30 cm) eluting with chloroform:methanol:concentrated ammonium hydroxide (30:10:1 by volume). Combine the like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined fractions and freeze dry the resultant residue to give 2',6' - di - N - tert - butoxycarbonylsisomicin, yield 258 mg, (40% theory); mass spectrum (M^+) m/e 647, also m/e 547; 530, 517, 499, 489, 471; 462; 350, 332, 322, 304; 191, 160.

10 C. 2',6' - Di - N - Tert - Butoxycarbonylsisomicin Via Cobalt (II) Acetate Complex or Via Nickel (II) Acetate Complex

In the procedure of Example 4B, by utilizing methanol as solvent (instead of dimethylsulfoxide) and either nickel (II) acetate or cobalt (II) acetate as transition metal salt (instead of cupric acetate) there is obtained 2',6' - di - N - tert - butoxycarbonylsisomicin in improved yields.

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EXAMPLE 5

1,3,2',6'-Tetra-N-Acetylsisomicin Via Cupric Acetate Complex

Add cupric acetate hydrate (49 gms, 24.5 mmol) to a stirred solution of sisomicin (22 gms, 49.2 mmol) in 91% aqueous methanol. Continue stirring the solution in an ice water bath for 15 minutes, then to the cupric salt complex thereby formed add dropwise over a period of 10 minutes 21 ml of acetic anhydride (226 mmol) keeping the temperature below 25°C. Stir the reaction mixture for 3 hours at room temperature, then bubble hydrogen sulfide through the solution. Remove the resultant precipitate of cupric sulfide by filtration through Celite and wash the residue with methanol. Combine the filtrate and washings and concentrate *in vacuo*. Chromatograph the resultant residue on 600 gms silica gel (60—200 mesh) eluting with a solvent mixture of chloroform:methanol:concentrated ammonium hydroxide (30:10:1). Combine like fractions containing the desired product as determined by thin layer chromatography and evaporate *in vacuo* and lyophilize the resultant residue to give 1,3,2',6' - tetra - N - acetylsisomicin, yield 23.5 gms (78% theory); $[\alpha]_D^{25} + 191^\circ$ (c, 0.2 in water); pmr (ppm) (D_2O): δ 1.18 ($C-CH_3$); 1.88—1.98 (N-acetyls); 2.48 ($N-CH_3$); 4.88 ($H-4'$); 5.07 ($H-1''$, $J_{1'',2''} = 4$ Hz); 5.48 ($H-1'$, $J_{1',2'} = 3$ Hz); mass spectrum: (M^+) m/e 615; also m/e 592, 550; 485, 467, 457, 439; 434, 416, 406, 388; 275, 257, 247, 229; 211; 160.

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EXAMPLE 6

1,3,2'-Tri-N-Acetylsisomicin

A. 6' - N - Trifluoroacetylsisomicin Via Cupric Acetate Complex

Add cupric acetate hydrate (17.48 gms, 87.3 mmol) to a stirred solution of sisomicin (40 gms, 89.5 mmol) in 95% aqueous methanol (3.12 liters). Stir for 5 minutes, then to the cupric salt complex thereby formed add dropwise over a period of 3 minutes ethyl trifluorothiol acetate (11.65 ml, 91.9 mmol). Continue stirring for 1 hour, then add an additional portion of ethyl trifluorothiol acetate (1.6 ml, 12.45 mmol). This reaction solution containing 6' - N - trifluoroacetylsisomicin is used as is in the procedure of Example 6B.

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B. 1,3,2'-Tri-N-Acetylsisomicin

Cool the reaction mixture prepared in Example 6A in an ice water bath and add dropwise acetic anhydride (28 gms, 280 mmol) at a rate of 25 drops per minute. Stir for 18 hours at room temperature, then bubble hydrogen sulfide through the solution for 10 minutes. Remove the precipitated cupric sulfide by filtration through a pad of Celite. Evaporate the filtrate *in vacuo*, dissolve the resultant residue in concentrated ammonium hydroxide (1 liter) and stir for 3 hours at room temperature. Concentrate the solution *in vacuo* and chromatograph the resultant residue on 4.5 kilograms of silica gel (60—200 mesh) eluting with a solvent system consisting of chloroform:methanol:concentrated ammonium hydroxide (30:10:1). Combine the like fractions containing the desired product as determined by thin layer chromatography, concentrate the combined fractions *in vacuo* and lyophilize the resultant residue to give 1,3,2' - tri - N - acetylsisomicin; $[\alpha]_D^{25} + 191.5^\circ$ (c, 0.3 in water); pmr (ppm) (D_2O): δ 1.31 ($C-CH_3$); 1.92, 1.94, 1.96 (N-acetyls); 2.82 ($N-CH_3$); 5.15 ($H-1''$, $J_{1'',2''} = 3.5$ Hz); 5.55 ($H-1'$, $J_{1',2'} = 2.5$ Hz); mass spectrum: (M^+) 573 also m/e 443, 425, 415, 397; 275, 257, 247, 229; 434, 416, 406, 388; 169, 160.

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EXAMPLE 7

6'-N-Hydrocarbonyloxycarbonylaminoglycosides

A. 6' - N - Benzyloxycarbonylgentamicin B Via Cupric Chloride Complex

Add cupric chloride dihydrate (0.34 gms, 2 mmol) to a stirred solution of gentamicin B (0.964 gms, 2 mmol) in water (3 ml) and dimethylsulfoxide (30 ml). Stir at room temperature for 20 minutes, then add dropwise to the cupric salt complex thereby formed *in situ* a solution of N - benzyloxycarbonyloxypthalimide (1.105 gms, 4 mmol) in dimethylsulfoxide (5 ml). Monitor the progress of the reaction via thin layer chromatography on silica gel using a solvent system consisting of chloroform:methanol:concentrated ammonium hydroxide (2:1:0.35). When the reaction is complete as evidenced by thin layer chromatography (about 3 hours) add water (10 ml) to the reaction mixture and bubble hydrogen sulfide through the solution. Remove the resultant precipitate of cupric sulfide by filtration through a pad of Celite and wash the residue with methanol. Stir the combined filtrate methanol wash with Amberlite IRA-401S (OH⁻) ion exchange resin. Filter off the resin and wash with methanol. Evaporate the combined filtrate and methanol wash *in vacuo* using benzene as a co-solvent until all the water is removed. Pour the resulting concentrated reaction solution into a large volume of methylene chloride with stirring. Separate the resulting precipitate by filtration and wash the precipitate with ether to obtain 6' - N - benzyloxycarbonylgentamicin B; yield 100%; pmr (ppm) (D₂O): δ 1.26 (4"-C-CH₃); 2.65 (3"-N-CH₃); 5.13 and 7.43 (O-CH₂-C₆H₅).

B. In the procedure of above Example 7A substitute for gentamicin B any aminoglycoside antibacterial agent having a primary carbinamine at C-5' to obtain the corresponding 6' - N - benzyloxycarbonylaminoglycoside, e.g. sisomicin, gentamicin C_{1a}, gentamicin A, Antibiotic JI-20A, Antibiotic 66-40B, Antibiotic 66-40D, the 5 - epi -, 5 - epi - azido - 5 - deoxy- and 5 - epi - amino - 5 - deoxy analogs of the foregoing, Antibiotic Mu-1, Antibiotic Mu-2, Antibiotic Mu-4, Antibiotic Mu-5, tobramycin, kanamycin A, kanamycin B, Antibiotic BBK-8, Antibiotic XK-88-3 and Antibiotic XK-88-5.

C. In the procedures of Examples 7A and 7B by substituting for N - benzyloxycarbonyloxypthalimide equivalent quantities of other phthalimide reagents, e.g. N - tert. - butoxycarbonyloxypthalimide or N - (2,2,2 - trichloroethoxycarbonyloxy)phthalimide, there is obtained the corresponding 6' - N - hydrocarbonyloxycarbonylaminoglycoside, e.g. the corresponding 6' - N - tert. - butoxycarbonylaminoglycoside and 6' - N - (2,2,2 - trichloroethoxycarbonyl) - aminoglycoside derivatives.

D. 6' - N - Tert. - Butoxycarbonylgentamicin B Via Cupric Acetate Complex

Add cupric acetate hydrate (24 gms 120 mmol) to a stirred solution of gentamicin B (19.28 gms, 40 mmol) in dimethylsulfoxide (1 liter). Continue stirring for 20 minutes, to the cupric salt complex thereby formed *in situ* add a solution of N - tert. - butoxycarbonyloxypthalimide (16 gms, 61 mmol) in dimethylsulfoxide (200 ml), over a period of 20 minutes. Stir for 18 hours at room temperature, then bubble hydrogen sulfide through the solution to precipitate cupric sulfide. Remove the solids by filtration through a pad of Celite and wash the residue with 200 ml of water. Stir the combined filtrate and washings with 200 ml of Amberlite IRA-401S (OH⁻) ion exchange resin for one hour. Remove the resin by filtration, wash with water, and concentrate the combined filtrate and washings *in vacuo* using benzene to azeotrope with water. Dissolve the resultant residue in methanol and pour the methanol solution into excess ether with stirring. Filter and air dry the resultant precipitate comprising 6' - N - tert. - butoxycarbonylgentamicin B, yield 23 gm (100% theory); $[\alpha]_D^{25} + 124^\circ$ (c, 1 in methanol). pmr (ppm) (D₂O): δ 1.21 (4"-C-CH₃); 1.42 (t-butyl); 2.55 (3"-N-CH₃); 5.06 (H-1", J_{1",2"} = 4.0 Hz); 5.21 (H-1', J_{1',2'} = 3.0 Hz). Analysis Calculated for C₂₄H₄₈N₄O₁₂·CO₂·H₂O: C, 46-57; H, 7.51; N, 8.68%. Found; C, 46.80; H, 7.82; N, 8.54%.

EXAMPLE 8

3,6'-Di-N-Hydrocarbonyloxycarbonyl Aminoglycosides

A. 3,6' - Di - N - Benzyloxycarbonylgentamicin B via Mixture of Cupric Acetate and Nickel (II) Acetate Complexes

Add cupric acetate hydrate (8 gms, 40 mmol) and nickel (II) acetate tetrahydrate (9.92 gms, 40 mmol) to a stirred solution of gentamicin B (9.64 gms, 20

- mmol) in dimethylsulfoxide (400 ml). Stir at room temperature for 30 minutes, then to the cupric-nickel (II) salt complex thereby formed add *N*-benzyloxycarbonyloxyphthalimide (14 gms, 47.2 mmol) in dimethylsulfoxide (70 ml) dropwise over a 10 minute period. Stir for one hour at room temperature, then pour the reaction mixture into ether (4 l) and shake for one minute. Allow the oil to settle and decant off the supernatant ether. Repeat this procedure two more times using 1500 ml and 1000 ml, respectively, of diethyl ether. Dissolve the resultant gummy residue thereby obtained in methanol (400 ml) and concentrated ammonium hydroxide (40 ml) and bubble hydrogen sulfide through the solution, separate the resultant precipitate comprising cupric sulfide and nickel sulfide by filtration through a pad of Celite. Wash the residue with methanol, then stir the combined filtrate and methanol wash with Amberlite IRA-401S (OH⁻) ion exchange resin (400 ml) to remove the *N*-hydroxyphthalimide. Filter the solution, wash the resin with methanol, then evaporate the combined filtrate and methanol wash *in vacuo*, and chromatograph the resultant residue on silica gel (900 gms) eluting with chloroform:methanol:concentrated ammonium hydroxide (30:10:1). Combine the like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined fractions *in vacuo* to a residue comprising the 3,6'-di-*N*-benzyloxycarbonylgentamicin B; yield 10.86 gms (75% theory); $[\alpha]_D^{25} + 105.3^\circ$ (c 4.07 in water). Analysis Calculated for: C₃₅H₅₀O₁₄N₄·CO₂·2H₂O; C, 52.04; H, 6.55; N, 6.74%. Found: C, 51.94; H, 6.33; N, 6.83%.
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- B. 3,6'-di-*N*-Benzyloxycarbonylgentamicin B Via Cobalt (II) Acetate Complex
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- Dissolve gentamicin B (4.82 gms, 10 mmol) in dimethylsulfoxide (195 ml) and water (5 ml). Add triethylamine (2 ml) and stir. Add cobalt (II) acetate tetrahydrate (7.08 gms, 28.5 mmol), continue stirring for 30 minutes, then to the gentamicin B-cobalt (II) acetate complex thereby formed add in a dropwise manner a solution of *N*-(benzyloxycarbonyloxy)phthalimide (6.5 gms, 20 mmol) in dimethylsulfoxide (20 ml) and stir for 3 hours, then pour into ether and isolate in the manner described in Example 8A. Purify the resultant residue by dissolving in a small amount of methanol and adding ethyl acetate followed by excess diethyl ether. Separate the resultant precipitate by filtration and air dry to obtain pure 3,6'-di-*N*-benzyloxycarbonylgentamicin B, yield 90% theory.
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- C. 3,6'-di-*N*-Benzyloxycarbonylgentamicin B Via Cadmium (II) Acetate Complex
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- Dissolve gentamicin B (4.82 gms, 10 mmol) in dimethylsulfoxide (195 ml) and add cadmium (II) acetate dihydrate (7.98 gms, 30 mmol). Stir for 30 minutes, then to the gentamicin B cadmium (II) acetate complex thereby formed add a solution of *N*-(benzyloxycarbonyloxy)phthalimide (6.5 gms, 20 mmol) in dimethylsulfoxide (20 ml). Stir for 3 hours at room temperature, then isolate and purify in a manner similar to that described in Example 8A to obtain 3,6'-di-*N*-benzyloxycarbonylgentamicin B.
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- D. 3,6'-Di-*N*-Benzyloxycarbonylkanamycin A Via Nickel (II) Acetate Complex
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- Add nickel (II) acetate tetrahydrate (24.8 gms, 10 mmol) to a stirred solution of kanamycin A (9.7 gms, 20 mmol) in dimethylsulfoxide (400 ml). Stir at room temperature for 30 minutes, then to the resulting nickel (II) salt complex thereby formed add *N*-benzyloxycarbonyloxyphthalimide (13.0—15.6 gms, 44—52 mmol) in dimethylsulfoxide (70 ml) over a 10 minute period. Stir at room temperature for one hour, then pour the reaction mixture into ether (2500 ml) and shake one minute. Allow the oil to settle and decant the supernatant dimethylsulfoxide ether. Repeat this procedure two more times using 1500 ml and 1000 ml, respectively, of ether. Dissolve the resultant gummy residue in methanol (1000 ml) and concentrated ammonium hydroxide (50 ml). Bubble hydrogen sulfide through the solution and separate the resultant precipitate of nickel (II) sulfide through a pad of Celite and wash the residue with methanol. Stir the combined filtrate and methanol wash with Amberlite IRA-401S (OH⁻) ion exchange resin (300 gms), filter off the resin and evaporate the filtrate *in vacuo*. Triturate the resultant gummy residue with a 50:50 mixture of acetonitrile:ether and filter the resultant white crystalline solid to obtain 3,6'-di-*N*-benzyloxycarbonylkanamycin A; yield 12.5 gms (83.5% theory); $[\alpha]_D^{25} + 78^\circ$ (c 4.84 in 50% aqueous methanol). Analysis Calculated for: C₃₄H₄₈O₁₅N₄·H₂O; C, 56.2; H, 6.75; N, 6.94%. Found: C, 51.02; H, 6.26; N, 6.85%.

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E. Treat each of the following aminoglycosides with a mixture of cupric acetate hydrate and nickel (II) acetate tetrahydrate or with cobalt (II) acetate tetrahydrate or with cadmium (II) acetate dihydrate according to the procedures of Examples 8A through 8D, respectively.

1) gentamicin B,
2) gentamicin A₃,
3) 6' - N - methylkanamycin A.

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

1) 3,6' - di - N - benzyloxycarbonylgentamicin B₁,
2) 3,6' - di - N - benzyloxycarbonylgentamicin A₃,
3) 3,6 - di - N - benzyloxycarbonyl - 6' - N - methylkanamycin A.

F. Preparation of 3,6' - Di - N - Tert. - Butoxycarbonylgentamicin B from 6' - Tert. - Butoxycarbonylgentamicin B Via Cupric Acetate Complex

To a solution of 6' - N - tert. - butoxycarbonylgentamicin B (10 gms, 17.1 mmol) in dimethylsulfoxide (300 ml), add cupric acetate monohydrate (6.9 gms, 34.5 mmol) and stir for 20 minutes. To the cupric salt complex thereby formed *in situ* add dropwise a solution of N - tert. - butoxycarbonyloxyphthalimide (4.5 gms, 17.1 mmol) in dimethylsulfoxide (20 ml). Continue stirring for 2 hours, then add an additional 2.4 gms, of N - tert. - butoxycarbonyloxyphthalimide and continue stirring the reaction mixture for 10 hours. Pour the reaction mixture into ether (1.5 liters) with stirring. Allow the resultant precipitate or oil to settle and carefully decant the supernatant liquid. Wash the residue two times with ether (300 ml). Dissolve the resultant residue in methanol (100 ml) and bubble hydrogen sulfide through the solution to precipitate cupric sulfide completely. Remove the solids by filtration through a pad of Celite and wash with methanol. Stir the methanolic solution with just enough Amberlite IRA-401S (OH⁶) ion exchange resin to bring the pH to about 9 and remove N - hydroxyphthalimide. Remove the resin by filtration, wash with methanol, and concentrate the combined filtrate and washings to a volume of about 50 ml containing 3,6' - di - N - tert. - butoxycarbonylgentamicin B, which may be used without further purification as starting compound in the procedure of Examples 15 and 16B. To isolate the compound, crystallize by triturating the residue after evaporation with ether to give 3,6' - di - N - tert. - butoxycarbonylgentamicin B (yield=90% theory). Alternatively, purify by chromatography on silica gel (ratio of compound: silica gel=1:40) using chloroform:methanol:ammonium hydroxide (2:1:0.35) as the developing solvent mixture. Pool the like fractions containing 3,6' - di - N - tert. - butoxycarbonylgentamicin B as determined by thin layer chromatography, concentrate and lyophilize to give 3,6' - di - N - tert. - butoxycarbonylgentamicin B; $[\alpha]_D^{25} + 113.3^\circ$ (c, 0.39 in water); pmr (ppm) (D₂O): δ 1.17 (4'' - C - CH₃); 1.38 (t - butyl); 2.5 (3'' - N - CH₃); 2.54 (H-3'', J_{2,3}'' = 10 Hz); 4.02 (H-5'' eq, J_{4,5}'' ax. eq = 12 Hz); 5.04 (H-1'', J_{1,2}'' = 3.5 Hz); 5.23 (H-1'', J_{1,2}'' = 3.0 Hz). Analysis calculated for C₂₉H₅₇N₄H₂O: C, 49.63; H, 8.19; N, 7.98%. Found: C, 49.83; H, 7.81; N, 7.68%.

G. 3,6' - Di - N - Tert. - Butoxycarbonylgentamicin B from Gentamicin B Via Cupric Acetate Complex

Add cupric acetate monohydrate (5.0 gms, 25 mmol) to a stirred solution of gentamicin B (4.82 gms, 10 mmol) in dimethylsulfoxide (250 ml). Stir for 30 minutes, then to the cupric salt complex thereby formed *in situ* add dropwise a solution of N - tert. - butoxycarbonyloxyphthalimide (10.5 gms, 40 mmol) in dimethylsulfoxide (20 ml). Stir at room temperature for 2 hours, then add additional N - tert. - butoxycarbonyloxyphthalimide (1.5 gms, 5.7 mmol). Stir for 16 hours, then again add an additional N - tert. - butoxycarbonyloxyphthalimide (0.35 gms, 1.33 mmol). Continue stirring for 6 hours, then pour the reaction mixture into 1.5 l of stirred ether. Stir for 30 minutes, allow the reaction mixture to stand, decant the supernatant solution, again add 500 ml of ether to the residue, let stand and decant the supernatant solution. Dissolve the resultant residue in 100 ml of methanol and bubble hydrogen sulfide for 15 minutes. Remove the cupric sulfide solid by filtration through a pad of Celite and wash the residue with methanol. Treat the methanolic solution with Amberlite IRA-401S (OH⁶) ion exchange resin to bring the pH of the solution to about 8.5-9.0, remove the solids by filtration and wash the solids with methanol. Evaporate the solution to near dryness and lyophilize from water. Crystallize 3,6' - di - N - tert. - butoxycarbonylgentamicin B by triturating with ether. Alternatively, the crude concentrate may be used as starting material in the procedure of Examples 15 and 16B.

EXAMPLE 9

Other 3,2',6'-Tri-*N*-AcylaminoglycosidesA. 3,2',6' - Tri - *N* - Benzyloxycarbonylgentamicin C₂ Via Mixture of Cupric Acetic Complex and Nickel (II) Acetate Complex

Add cupric acetate hydrate (0.6 gms, 3 mmol) and nickel (II) acetate tetrahydrate (0.743 gms, 3 mmol) to a stirred solution of gentamicin C₂ (0.925 gms, 2 mmol) in dimethylsulfoxide (40 ml). Stir at room temperature for 30 minutes, then to the mixed cupric and nickel salt complex thereby formed add *N* - benzyloxycarbonyloxyphthalimide (1.8 gms, 6.5 mmol) in dimethylsulfoxide (7 ml) over a 10 minute period. Continue stirring for one hour, then pour the reaction mixture into ether (400 ml) with stirring. Decant the supernatant ether, repeat the foregoing process using 200 ml and 100 ml of ether, respectively. Dissolve the resulting gummy residue in methanol (100 ml) and concentrated ammonium hydroxide (5 ml), then bubble hydrogen sulfide through the solution. Remove the resultant precipitate of cupric sulfide and nickel (II) sulfide by filtration through a pad of Celite, wash the precipitate with methanol then stir the combined filtrate and methanol wash with Amberlite IRA-401S (OH⁻) ion exchange resin. Separate the resin by filtration and wash with methanol. Evaporate the combined filtrate and methanol wash *in vacuo*, then chromatograph the resultant gummy residue on silica gel (50 gms) eluting with chloroform:methanol:concentrated ammonium hydroxide (30:10:1). Combine the like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined eluates to a residue comprising 3,2',6' - tri - *N* - benzyloxycarbonylgentamicin C₂; yield 1.4 gms (81% theory); mass spectrum: m/e 484; 411; 325; 160.

B. In a manner similar to that described in Example 9A, treat gentamicin C₁ with cupric acetate hydrate and nickel (II) acetate tetrahydrate in dimethylsulfoxide followed by treatment of the salt complex thereby formed with *N* - benzyloxycarbonyloxyphthalimide and thence treatment with hydrogen sulfide and isolation and purification of the resultant product to obtain 3,2',6' - tri - *N* - benzyloxycarbonylgentamicin C₁, yield 1.3 gms, 72.5% theory. Mass spectrum: m/e 749; 615; 484, 466, 456; 425, 407; 325, 307, 297; 291; 160.

C. Mixed 3,2',6' - Tri - *N* - Acylaminoglycosides and Conversion to 3,6' - Di - *N* - Acylaminoglycoside(1) 6' - *N* - Acetyl - 2' - *N* - (2,2,2 - Trichloroethoxycarbonyl)sisomicin Via Cupric Acetate Complex

In a manner similar to that described in Example 4A treat 6' - *N* - acetylisisomicin with about 7 equivalents of cupric acetate hydrate in methanol, then to the cupric salt complex thereby formed add in portions about 1 equivalent of *N* - (2,2,2 - trichloroethoxycarbonyl)succinimide. Stir the reaction mixture for one hour, then treat with ammonium hydroxide, and isolate and purify the resultant product in a manner similar to that described in Example 4A to obtain 2' - *N* - (2,2,2 - trichloroethoxycarbonyl) - 6' - *N* - acetylisisomicin.

(2) 3,6' - Di - *N* - Acetyl - 2' - *N* - (2,2,2 - Trichloroethoxycarbonyl)sisomicin Via Cupric Acetate Complex

Add cupric acetate hydrate (24 gms, 120 mmol) to a stirred solution of 2' - *N* - (2,2,2 - trichloroethoxycarbonyl) - 6' - *N* - acetylisisomicin (26.5 gms, 40 mmol) in dimethylsulfoxide (1 liter). Continue stirring for 20 minutes, then to the cupric salt complex thereby formed add dropwise at the rate of about 25 drops per minute 40 ml of a 1 molar solution of acetic anhydride in tetrahydrofuran (40 mmol). Stir the reaction mixture for an additional 30 minutes, then pour into ether (8 l). Shake well and set aside. Decant the ether layer and wash the residue twice more with 1 liter ether each time. Dissolve the residue in methanol (800 ml) and bubble hydrogen sulfide for 15 minutes. Stir the mixture for an additional 30 minutes, then filter the solution through a pad of Celite and wash the cupric sulfide residue with water. Concentrate the combined filtrate and water washings and chromatograph the resultant residue on silica gel eluting with chloroform:methanol:ammonium hydroxide (30:10:1). Combine like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined fractions *in vacuo* to a residue comprising 3,6' - di - *N* - acetyl - 2' - *N* - (2,2,2 - trichloroethoxycarbonyl)sisomicin.

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18	19	1,575,982	19
5	5	(3) 3,6' - Di - N - Acetylsisomicin	5
10	10	Add 3.9 molar equivalents of zinc powder to a solution of 3,6' - di - N - acetyl - 2' - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin in 10% acetic acid in methanol. Heat the solution at reflux temperature for two hours monitoring the reaction by thin layer chromatography on silica gel using chloroform:methanol:ammonium hydroxide (30:10:1) as solvent system. When the reaction is complete as determined by thin layer chromatography, filter the solution, add sodium carbonate to the filtrate, filter and concentrate the filtrate <i>in vacuo</i> . Purify by chromatographing the resultant residue on silica gel eluting with chloroform:methanol:ammonium hydroxide (30:10:1). Combine the like fractions containing the desired product as determined by thin layer chromatography, then evaporate the combined fractions <i>in vacuo</i> and lyophilize the resultant aqueous mixture to a residue comprising 3,6' - di - N - acetylsisomicin.	10
15	15	EXAMPLE 10	15
20	20	1,3,2',3" - Tetra - N - Acetylsisomicin Via Cupric Acetate Complex	20
25	25	A. 6' - N - Trifluoroacetylsisomicin Via Cupric Acetate Complex	25
30	30	Dissolve sisomicin (39.1 gms, 87.5 mmol) in methanol (2950 ml) and water (200 ml). While stirring add cupric acetate hydrate (8.92 gms, 44.7 mmol). Stir for 15 minutes, then add dropwise over a period of three minutes ethyl trifluorothiolacetate (11.55 ml, 14.2 gms, 90 mmol) followed by an additional 1.3 ml (1.6 gms, 10 mmol) of ethyl trifluoroethiolacetate. Remove the methanol by evaporation <i>in vacuo</i> , then add water until the reaction volume is 300 ml. Pass hydrogen sulfide through the reaction solution, then filter the resultant cupric sulfide precipitate through a Celite pad to obtain a solution containing 6' - N - trifluoroacetylsisomicin, which is used as is in the procedure of Example 10B.	30
35	35	B. 1,3,2',3" - Tetra - N - Acetyl - 6' - N - Trifluoroacetylsisomicin	35
40	40	To the solution obtained in Example 10A add 300 ml of methanol, then add acetic anhydride (109 ml, 1.16 Mol, 13.2 equivalents) adjusting the pH of the reaction mixture to 8.5 with triethylamine prior to adding the last 10 ml of acetic anhydride. Stir for 18 hours at room temperature, then evaporate <i>in vacuo</i> to a residue comprising 1,3,2',3" - tetra - N - acetyl - 6' - N - trifluoroacetylsisomicin.	40
45	45	C. 1,3,2',3" - Tetra - N - Acetylsisomicin	45
50	50	Dissolve the 1,3,2',3" - tetra - N - acetyl - 6' - N - trifluoroacetylsisomicin prepared in Example 10B in 500 ml of concentrated ammonium hydroxide. Stir at room temperature overnight and evaporate <i>in vacuo</i> . Dissolve the resultant residue in water and stir the solution with Amberlite IRA-401S (OH ⁻) ion exchange resin. Remove the resin by filtration, wash with methanol, then evaporate the combined filtrate and methanol wash <i>in vacuo</i> followed by chromatography of the resultant residue on a 1.7 kilogram silica gel column (7.5x165 cm) eluting with the lower phase of a chloroform:methanol:ammonium hydroxide (28%) (2:1:1) solvent system. Combine the like fractions containing the desired product as determined by thin layer chromatography and evaporate the combined eluates to a residue comprising 1,3,2',3" - tetra - N - acetylsisomicin, yield 29.9 gms, (55% theory); $[\alpha]_D^{25} +207.4^{\circ}$ (c, 0.3 in water); pmr (ppm) (D ₂ O): δ 1.07, 1.17 (4"-C-CH ₃); 1.95, 1.98, 2.03 (N-acetyls); 3.13, 3.00 (3"-N-CH ₃); 5.29 (H-1", J _{1,2} "=4.0 Hz); 5.64 (H-1', J _{1,2} '=2.5 Hz); mass spectrum: (M ⁺) m/e 615, also m/e 598; 592; 550; 443, 425, 415, 397; 453, 411; 275, 257, 247, 229; 202; 169. Analysis calculated for: C ₂₇ H ₄₅ O ₁₁ N ₅ ·1.5 H ₂ O: C, 50.43; H, 7.52; N, 10.89%. Found: C, 50.61; H, 7.36; N, 10.79%.	50
55	55	EXAMPLE 11	55
60	60	Isolation of Aminocyclitol - Aminoglycoside Transition Metal Salt Complexes	60
		A. Isolation of Gentamicin B-dicupric acetate Complex	
		Add cupric acetate hydrate (0.4 gms, 2 mmol) to a solution of gentamicin B (0.482 gms, 1 mmol) in methanol (25 ml), stir for 30 minutes at room temperature then evaporate the solvent <i>in vacuo</i> and dry the resultant residue at 60°C <i>in vacuo</i> to obtain gentamicin B-dicupric acetate complex as a deep blue coloured solid (in contrast to green coloured cupric acetate dihydrate); yield 0.8 gms; λ_{max}^{Nujol} 6.42, 8.72, 13.90 μ . ("Nujol" is a Registered Trade Mark)	

B. Isolation of Gentamicin B-dicobalt (II) Acetate Complex

(1) Dissolve gentamicin B (0.482 gms, 1 mmol) in dimethylsulfoxide (20 ml) and add cobalt (II) acetate tetrahydrate (0.598 gms, 2 mmol). Stir for 30 minutes at room temperature and then pour into ether (30 ml). Decant the ether layer and triturate the residue with fresh ether (20 ml). Repeat this process once more, then triturate with ethyl ether. Isolate the resultant solid by filtration, wash with ethyl ether and dry to obtain gentamicin B-dicobalt (II) acetate complex as a lavender colored solid (in contrast to pink colored cobalt (II) acetate tetrahydrate), yield 0.8 gms. $\lambda_{\text{Max}}^{\text{NaJol}}$ 5.88, 6.36, 10.59, 12.42, 12.92, 13.90 μ .

(2) Dissolve the above isolated gentamicin B-dicobalt (II) acetate complex in dimethylsulfoxide and treat with *N*-(benzyloxycarbonyloxy)succinimide in a manner similar to that described in Example 8B followed by isolation and purification in the described manner to obtain 3,6' - di - *N* - benzyloxycarbonylgentamicin B.

C. In a manner similar to that described in Example 11A and 11B, treat gentamicin B with nickel (II) acetate tetrahydrate and with cadmium (II) acetate dihydrate and isolate and a purify the complexes thereby formed to obtain gentamicin B-dinickel (II) acetate complex and gentamicin B-dicadmium (II) acetate complex.

In similar manner each of the aminocyclitolaminoglycoside transition metal salt complexes prepared *in situ* in the preceding Examples may be isolated.

EXAMPLE 12

Reaction of 1,3 - Di - De - *N* - Amidinodihydrostreptomycin with *N* - benzyloxycarbonyloxyphthalimide with and without cupric Acetate Complex

A. 1,3 - Di - De - *N* - Amidinohydrostreptomycin

Add a solution of dihydrostreptomycin sulfate (25 gms, 17.1 mmol) in water (100 ml) to a solution of barium hydroxide (54 gms) dissolved in water (600 ml). Separate the barium sulfate thereby formed by filtration and heat the filtrate at reflux temperature under an atmosphere of nitrogen for 62 hours. To the cooled reaction mixture add 4 N sulfuric acid dropwise until the reaction mixture reaches a pH of 7. Separate the barium sulfate thereby formed by filtration, add Amberlite IRA-401S (OH⁻) resin to the filtrate and heat the mixture until the filtrate reaches a pH of 10. Remove the water by distillation and chromatograph the resultant residue on silica gel (600 gms) eluting with a solvent mixture of chloroform/methanol/28% ammonium hydroxide (3:4:2). Collect the like fractions containing 1,3 - di - de - *N* - amidinodihydrostreptomycin as determined by thin layer chromatography and evaporate the combined eluates to a residue of 1,3 - di - de - *N* - amidinohydrostreptomycin (yield—10.9 gms).

B. Reaction of 1,3 - Di - De - *N* - Amidinodihydrostreptomycin Via Cupric Acetate Complex with *N* - Benzyloxycarbonyloxyphthalimide to Produce 2'' - *N* - Benzyloxycarbonyl - 1,3 - di - de - *N* - Amidinodihydrostreptomycin

To a solution of 1,3 - di - de - *N* - amidinodihydrostreptomycin (5 gms, 10 mmol) in dimethylsulfoxide (200 ml) add cupric acetate monohydrate (2 gms, 10 mmol) and stir at room temperature for 15 minutes, then to the cupric salt complex thereby formed add dropwise with continuous stirring of the reaction mixture *N* - benzyloxycarbonyloxyphthalimide (5.5 gms) in dimethylsulfoxide (20 ml). Continue stirring for 2 hours, then pour the reaction mixture into diethyl ether (2 liters). Decant the supernatant ether and repeat the foregoing process using 1 liter and 500 ml of diethyl ether, respectively. Dissolve the resultant gummy residue in a solvent mixture comprising methanol (150 ml) and 28% ammonium hydroxide (10 ml). Bubble hydrogen sulfide through the solution and separate the resultant precipitate of cupric sulfide by filtration through a pad of Celite, evaporate the filtrate and chromatograph the resultant residue on silica gel (400 gms) eluting with a solvent mixture of chloroform/methanol/28% ammonium hydroxide (2:1:0.35). Combine the like eluates containing the desired compound as determined by thin layer chromatography and concentrate the combined eluates to a residue comprising 2'' - *N* - benzyloxycarbonyl - 1,3 - di - de - *N* - amidinohydrostreptomycin, yield 6 gms. (98% theory); $[\alpha]_D^{25}$ -96.5° (c 0.5 in water). pmr (ppm) (D₂O): δ 1.21 (-d, 4'-C-CH₃, J=6 Hz); 3.04 (s, 2''-N-CH₃); 5.13 (s, C₆H₅CH₂ OCONH); 7.41 (s,

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$C_6H_5CH_2OCONH$); Analysis calculated for: $C_{27}H_{43}O_{14}N_3 \cdot H_2O$; Calc: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.69; H, 6.78; N, 6.27. CD: $[\theta]_{285}^{25} = +7,320$.

C. Reaction of 1,3-Di-De-*N*- Amidinodihydrostreptomycin with *N*-Benzyloxycarbonyloxypthalimide without Cupric Acetate Complex whereby is produced 1-*N*-Benzyloxycarbonyl-1,3-Di-De-*N*-Amidinodihydrostreptomycin

To a solution of 1,3-di-de-*N*-amidinodihydrostreptomycin (1 gm, 2.0 mmol) in *N,N*-dimethylformamide (40 ml) add a solution of *N*-benzyloxycarbonyloxypthalimide (0.8 gms, 2.7 mmol) in *N,N*-dimethylformamide (4 ml). Stir the reaction mixture for 1 hour, then add water (10 ml) and Amberlite IRA-401S (OH⁻) resin until the pH of the reaction mixture reaches 8.5. Separate the resin by filtration, remove the solvents by distillation, then chromatograph the resultant residue on silica gel (100 gms) eluting with a solvent mixture of chloroform/methanol/28% ammonium hydroxide (2:1:0.35). Collect the like eluates containing 1-*N*-benzyloxycarbonyl-1,3-di-de-*N*-amidinodihydrostreptomycin as determined by thin layer chromatography and evaporate the combined eluates to a residue of 1-*N*-benzyloxycarbonyl-1,3-di-de-*N*-amidinodihydrostreptomycin, yield 0.417 gms, (33% theory); $[\alpha]_D^{25} -93.6^\circ$ (c 0.55% in water). pmr (ppm) (D_2O): δ 1.22 (d, 4'-C-CH₃, J=6 Hz); 2.42 (s, 2"-N-CH₃); 5.12 (s, $C_6H_5CH_2OCONH$); 7.40 (s, $C_6H_5CH_2OCONH$); Analysis calculated for: $C_{27}H_{43}O_{14}N_3 \cdot H_2O$; Calc.: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.41; H, 6.55; N, 6.21. CD: $[\theta]_{282}^{25} = +11,100$.

EXAMPLE 13

Conversion of 3,2',6'-Tri-*N*-acetylisisomicin to 1-*N*-Ethylisisomicin

A. 3,2',6'-Tri-*N*-Acetyl-1-*N*-Ethylisisomicin

Add 0.1 N hydrochloric acid to a stirred solution of 3,2',6'-tri-*N*-acetylisisomicin (1.146 gms, 2 mmol) in 20 ml of water so as to bring the pH of the solution to 2.7. Cool the reaction mixture to 3°C and add 2.2 ml of a 1 molar solution of acetaldehyde in tetrahydrofuran (2.2 mmol), then add dropwise a solution of sodium cyanoborohydride (0.16 gms, 2.6 mmol) in 2 ml of water. Stir the reaction mixture for one hour maintaining the temperature below 5°C and the pH at about 2.7 by the addition of 0.1 N hydrochloric acid, then add 0.44 ml of a 1 molar solution of acetaldehyde in tetrahydrofuran (0.44 mmol) followed by a solution of sodium cyanoborohydride (35 mg, 0.56 mmol) in a few drops of water, maintaining the pH at 2.7 by the addition of 0.1 N hydrochloric acid. Continue stirring the reaction mixture for one hour and repeat 2 times the foregoing addition of 0.22 ml of acetaldehyde solution and 10 mg of sodium cyanoborohydride. Continue stirring at room temperature for 18 hours, then bring the solution to a pH of 9 by stirring the solution with Amberlite IRA-401S ion exchange resin in the hydroxide form. Remove the resin by filtration, wash with water and concentrate the combined filtrate and washings *in vacuo*, and chromatograph the resultant residue on 100 gms of silica gel (60-200 mesh) eluting with chloroform:methanol:ammonium hydroxide (30:10:0.2). Combine the like fractions as determined by thin layer chromatography on silica gel or using the same solvent system as above but in the proportion of 30:10:1 and concentrate *in vacuo* the combined eluates containing the major product and lyophilize the resultant aqueous mixture to a residue comprising 3,2',6'-tri-*N*-acetyl-1-*N*-ethylisisomicin, yield 0.84 gms (70% theory); $[\alpha]_D^{25} +165^\circ$ (c, 0.8 in water); pmr (ppm) (D_2O): δ 1.05 (CH₂CH₃, J=7.0 Hz); 1.17 (4"-C-CH₃); 1.84, 1.9, 1.91 (N-acetyls); 2.52 (3"-N-CH₃); 4.80 (H-4'); 4.92 (H-1", J_{1",2"}=4.0 Hz); 5.4 (H-1', J_{1',2'}=2.5 Hz); mass spectrum: (M⁺) m/e 601, (M+1)+m/e 602; also m/e 402, 392, 374, 160; 471, 453, 443, 425; 211; and 261. Analysis calculated for $C_{27}H_{43}N_3O_{10} \cdot 1.5 H_2O$: C, 51.58; H, 8.02; N, 11.14%. Found: C, 51.47; H, 8.89; N, 10.91%.

B. 1-*N*-Ethylisisomicin

Add 3,2',6'-tri-*N*-acetyl-1-*N*-ethylisisomicin (0.1 gm) to 10 ml of 1 N sodium hydroxide and heat the solution under an atmosphere of nitrogen at reflux temperature until thin layer chromatographic analysis of an aliquot on silica gel using a 2:1:0.35 mixture of chloroform:methanol:ammonium hydroxide as the developing system indicates essential completion of de-*N*-acetylation (about 48 hours). Cool the reaction solution, add water until the volume is at about 50 ml, stir with Amberlite IRC-50 ion exchange resin in the proton form (which has been

5 washed with water) until the pH is at about 5.5. Remove the resin by filtration, wash with water and stir the resin with 100 ml of 7% aqueous ammonium hydroxide for about 30 minutes, decant the supernatant solution, and repeat the foregoing procedure twice using 50 ml of 7% aqueous ammonium hydroxide each time then remove the resin by filtration. Combine the ammonium hydroxide filtrate and decantations, concentrate *in vacuo* and extract the resultant residue with methanol. Combine the methanol extracts and concentrate *in vacuo*, dissolve the resultant residue in a solvent mixture consisting of chloroform:methanol:ammonium hydroxide (2:1:0.35) and pass the solution through a column of alumina (8 gms, 0.8 cm x 30 cm column), eluting with the same chloroform:methanol:ammonium hydroxide solvent mixture. Combine the like eluates as determined by thin layer chromatography and evaporate the combined eluates containing 1 - *N* - ethylsisomicin and lyophilize the resultant aqueous mixture to a residue comprising 1 - *N* - ethylsisomicin (71 mg) (90% yield).

15 C. Conversion of Sisomicin to 1 - *N* - Ethylsisomicin without Purification of the Intermediates

(1) 3,2',6' - Tri - *N* - Acetylsisomicin

20 Add cupric acetate hydrate (3 gms, 15 mmol) to a stirred solution of sisomicin (0.447 gms, 1 mmol) in water (5.33 ml) and dimethylformamide (18 ml). Stir at room temperature for 30 minutes, then add dropwise at the rate of about a drop every three seconds 3.1 ml of a 1 molar solution of acetic anhydride in dimethylformamide (3.1 mmol). Stir an additional 30 minutes, then add 10 ml of water and bubble hydrogen sulfide through the solution. Stir the reaction mixture for 30 minutes, then filter the precipitated cupric sulfide through a pad of Celite, wash the resultant cupric sulfide residue with water, and bring the combined filtrate to pH 9 by stirring with Amberlite IRA-401S ion exchange resin in the hydroxide cycle. Remove the resin by filtration, wash with water and concentrate the combined filtrate and washings to a residue comprising 3,2',6' - tri - *N* - acetylsisomicin.

30 (2) 3,2',6' - Tri - *N* - Acetyl - 1 - *N* - Ethylsisomicin

Dissolve the product of Example 13C (1) in 10 ml of water and add 0.1 N hydrochloric acid until the solution is at a pH of about 2.7. Cool the reaction mixture to about 3°C and add 0.73 ml of 1 molar solution of acetaldehyde in tetrahydrofuran (0.73 mmol), then add dropwise a solution of 0.55 gms of sodium cyanoborohydride in 0.7 ml of water. Stir for one hour maintaining the pH of the solution at about 2.7 by the addition of 0.1 N hydrochloric acid, then add 0.15 ml of a 1 molar solution of acetaldehyde in tetrahydrofuran (0.15 mmol) followed by a solution of 12 mg of sodium cyanoborohydride in a few drops of water, again maintaining the pH at about 2.7. Repeat the foregoing procedure twice using each time 0.7 ml of a 1 molar solution of acetaldehyde in tetrahydrofuran and 3 mg of sodium cyanoborohydride. Stir at room temperature overnight, then bring the solution to a pH of about 9 with 1 N sodium hydroxide. Concentrate the solution *in vacuo* to a residue comprising 3,2',6' - tri - *N* - acetyl - 1 - *N* - ethylsisomicin.

45 (3) 1 - *N* - Ethylsisomicin

50 Dissolve the 3,2',6' - tri - *N* - acetyl - 1 - *N* - ethylsisomicin obtained in Example 13C(2) in 60 ml of 1 N sodium hydroxide and heat at reflux temperature under an atmosphere of nitrogen for 48 hours. Cool and bring the reaction mixture to a pH of about 5.5 by adding Amberlite IRC-50 ion exchange resin in the proton form. Remove the resin by filtration and wash with water. Stir the resin with 100 ml of 7% aqueous ammonium hydroxide solution for 30 minutes, decant the solution and repeat the foregoing procedure twice using 100 ml of 7% ammonium hydroxide each time, then filter. Combine the ammonium hydroxide filtrate with the decantations and evaporate *in vacuo*, and chromatograph the resultant residue on a column of silica gel (25 gms) eluting with a solvent mixture of chloroform:methanol:ammonium hydroxide (2:1:0.34). Combine the like fractions as determined by thin layer chromatography on silica gel using the same solvent system and concentrate *in vacuo* the combined eluates containing 1 - *N* - ethylsisomicin and lyophilize the resultant aqueous mixture to a residue of 1 - *N* - ethylsisomicin, yield, 0.233 gms (49% theory).

EXAMPLE 14

Conversion of 3,2',6' - Tri - N - Acetylaminoglycosides to 1 - N - Ethylaminoglycosides

A. 3,2',6' - Tri - N - Acetyl - 1 - N - Ethylverdamicin

Dissolve 3,2',6' - tri - N - acetylverdamicin (880 mg, 1.5 mmol) in water (15 ml) and adjust the pH to 2.7 using 0.1 N hydrochloric acid (approximately 28 ml). Cool in an ice bath to 8-10°C, then while stirring add aqueous acetaldehyde (4.1 ml of 0.73 molar acetaldehyde) in water (2 equivalents). Stir for 5 minutes at 8°C, then add dropwise a solution of sodium cyanoborohydride (182 mg, 3 mmol, 2 equivalents) in 2.5 ml of water, adjusting the pH of the solution to 2.7 with 0.1 N hydrochloric acid. Continue maintaining the pH at about 2.7 by addition of 0.1 N hydrochloric acid until the reaction is complete as determined by thin layer chromatography. Allow the reaction mixture to stand at room temperature for 18 hours, then freeze dry the reaction mixture and chromatograph the resultant residue on silica gel (90 gm) in a 1.5x126 cm column eluting with chloroform:methanol:ammonium hydroxide (28%) (30:10:1). Combine the like fractions containing 3,2',6' - tri - N - acetyl - 1 - N - ethylverdamicin as determined by thin layer chromatography. Evaporate the combined eluates *in vacuo* to a residue of 3,2',6' - tri - N - acetyl - 1 - N - ethylverdamicin, yield 550 mg (0.84 mmol, 56% theory); mass spectrum: m/e 485, 467, 457, 439; 420, 402, 392, 374; 261, 243, 233, 215, 183.

B. 1 - N - Ethylverdamicin

Treat 3,2',6' - tri - N - acetyl - 1 - N - ethylverdamicin (200 mg, 0.304 mmol) with 1 N sodium hydroxide (20 ml) at reflux temperature under an atmosphere of nitrogen, then isolate and purify the resulting product in a manner similar to that described in Example 13B to obtain 1 - N - ethylverdamicin, yield 88 mg (59% theory).

EXAMPLE 15

Conversion of 3,6'-Di-N-substituted Aminoglycosides to the corresponding 3,6'-Di-N-unsubstituted-1-N-Alkylaminoglycosides

Conversion of 3,6' - Di - N - Tert. - Butoxycarbonylgentamicin B to 1 - N - Ethylgentamicin B

To a stirred solution of 3,6' - di - N - tert. - butoxycarbonylgentamicin B (1.36 gms) in water (15 ml) add a 1 molar solution of acetaldehyde in tetrahydrofuran (2 ml). Adjust the pH of the solution to about 4.9 with 0.1 N hydrochloric acid. Add sodium cyanoborohydride (0.2 gm) and periodically adjust the pH to about 4.9. Stir for 5 hours at room temperature, then evaporate the solution to dryness, add trifluoroacetic acid (10 ml) to the resultant residue and let stand for 5 minutes. Pour the reaction solution into ether, isolate the resultant precipitate by filtration and wash with ether. Chromatograph the precipitate on 100 gm of silica gel using chloroform:methanol:ammonium hydroxide (3:4:2) as the solvent mixture. Combine the like fractions containing 1 - N - ethylgentamicin B as determined by thin layer chromatography, evaporate *in vacuo*, dissolve the resultant residue in water and lyophilize; yield 40% theory; $[\alpha]_D^{25} + 126.2^\circ$ (c, 1 in water); pmr (ppm) (D₂O): 85.55 (H-1', J_{1',2'} = 3.0 Hz); 5.05 (H-1'', J_{1'',2''} = 4 Hz); 2.9 (3''-N-CH₃); 1.05-1.5 (2 C-CH₃); mass spectrum: [M+1]⁺ 511; also m/e 380, 352, 334; 378, 350, 332; 219, 191, 173. Analysis calculated for: C₂₁H₄₂O₁₀N₄·2CO₂·3H₂O; C, 42.33; H, 7.41; N, 8.58%. Found: C, 42.37; H, 7.43; N, 8.81%.

EXAMPLE 16

Conversion of 3,6'-Di-N-Tert-Butoxycarbonylgentamicin B to 1-N-(p-Amino-α-Hydroxybutyryl) Gentamicin B

A. 1-N-(S-p-amino-α-hydroxybutyryl)gentamicin B

(1) To a solution of 68.2 gm (0.1 mmol) of 3,6' - di - N - tert. - butoxycarbonylgentamicin B (purified product of Example 8F) in water (1 ml), add methanol (1 ml), then add dropwise with stirring a solution (0.25 ml) of N - (S - p - benzyloxycarbonylamino - α - hydroxybutyryloxy) succinimide (0.1 mmol) in dimethylformamide (0.4 molar solution). Stir for 1 hour, then add additional N - (S - p - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide (0.125 ml). Continue stirring the reaction mixture at room temperature for 18 hours, then concentrate *in vacuo* and dissolve the resultant residue in methanol (4 ml) and water

(16 ml). Add 5% palladium-on-carbon catalyst (0.05 gm) and hydrogenate at 4 atmospheres overnight. Remove the solids by filtration through a pad of Celite, wash the Celite pad with water and concentrate the combined filtrates and washings to a small volume and lyophilize. Dissolve the resultant residue in trifluoroacetic acid (0.5 ml) and set aside for 3 minutes. Pour the solution into excess ether, collect the precipitate by filtration, wash with ether and dry to give 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B as the trifluoroacetate salt. Obtain the free base by dissolving the foregoing salt in water and stirring the solution with Amberlite IRA-401S (OH⁻) ion exchange resin. Filter and lyophilize the filtrate to obtain 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B, yield 49.7 mg (83% theory).

(2) From Crude 3,6' - Di - N - Tert. - Butoxycarbonylgentamicin B

To the 50 ml solution comprising 3,6' - di - N - tert - butoxycarbonylgentamicin B prepared in Example 8F, add 50 ml of methanol. To this solution add dropwise with stirring 10 ml of a 1 molar solution of 1 - N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide in dimethylformamide. Stir for 1 hour and monitor the reaction by thin layer chromatography using chloroform:methanol:ammonium hydroxide (2:1:0.35). Add an additional 5 ml of the 1 molar solution of 1 - N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide and stir the reaction mixture for 10 hours. Concentrate the solution *in vacuo* and chromatograph the resultant residue on 300 gm of silica gel using chloroform:methanol:ammonium hydroxide (30:10:0.5) as the eluting solvent. Combine the like fractions containing the 1 - N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - tert - butoxycarbonylgentamicin B and evaporate to a residue. Dissolve the residue in water (50 ml) and methanol (18 ml) and hydrogenate in the presence of 5% palladium-on-carbon (600 mg) at 4 atmospheres for 10 hours. Remove the catalyst by filtration through a pad of Celite and wash the residue with water. Combine the filtrate and washings and evaporate *in vacuo* to remove the methanol. Wash the resulting aqueous layer 3 times with chloroform (25 ml) and concentrate the aqueous solution *in vacuo* and dry the resultant residue over phosphorous pentoxide *in vacuo*. Dissolve the resultant residue in a minimum amount of trifluoroacetic acid and set the solution aside for 3 minutes. Add ether and isolate the resultant precipitate by filtration. Wash the precipitate with ether and dry. Dissolve the solid in 30 ml of water and bring the solution to about pH 8.5 by stirring with Amberlite IRA-401S (OH⁻) ion exchange resin. Remove the solid by filtration, wash with water, combine the filtrate and washings and lyophilize to give 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B.

Alternatively, add the sulfuric acid to bring the foregoing aqueous solution to about pH 4.5 and lyophilize to obtain the sulfate salt of 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B; pmr (ppm) (D₂O): δ 1.3 (4" - C - CH₃); 2.9 (3" - N - CH₃); 4.28 (H-2", J=9 Hz and 4 Hz); 5.16 (H-1", J_{1,2}"=3.5 Hz); 6.12 (H-1', J_{1,2}'=3.0 Hz).

B. 1 - N - (R - γ - Amino - α - hydroxybutyryl)gentamicin B

(1) To a solution of 3,6' - di - N - tert. - butoxycarbonylgentamicin B (3.41 gms, 5 mmol) in methanol (25 ml) and water (25 ml) add with stirring a solution of N - (R - γ - N - benzyloxycarbonylamino - α - hydroxybutyryloxy) succinimide (1.8 gms, 5 mmol) in dimethylformamide (10 ml). Stir the reaction mixture for 6 hours, concentrate *in vacuo* and chromatograph the resultant residue on silica gel (60-200 mesh, 300 gms) using a solvent system comprising chloroform:methanol:ammonium hydroxide (30:10:0.25). Combine the like fractions containing the desired product as determined by thin layer chromatography, concentrate *in vacuo* and rechromatograph the residue on the same column using the same solvent system. Combine the like rechromatographed fractions and evaporate *in vacuo* to a residue comprising 1 - N - (R - γ - N - benzyloxycarbonylamino - α - hydroxybutyryloxy) - 3,6' - di - N - tert - butoxycarbonylgentamicin B.

(2) Dissolve the product of Example 16B(1) in 50% aqueous methanol (20 ml) and hydrogenate over 5% palladium-on-carbon catalyst (0.2 gms) at room temperature and 4 atmospheres for 24 hours. Remove the catalyst by filtration through a pad of Celite, wash the water and evaporate the combined filtrates *in*

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vacuo. Dissolve the resultant residue in trifluoroacetic acid (25 ml) and set aside for 3 minutes. Add ether and isolate the resultant precipitate comprising the trifluoroacetic acid addition salt of 1 - *N* - (*R* - γ - amino - α - hydroxybutyryl)gentamicin B, wash with ether and dry. Dissolve the dry precipitate in water (10 ml) and stir with Amberlite IRA-401S (OH⁻) ion exchange resin, sufficient to place the pH to 9.5. Remove the resin by filtration, wash with water and lyophilize the combined filtrates to a residue comprising pure 1 - *N* - (*R* - γ - amino - α - hydroxybutyryl)gentamicin B, yield 0.6 gms; $[\alpha]_D^{25} + 118.1^\circ$ (c 1 in water) pmr (ppm) (D₂O): 81.23 (4''-C-CH₃); 2.66 (3''-N-CH₃); 4.2 (H-2''', J=5 Hz and 3 Hz); 5.03 (H-1'', J=4 Hz); 5.4 (H-1', J=3.5 Hz). Analysis calculated for: C₂₃H₄₅O₁₂N₅·3CO₂·4H₂O; C, 39.64; H, 6.78; N, 8.89%. Found: C, 39.88; H, 6.58; N, 9.28%.

EXAMPLE 17

Conversion of 3,6' - Di - *N* - *Tert* -
Butoxycarbonylgentamicin B to 1 - *N* - (*S* - β - Amino - α -
Hydroxypropionyl)gentamicin B

A. 1 - *N* - (*S* - β - *N* - Benzyloxycarbonylamino - α - Hydroxypropionyl) - 3,6' -
Di - *N* - *Tert* - Butoxycarbonylgentamicin B

Add to a stirring solution of 3,6' - di - *N* - *tert* - butoxycarbonylgentamicin B (11.28 g, 16.4 mmol) in methanol (100 ml) and water (100 ml), a solution of *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide (10 g, 30 mmol) in 40 ml of *N,N* - dimethylformamide over a fifteen minute period. After a period of two hours, evaporate off the solvents *in vacuo* at 50°C. Chromatograph the resultant residue on 500 g of silica gel using a 30:10:0.5 ratio of chloroform:methanol:concentrated ammonium hydroxide to obtain 13.22 g of 1 - *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyl) - 3,6' - di - *N* - *tert* - butoxycarbonylgentamicin B.

B. 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B

Hydrogenate the product of Example 17A at 4 atmospheres at room temperature in a mixture of water (730 ml) and methanol (240 ml) in the presence of 0.7 g, 5% palladium-on-carbon catalyst for 24 hours. Remove the catalyst by filtration through a pad of Celite and wash with water. Concentrate the combined filtrates and dry the residue thoroughly. Dissolve the product in trifluoroacetic acid (80 ml) and set aside for 3 minutes. Pour the solution into excess ether (2 l) to precipitate the trifluoroacetic acid salt of pure 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B. Isolate by filtration, wash with ether and dry. Treat the product with Amberlite IRA-401S (OH⁻) resin in water to pH 9, filter the solution, wash the resin and lyophilize the combined solution to give 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B; $[\alpha]_D^{25} + 112.5^\circ$ (c 0.4 in water); pmr (ppm) (D₂O): 81.15 (4''-C-CH₃); 1.36 (H-2ax, J_{1,2}=J_{2,3}=J_{ax,eq}=12.5 Hz); 1.90 (H-2eq); 2.45 (3''-N-CH₃); 4.06 (H-5'' eq, J_{5''} eq, ax=13.0 Hz); 4.13 (H-2''', J_{2'''}, 3'''=4.0 and 7.0 Hz); 5.05 (H-1'', J_{1''}, 2''=4.0 Hz); 5.32 (H-1', J_{1'}, 3, 3'=3.0 Hz). Yield=near quantitative.

C. Carry out the procedures of Examples 17A and 17B utilizing *N* - (*S* - δ - benzyloxycarbonylamino - α - hydroxyvaleryloxy)succinimide instead of the succinimide reagent specified in Example 17A, whereby is obtained 1 - *N* - (*S* - δ - amino - α - hydroxyvaleryl)gentamicin B.

EXAMPLE 18

Conversion of 3,6' - Di - *N* - Benzyloxycarbonyl-
aminoglycosides to 1 - *N* - (*S* - γ - Amino - α -
Hydroxybutyryl)Aminoglycosides

A. Preparation of 1 - *N* - (*S* - γ - Amino - α - Hydroxybutyryl)kanamycin A

(1) To a stirred solution of 3,6' - di - *N* - benzyloxycarbonylkanamycin A (1.54 gms, 2 mmol) in tetrahydrofuran (20 ml) and water (20 ml) add dropwise a solution of *N* - (*S* - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide (1.163 gms, 3 mmol) in 2 ml of *N,N* - dimethylformamide. Stir for two hours, then concentrate *in vacuo* and chromatograph the resultant syrupy residue on silica gel (150 gms), eluting with chloroform:methanol:concentrated ammonium hydroxide (2:1:0.35) solvent mixture. Combine the like fractions containing the desired product as determined

by thin layer chromatography and evaporate the combined eluates *in vacuo* to a residue comprising 1 - *N* - (*S* - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - *N* - benzyloxycarbonylkanamycin A; yield 1.3 gm.

(2) Hydrogenate the product of Example 18A (1) in aqueous methanol (1:1) (20 ml) in the presence of 5% palladium-on-charcoal (0.10 gms) for 3 hours. Separate the catalyst by filtration and evaporate the resultant filtrate *in vacuo* to a residue comprising 1 - *N* - (*S* - γ - amino - α - hydroxybutyryl)kanamycin A in quantitative yields.

B. In the procedure of Example 18A, substitute *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide for *N* - (*S* - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide to obtain 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)kanamycin A in an overall yield of greater than 50% theory.

EXAMPLE 19

Conversion of 3,6'-Di-*N*-Benzyloxycarbonyl-gentamicin B to 1-*N*-(*S*- β -Amino- α -Hydroxypropionyl)gentamicin B and to 1-*N*-(*R*- β -Amino- α -Hydroxypropionyl)gentamicin B

A. 1 - *N* - (*S* - β - Amino - α - Hydroxypropionyl)gentamicin B

To a solution of 3,6' - di - *N* - benzyloxycarbonylgentamicin B (3.75 gms, 5 mmol) in methanol (25 ml) and water (25 ml), add with stirring a solution of *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide (2.18 gms, 6.5 mmol) in dimethylformamide (12 ml). Stir the reaction mixture for 2 hours, concentrate *in vacuo*, and dissolve the resultant residue in water (75 ml) and methanol (75 ml). Hydrogenate in the presence of 5% palladium on charcoal (5 gms) at 4 atmospheres for 24 hours. Remove the catalyst by filtration through a pad of Celite and wash with water. Concentrate the combined filtrate and washings and chromatograph the resultant residue on Dowex-1 $\times$ 2 ion exchange resin (200 gm, 200-400 mesh) in the hydroxide form eluting with water. Combine the like fractions containing 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B as determined by thin layer chromatography on silica gel using chloroform:methanol:ammonium hydroxide (3:4:2) as the solvent system. Concentrate to a small volume, dilute with water and lyophilize to obtain 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B, yield 1.7 gms. (60% theory). ("Dowex" is a Registered Trade Mark).

B. 1-*N*-(*R*- β -Amino- α -Hydroxypropionyl)gentamicin B

In a manner similar to that described in Example 19A treat 3,6' - di - *N* - benzyloxycarbonylgentamicin B with 1 - *N* - (*R* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide and isolate and purify the resultant product in a manner similar to that described to obtain 1 - *N* - (*R* - β - amino - α - hydroxypropionyl)gentamicin B.

C. Conversion of 3,6' - Di - *N* - Benzyloxycarbonylgentamicin B to 1 - *N* - (*S* - β - Amino - α - Hydroxypropionyl)gentamicin B and 1 - *N* - (*R* - β - Amino - α - Hydroxypropionyl)gentamicin B using Racemic β - *N* - Benzyloxycarbonylamino - α - Hydroxypropionic Acid

(1) To a stirred solution of 3,6' - di - *N* - benzyloxycarbonylgentamicin B (1 gm, 1.33 mmol) in methanol (5 ml) and water (5 ml) add a solution of racemic *N* - (β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide (0.674 gms, 0.2 mmol) in dimethylformamide (2 ml). Stir for 2 hours, then evaporate the solvent *in vacuo*. Chromatograph the resultant residue on silica gel (60-100 mesh, 100 gms) eluting with a mixture of chloroform:methanol:ammonium hydroxide (30:10:1). Monitor the fractions by thin layer chromatography on silica gel using chloroform:methanol:ammonium hydroxide (2:1:0.35) as solvent system. Combine the like fractions containing 1 - *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyl) - 3,6' - di - *N* - benzyloxycarbonylgentamicin B, evaporate *in vacuo*, add water to the resultant residue and lyophilize from water, yield 0.42 gms (65% theory). Similarly, combine the like fractions containing 1 - *N* - (*R* - β - benzyloxycarbonylamino - α - hydroxypropionyl)gentamicin B, evaporate the solvents *in vacuo*, add water and lyophilize, yield 0.36 gms (55.8 % theory).

(2) Hydrogenate each of the two fractions separately in methanol (5 ml) and water (5 ml) in the presence of 5% palladium on charcoal catalyst at 4 atmospheres

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for 24 hours. In each instance remove the catalyst by filtration through Celite, wash with water and evaporate the combined filtrates to the residue. Add water to the residue and lyophilize to obtain pure 1 - N - (S - β - amino - α - hydroxypropionyl)gentamicin B and 1 - N - (R - β - amino - α - hydroxypropionyl)gentamicin B, respectively, in near quantitative yields.

EXAMPLE 20

Conversion of 3,6'-Di-N-Benzoyloxycarbonylgentamicin B to 1-N-(S-γ-Amino-α-Hydroxybutyryl)gentamicin B and 1-N-(R-γ-Amino-α-Hydroxybutyryl)gentamicin B using Racemic N-γ-Benzoyloxycarbonyl-α-Hydroxybutyric acid

In a manner similar to that described in Example 19C treat 3,6' - di - N - benzyloxycarbonylgentamicin B in aqueous methanol with a solution of racemic N - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide in dimethylformamide, then evaporate the reaction mixture *in vacuo* to a residue comprising 1 - N - (R,S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B. Chromatograph on silica gel in a manner similar to that described in Example 19C and combine the like fractions as determined by thin layer chromatography and evaporate each of the combined fractions *in vacuo* to a residue comprising 1 - N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B and 1 - N - (R - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B, respectively. Hydrogenate each of the two fractions in the presence of 5% palladium-on-charcoal catalyst similar to that described in Example 19C (2) to obtain 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B and 1 - N - (R - γ - amino - α - hydroxybutyryl)gentamicin B, respectively.

WHAT WE CLAIM IS:—

1. Process for producing an organic compound having at least one amino group having an available neighboring hydroxyl group and at least one amino group blocked with an acyl blocking group Y and at least one of said amino groups being either devoid of or sterically less available to, an available neighboring hydroxyl group; which comprises the reaction of said polyamino-organic compound in an inert organic solvent, with a salt of a divalent transition metal cation selected from copper (II), nickel (II), cobalt (II) and cadmium (II) or with mixtures thereof, whereby is formed a complex of said polyamino-organic compound between said transition metal salt and at least one pair of groups consisting of said amino group having an available neighboring hydroxyl group and the said neighbouring hydroxyl group; followed by the reaction of the resulting polyamino-organic compound-transition metal salt complex with an amine blocking reagent having an acyl blocking group Y; then reaction of the resulting polyamino-organic compound-transition metal salt complex having acyl blocking groups, Y, on non-complexed amino groups, with a transition metal precipitating reagent or with ammonium hydroxide, whereby said transition metal cation is removed.

2. Process according to Claim 1, wherein the reaction of the complex with the amine blocking reagent is carried out *in situ*.

3. Process according to Claim 1 or 2, wherein said transition metal precipitating reagent is selected from acetyl-acetone, dioxime of dimethylglyoxal, and hydrogen sulfide.

4. Process according to Claim 1 or 2, wherein said transition metal cation is removed by reaction with hydrogen sulfide or with ammonium hydroxide.

5. Process according to any one of Claims 1 to 4, wherein said divalent transition metal cation is selected from copper (II), nickel (II) and cobalt (II).

6. Process according to any one of Claims 1 to 5, wherein said salt of said divalent transition metal cation is cupric acetate, nickel (II) acetate or cobalt (II) acetate.

7. Process according to any one of Claims 1 to 6, wherein the total molar quantity of said transition metal salt is at least equal to the number of said amino groups having an available neighboring hydroxyl group, and the molar quantity of said acyl blocking reagent is equal to the number of amino groups devoid of a transition metal salt complex and to be selectively blocked.

8. Process according to any one of Claims 1 to 7, wherein said polyamino-organic compound is an amino-cyclitol-aminoglycoside.



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for 24 hours. In each instance remove the catalyst by filtration through Celite, wash with water and evaporate the combined filtrates to the residue. Add water to the residue and lyophilize to obtain pure 1 - N - (S - β - amino - α - hydroxypropionyl)gentamicin B and 1 - N - (R - β - amino - α - hydroxypropionyl)gentamicin B, respectively, in near quantitative yields.

EXAMPLE 20

Conversion of 3,6'-Di-N-Benzyloxycarbonylgentamicin B to
1-N-(S- γ -Amino- α -Hydroxybutyryl)gentamicin B and
1-N-(R- γ -Amino- α -Hydroxybutyryl)gentamicin

B using Racemic N- γ -Benzyloxycarbonyl- α -Hydroxybutyric acid

In a manner similar to that described in Example 19C treat 3,6' - di - N - benzyloxycarbonylgentamicin B in aqueous methanol with a solution of racemic N - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide in dimethylformamide, then evaporate the reaction mixture *in vacuo* to a residue comprising 1 - N - (R,S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B. Chromatograph on silica gel in a manner similar to that described in Example 19C and combine the like fractions as determined by thin layer chromatography and evaporate each of the combined fractions *in vacuo* to a residue comprising 1 - N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B and 1 - N - (R - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - N - benzyloxycarbonylgentamicin B, respectively. Hydrogenate each of the two fractions in the presence of 5% palladium-on-charcoal catalyst similar to that described in Example 19C (2) to obtain 1 - N - (S - γ - amino - α - hydroxybutyryl)gentamicin B and 1 - N - (R - γ - amino - α - hydroxybutyryl)gentamicin B, respectively.

WHAT WE CLAIM IS:—

1. Process for producing an organic compound having at least one amino group having an available neighboring hydroxyl group and at least one amino group blocked with an acyl blocking group Y and at least one of said amino groups being either devoid of or sterically less available to, an available neighboring hydroxyl group; which comprises the reaction of said polyamino-organic compound in an inert organic solvent, with a salt of a divalent transition metal cation selected from copper (II), nickel (II), cobalt (II) and cadmium (II) or with mixtures thereof, whereby is formed a complex of said polyamino-organic compound between said transition metal salt and at least one pair of groups consisting of said amino group having an available neighboring hydroxyl group and the said neighbouring hydroxyl group; followed by the reaction of the resulting polyamino-organic compound-transition metal salt complex with an amine blocking reagent having an acyl blocking group Y; then reaction of the resulting polyamino-organic compound-transition metal salt complex having acyl blocking groups, Y, on non-complexed amino groups, with a transition metal precipitating reagent or with ammonium hydroxide, whereby said transition metal cation is removed.

2. Process according to Claim 1, wherein the reaction of the complex with the amine blocking reagent is carried out *in situ*.

3. Process according to Claim 1 or 2, wherein said transition metal precipitating reagent is selected from acetyl-acetone, dioxime of dimethylglyoxal, and hydrogen sulfide.

4. Process according to Claim 1 or 2, wherein said transition metal cation is removed by reaction with hydrogen sulfide or with ammonium hydroxide.

5. Process according to any one of Claims 1 to 4, wherein said divalent transition metal cation is selected from copper (II), nickel (II) and cobalt (II).

6. Process according to any one of Claims 1 to 5, wherein said salt of said divalent transition metal cation is cupric acetate, nickel (II) acetate or cobalt (II) acetate.

7. Process according to any one of Claims 1 to 6, wherein the total molar quantity of said transition metal salt is at least equal to the number of said amino groups having an available neighboring hydroxyl group, and the molar quantity of said acyl blocking reagent is equal to the number of amino groups devoid of a transition metal salt complex and to be selectively blocked.

8. Process according to any one of Claims 1 to 7, wherein said polyamino-organic compound is an amino-cyclitol-aminoglycoside.



9. Process according to Claim 8, wherein said amino-cyclitol-aminoglycoside is a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol which has available hydroxyl groups neighboring to every amino group except those at the 3,2' and 6' positions; and wherein about three moles of the acylating reagent having an acyl group, Y, are used, whereby is produced the corresponding 3,2',6' - tri - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with Y being as defined in Claim 1.

10. Process according to Claim 9, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is selected from sisomicin, verdamycin, tobramycin, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, the 5 - epi - and 5 - epi - azido - 5 - deoxy analogs of the foregoing; kanamycin B, 3',4' - dideoxykanamycin B, nebramycin factor 4, nebramycin factor 5',3',4' - dideoxy - 3',4' - dehydrokanamycin B, 3',4' - dideoxy - 6' - N - methylkanamycin B and 5 - deoxysisomicin, and wherein the molar quantity of the transition metal salt or mixture thereof is at least two times that of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

11. Process according to Claim 9 or 10, wherein the acylating reagent is acetic anhydride whereby is produced the corresponding 3,2',6' - tri - N - acetyl - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

12. Process according to Claim 11, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is sisomicin whereby is formed 3,2',6' - tri - N - acetylsisomicin.

13. Process according to Claim 9 or 10, wherein the acylating reagent is N - (2,2,2 - trichloroethoxycarbonyloxy)succinimide whereby is formed the corresponding 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl) - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

14. Process according to Claim 13, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is sisomicin whereby is formed 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl)sisomicin.

15. Process according to Claim 8, wherein said aminocyclitol - aminoglycoside is a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol which has available hydroxyl groups neighboring to every amino function except those at the 3 and 6' positions and is selected from gentamicin B, gentamicin B₁, gentamicin A₃, kanamycin A and 6' - N - methylkanamycin A; and wherein the molar quantity of the transition metal salt or mixture thereof is at least two times that of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol, and wherein about two moles of the acylating reagent having an acyl group, Y, are used, whereby is produced the corresponding 3,6' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with Y being as defined in Claim 1.

16. Process according to Claim 15, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is selected from gentamicin B, gentamicin B₁, and kanamycin A, and wherein the acylating reagent is selected from acetic anhydride, N - tert - butoxycarbonyloxypthalimide and N - benzyloxycarbonyloxypthalimide, whereby are formed the corresponding 3,6' - di - N - acetyl, 3,6' - di - N - tert - butoxycarbonyl and 3,6' - di - N - benzyloxycarbonyl derivatives of gentamicin B, gentamicin B₁, or kanamycin A.

17. Process according to Claim 8, wherein said aminocyclitol-aminoglycoside is a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol which has available hydroxyl groups neighboring to every amino function except the 2' and the 3-amino groups, and is selected from kanamycin C, Antibiotic G-418, gentamicins A, A₁, and X₂, and wherein the molar quantity of the transition metal salt or mixture thereof is at least about two times the molar quantity of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol; and wherein about two moles of the acylating reagent having an acyl group, Y, are used, whereby is produced the corresponding 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with Y being as defined in Claim 1.

18. Process according to Claim 8, wherein said aminocyclitol-aminoglycoside is a 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol which has available hydroxyl groups neighboring to every amino function except those at the 3,2' and 6' positions; and wherein the molar quantity of the transition metal salt or mixture thereof is at least about two times that of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol; and wherein about two moles of the acylating reagent having an acyl group, Y, are used, whereby is produced the corresponding 2',6' - di - N - Y -



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4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with Y being as defined in Claim 1.

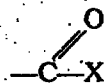
19. A process as claimed in Claim 1, substantially as herein described with particular reference to the Examples.

20. A compound whenever obtained in the process as claimed in any one of Claims 1 to 19.

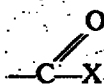
21. 3,2',6' - tri - N - Acetylsisomicin whenever obtained in the process as claimed in any one of Claims 1 to 12.

22. 3,2',6' - tri - N - (2,2,2 - trichloroethoxycarbonyl) sisomicin whenever obtained in the process as claimed in any one of Claims 1 to 10, 13 and 14.

23. Process for the preparation of 1 - N - substituted derivatives of the 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols sisomicin, verdamicin, tobramycin, gentamicin C₁, gentamicin C_{1a}, gentamicin C₂, gentamicin C_{2a}, gentamicin C_{2b}, Antibiotic 66-40B, Antibiotic 66-40D, Antibiotic JI-20A, Antibiotic JI-20B, Antibiotic G-52, of the 5-epi- and 5 - epi - azido - 5 - deoxy analogs of the foregoing; kanamycin B, 3',4' - dideoxykanamycin B, nebramycin factor 4, nebramycin factor 5', 3',4' - dideoxy - 3',4' - dehydrokanamycin B, 3',4' - dideoxy - 6' - N - methylkanamycin B, 5 - deoxysisomicin, gentamicin B, gentamicin B₁, gentamicin A₃, kanamycin A, 6' - N - methylkanamycin A, kanamycin C, Antibiotic G-418, gentamicin A, gentamicin A₁, and gentamicin X₂; wherein the 1 - N - substituent is —CH₂X or



with X being hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, hydroxyalkyl, aminoalkyl, mono- or di - N - alkylaminoalkyl, aminohydroxyalkyl, mono- or di - N - alkylaminohydroxyalkyl, phenyl, benzyl, or tolyl, said aliphatic radicals having up to seven carbon atoms and, if substituted by amino and hydroxy, bearing the substituents on different carbon atoms and of the pharmaceutically acceptable acid addition salts thereof, which comprises the reaction of any of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitols in an inert organic solvent with a salt of a divalent transition metal cation selected from copper (II), nickel (II), cobalt (II) and cadmium (II) or with mixtures thereof, whereby is formed a complex of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol between said transition metal salt and pairs of amino and hydroxy groups; followed by the reaction *in situ* of the resulting complex with an amine blocking reagent having an acyl blocking group Y; then reaction of the resulting complex having acyl blocking groups, Y, on non-complexed amino groups, with a transition metal precipitating reagent or with ammonium hydroxide, whereby said transition metal cation is removed and a 3,2',6' - tri - N - Y - or a 3,6' - di - N - Y - or a 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is obtained; followed by the introduction of the 1 - N - substituent —CH₂X or



wherein X is as defined above; and then removal of all amino blocking groups present in the molecule and isolating the 1 - N - substituted derivatives as such or as a pharmaceutically acceptable acid addition salt.

24. Process according to Claim 23, wherein said transition metal cation is removed by reaction with hydrogen sulfide or with ammonium hydroxide.

25. Process according to Claim 23 or 24, wherein said salt of said divalent transition metal cation is cupric acetate, nickel (II) acetate, or cobalt (II) acetate.

26. Process according to any one of Claims 23 to 25, wherein the molar quantity of the transition metal salt or mixture thereof is at least two times that of said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

27. Process according to any one of Claims 23 to 26, wherein introduction of the 1 - N - substituent —CH₂X comprises the reaction of an acid addition salt of the 3,2',6' - tri - N - Y - or the 3,6' - di - N - Y - or the 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol with about one equivalent of a hydride-donor reducing reagent selected from the group consisting of dialkylaminoborane, tetraalkylammonium cyanoborohydride, alkali metal



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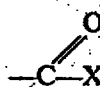
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cyanoborohydride and alkali metal borohydride in an inert solvent and with at least one equivalent of an aldehyde having the formula $XCHO$ wherein X is as defined in claim 23.

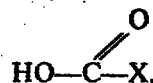
28. Process according to any one of Claims 23 to 27, wherein said amine blocking reagent is acetic anhydride, the aldehyde of the formula $XCHO$ is acetaldehyde, the reducing agent is sodium cyanoborohydride, and the protecting groups in the thereby formed 1 - N - ethyl - 3,2',6' - tri - N - acetyl- or 1 - N - ethyl - 3,6' - di - N - acetyl- or 1 - N - ethyl - 3,2' - di - N - acetyl - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol are removed by treatment with base whereby is obtained the corresponding 1 - N - ethyl - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol.

29. Process according to Claim 28, wherein the 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is sisomicin whereby is obtained 1 - N - ethylsisomicin.

30. Process according to any one of Claims 23 to 26, wherein introduction of the 1 - N - substituent

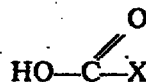


comprises the reaction of the 3,2',6' - tri - N - Y - or the 3,6' - di - N - Y - or the 3,2' - di - N - Y - 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol wherein Y is a blocking group removable without affecting the 1 - N - acyl substituent, with an acid of the formula



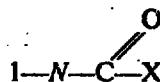
wherein X is as defined in Claim 23 with any primary or secondary amino group being protected, in the presence of a carbodiimide, or with a reactive derivative of said acid.

31. Process according to Claim 30, wherein the 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is selected from kanamycin A, gentamicin B and gentamicin B₁; Y is *tert.* - butoxycarbonyl or benzyloxycarbonyl, and the acyl moiety of the acid



is β - amino - α - hydroxypropionyl, γ - amino - α - hydroxybutyryl or δ - amino - α - hydroxyvaleryl the amino group therein being protected.

32. Process according to any one of Claims 23 to 26, 30 and 31, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is kanamycin A, Y is *tert.* - butoxy - carbonyl or benzyloxycarbonyl and the



substituent is introduced into the 3,6' - di - N - Y - kanamycin A by reaction with N - (S - γ - benzyloxycarbonylamino - α - hydroxybutyryl)succinimide, whereby, after removal of the blocking groups, is obtained 1 - N - (S - γ - amino - α - hydroxybutyryl)kanamycin A.

33. Process according to any one of Claims 23 to 26, 30 and 31, wherein said 4,6 - di - O - (aminoglycosyl) - 1,3 - diaminocyclitol is gentamicin B, Y is *tert.* - butoxycarbonyl or benzyloxycarbonyl and the



substituent is introduced into the 3,6' - di - N - Y - gentamicin B by reaction with N - (β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide, N -



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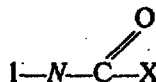
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(γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide, or *N* - (δ - benzyloxycarbonylamino - α - hydroxyvaleryloxy)succinimide, whereby, after removal of the blocking groups, is obtained 1 - *N* - (β - amino - α - hydroxypropionyl)gentamicin B, 1 - *N* - (γ - amino - α - hydroxybutyryl)gentamicin B and 1 - *N* - (δ - amino - α - hydroxyvaleryl)gentamicin B, respectively.

34. Process according to Claim 33, wherein *N* - (*S* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide is used whereby is formed 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B.

35. Process according to any one of Claims 23 to 26, 30 and 31, wherein said 4,6 - di - *O* - (aminoglycosyl) - 1,3 - diaminocyclitol is gentamicin B, Y is *tert* - butoxycarbonyl or benzyloxycarbonyl and the



substituent is introduced into the 3,6' - di - *N* - Y - gentamicin B by reaction with *N* - (*S,R* - β - benzyloxycarbonylamino - α - hydroxypropionyloxy)succinimide or *N* - (*S,R* - γ - benzyloxycarbonylamino - α - hydroxybutyryloxy)succinimide followed by separation of the diastereoisomers in the thereby formed 1 - *N* - (*S,R* - β - benzyloxycarbonylamino - α - hydroxypropionyl) - 3,6' - di - *N* - Y - gentamicin B or 1 - *N* - (*S,R* - γ - benzyloxycarbonylamino - α - hydroxybutyryl) - 3,6' - di - *N* - Y - gentamicin B, respectively, whereby, after removal of the blocking groups, is obtained 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B and 1 - *N* - (*R* - β - amino - α - hydroxypropionyl)gentamicin B and 1 - *N* - (*S* - γ - amino - α - hydroxybutyryl)gentamicin B and 1 - *N* - (*R* - γ - amino - α - hydroxybutyryl)gentamicin B, respectively.

36. A process as claimed in Claim 23, substantially as herein described with particular reference to the Examples.

37. A compound whenever obtained in the process as claimed in any one of Claims 23 to 36.

38. 1 - *N* - ethylsisomicin whenever obtained in the process as claimed in any one of Claims 23 to 29.

39. 1 - *N* - (*S* - γ - amino - α - hydroxybutyryl)kanamycin A whenever obtained in the process as claimed in any one of Claims 23 to 26 and 30 to 32.

40. 1 - *N* - (*S* - β - amino - α - hydroxypropionyl)gentamicin B whenever obtained in the process as claimed in any one of Claims 23 to 26 and 33 to 35.

41. A 4,6 - di - *O* - (aminoglycosyl) - 1,3 - diaminocyclitol being selectively blocked in positions 3,2',6' which is selected from 3,2',6' - tri - *N* - Y - sisomicin, 3,2',6' - tri - *N* - Y - verdamicin, 3,2',6' - tri - *N* - Y - tobramycin, 3,2',6' - tri - *N* - Y - gentamicin C₁, 3,2',6' - tri - *N* - Y - gentamicin C_{1a}, 3,2',6' - tri - *N* - Y - gentamicin C₂, 3,2',6' - tri - *N* - Y - gentamicin C_{2a}, 3,2',6' - tri - *N* - Y - gentamicin C_{2b}, 3,2',6' - tri - *N* - Y - Antibiotic 66-40B, 3,2',6' - tri - *N* - Y - Antibiotic 66-40D, 3,2',6' - tri - *N* - Y - Antibiotic JI-20A, 3,2',6' - tri - *N* - Y - Antibiotic JI-20B, 3,2',6' - tri - *N* - Y - Antibiotic G-52, the 5-*epi*- and 5 - *epi*-azido - 5 - deoxy analogs of the foregoing;

3,2',6' - tri - *N* - Y - kanamycin B, 3,2',6' - tri - *N* - Y - 3',4' - dideoxykanamycin B, 3,2',6' - tri - *N* - nebramycin factor 4, 3,2',6' - tri - *N* - Y - nebramycin factor 5', 3,2',6' - tri - *N* - Y - 3',4' - dideoxy - 3',4' - dehydrokanamycin B, 3,2',6' - tri - *N* - Y - 3',4' - dideoxy - 6' - *N* - methylkanamycin B, and 3,2',6' - tri - *N* - Y - 5 - deoxysisomicin; wherein Y is an acyl group.

42. A compound of Claim 41, wherein Y is acetyl, 2,2,2 - trichloroethoxycarbonyl or benzyloxycarbonyl.

43. 3,2',6' - tri - *N* - acetylsisomicin.

44. A 4,6 - di - *O* - (aminoglycosyl) - 1,3 - diaminocyclitol being selectively blocked in positions 3,6' which is selected from 3,6' - di - *N* - Y - gentamicin B, 3,6' - di - *N* - Y - gentamicin B₁, 3,6' - di - *N* - Y - gentamicin A₃, 3,6' - di - *N* - Y - kanamycin A, 3,6' - di - *N* - Y - 6' - *N* - methylkanamycin A wherein Y is an acyl group.

45. A compound of Claim 44, wherein Y is selected from acetyl, *tert* - butoxycarbonyl, benzyloxycarbonyl and 2,2,2 - trichloroethoxycarbonyl.

46. 3,6' - di - *N* - *tert* - butoxycarbonylkanamycin A and 3,6' - di - *N* - benzyloxycarbonylkanamycin A.

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47. 3,6' - di - *N* - *tert.* - butoxycarbonylgentamicin B and 3,6' - di - *N* - benzyloxycarbonylgentamicin B.

5 48. A 4,6 - di - *O* - (aminoglycosyl) - 1,3 - diaminocyclitol being selectively blocked in positions 2',6' which is selected from 2',6' - di - *N* - *Y* - sisomicin, 2',6' - di - *N* - *Y* - verdamicin, 2',6' - di - *N* - *Y* - tobramycin, 2',6' - di - *N* - *Y* - gentamicin C₁, 2',6' - di - *N* - *Y* - gentamicin C₂, 2',6' - di - *N* - *Y* - gentamicin C_{2b}, 2',6' - di - *N* - *Y* - Antibiotic 66-40B, 2',6' - di - *N* - *Y* - Antibiotic 66-40D, 2',6' - di - *N* - *Y* - Antibiotic JI-20A, 2',6' - di - *N* - *Y* - Antibiotic JI-20B, 2',6' - di - *N* - *Y* - Antibiotic G-52, the 5 - epi - and 5 - epi - azido - 5 - deoxy analogs of the foregoing and of 2',6' - di - *N* - *Y* - gentamicin C_{2b}; 2',6' - di - *N* - *Y* - kanamycin B, 2',6' - di - *N* - *Y* - 3',4' - dideoxykanamycin B, 2',6' - di - *N* - *Y* - nebramycin factor 4, 2',6' - di - *N* - *Y* - nebramycin factor 5, 2',6' - di - *N* - *Y* - 3',4' - dideoxy - 3',4' - dehydrokanamycin B, 2',6' - di - *N* - *Y* - 3',4' - dideoxy - 6' - *N* - methylkanamycin B, and 2',6' - di - *N* - *Y* - 5 - deoxysisomicin; wherein *Y* is an acyl group.

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49. A compound of Claim 48, wherein *Y* is a member selected from the group consisting of 2,2,2 - trichloroethoxycarbonyl, *tert.* - butoxycarbonyl and benzyloxycarbonyl.

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SPECIFICATION NO 1575982

The following amendments were allowed under Section 29 on 19 October 1982:-

Page 2, line 28, Page 27, line 32, *delete* said *insert* the corresponding non-blocked
Page 2, line 31, Page 27, line 35, *after* said first occurrence *insert* non-blocked

THE PATENT OFFICE
20 December 1982

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