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PATENT SPECIFICATION

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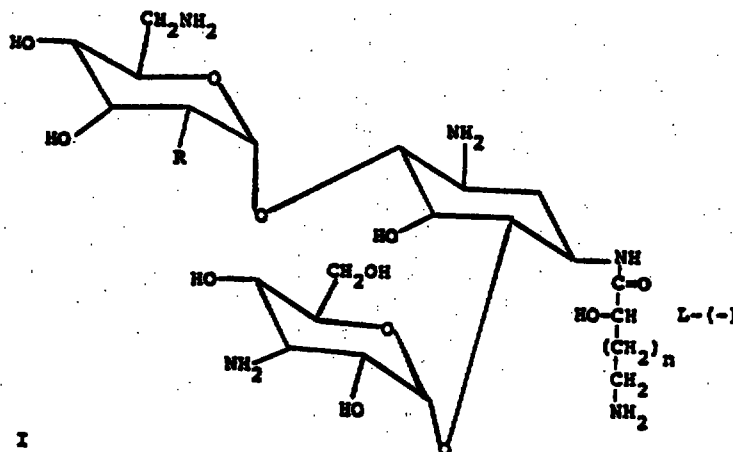
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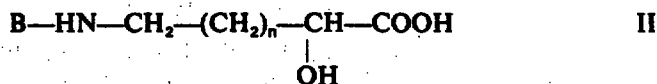
(54) PROCESS FOR THE PREPARATION OF KANAMYCIN DERIVATIVES

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, of 345 Park Avenue, State of New York, 10022, United States of America, 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 an improved process for the preparation of compounds of the formula



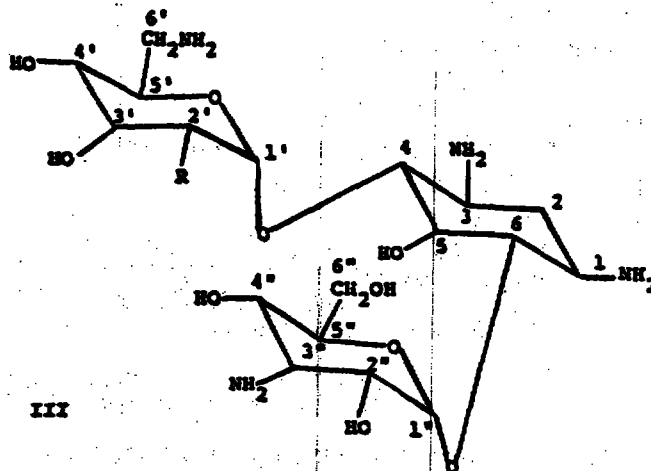
wherein R is OH or NH₂, and n is 0 or an integer of 1 or 2, or a nontoxic pharmaceutically acceptable acid addition salt thereof which process comprises acylating polysilylated kanamycin A or B or polysilylated kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety, in a substantially anhydrous organic solvent, with an acylating derivative of an acid of the formula



in which n is 0 or an integer of 1 or 2 and B is an amino-blocking group, and subsequently removing all blocking groups.

The kanamycins are well-known antibiotics, having been described, for example in the Merck Index, 8th edition, pp. 597—8. Numerous derivatives of the kanamycins also are known. The structural formulae of kanamycins A and B are given below, along with the standard numbering system used in the art.

Hereinafter, where readily understandable, the various kanamycin derivatives will be referred to as derivatives of kanamycin A or B rather than by structural formula, so as to avoid the necessity of comparing complex structures to determine differences.



Kanamycin A: R = OH

Kanamycin B: R = NH₂

U.S. Patent 3,781,268 discloses and claims 1-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A (amikacin) and B, as well as their mono- and dicarbobenzyloxy protected derivatives. For lower and higher homologs see U.S. Patents 3,886,139 and 3,904,597. The compounds are prepared by acylating a 6'-N-protected kanamycin A or B with an acylating derivative of an N'-protected L-(-)- γ -amino- α -hydroxybutyric acid, in an aqueous medium, followed by removal of one or both N-protecting groups.

U.S. Patent 3,974,137 discloses and claims a process for preparing 1-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A which comprises reacting 6'-carbobenzyloxykanamycin A with at least three moles of benzaldehyde, a substituted benzaldehyde or pivaldehyde, to produce 6'-N-carbobenzyloxykanamycin A containing Schiff base moieties on the 1,3 and 3''-positions, acylating this tetra-protected kanamycin A derivative with the N-hydroxysuccinimide ester of L-(-)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid, and subsequently removing the protecting groups.

Belgian Patent 828,192 discloses and claims a process for preparing 1-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A by preparation of the same tetra-protected kanamycin A derivative as in U.S. 3,974,137, acylating with the N-hydroxy-5-norbornene-2,3-dicarboximide ester of L-(-)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid, and subsequently removing the protecting groups.

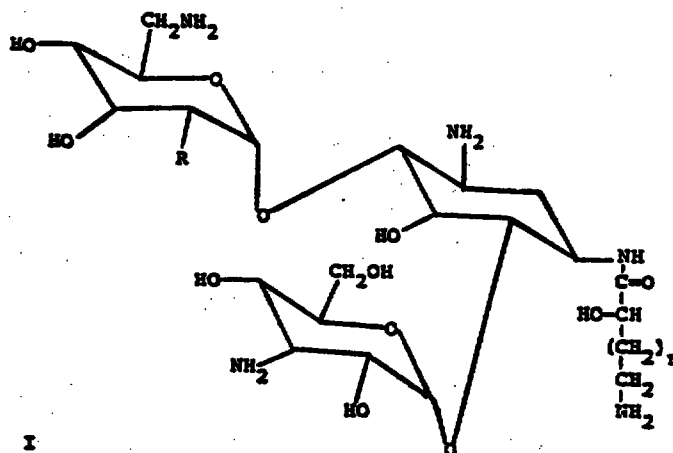
The present invention provides an improved and commercially attractive process for the preparation of compounds of formula I. The use of a polysilylated kanamycin A or B as a starting material gives high solubility in the organic solvent system, thus permitting reaction at high concentrations. Although the reaction is usually conducted in solutions containing about 10-20% polysilylated kanamycin starting material, excellent results have been obtained at concentrations of about 50 gms./100 ml. of solvent.

As with prior art processes, the present process gives a mixture of acylated products. The desired 1-N-acylated product is separated from the other products by chromatography and, if desired, the by-products may be hydrolyzed to the starting kanamycin for recycling. In prior art processes it was found that any 3''-N-acylated material which was produced caused a loss of about an equal amount of the desired 1-N-acylated product, due to the great difficulty of separating the latter from the former. A particularly desirable feature of the present process is the extremely low amount of undesirable 3''-N-acylated product which is produced (typically, none is detected).

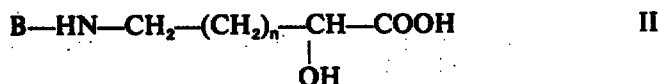
In preparing 1-[L-(-)- α -amino- α -hydroxybutyryl]kanamycin A, amikacin by various prior art procedures, there is typically also produced the 3''-N-

acylated product (BB-K11), the 3-N-acylated product (BB-K29), the 6'-N-acylated product (BB-K6) and polyacylated material, as well as unreacted kanamycin A. Thus, in commercial production of amikacin by acylation of 6'-N-carbobenzyloxy kanamycin A in an aqueous medium, followed by removal of the protecting group we found that about 10% of the desired amikacin (2.5 kg. in a 25 kg. batch) usually was lost because of the presence of BB-K11 as a co-product. When preparing amikacin by the present process, BB-K11 typically is not detected in the reaction mixture.

The present invention provides the process for the preparation of a 1 - N - [ω - amino - α - hydroxyalkanoyl]kanamycin A or B having the formula



in which R' is OH or NH_2 and n is 0 or an integer of 1 or 2, or a nontoxic pharmaceutically acceptable acid addition salt thereof, which comprises acylating polysilylated kanamycin A or B or polysilylated kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety with an acylating derivative of the acid of the formula



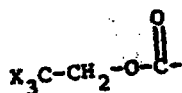
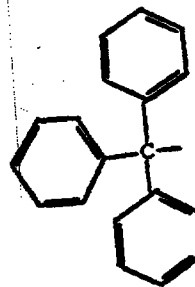
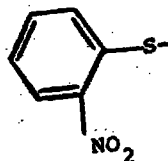
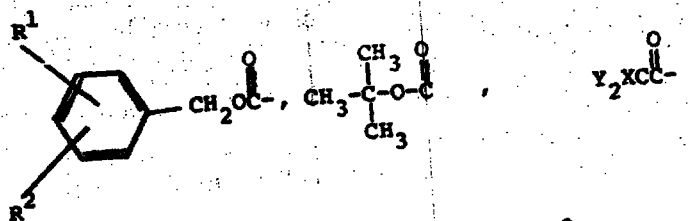
in which n is 0 or an integer of 1 or 2 and B is an amino-blocking group, in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

The blocking groups which may be used to protect the 6'-amino moiety of the kanamycin and the amino group of the acylating acid (group B in Formula II) are conventional blocking groups for the protection of primary amino groups and are well known to those skilled in the art. Suitable blocking groups include alkoxycarbonyl groups such as *t*-butoxycarbonyl and *t*-amyloxycarbonyl; aralkoxycarbonyl groups such as benzyloxycarbonyl; cycloalkyloxycarbonyl groups such as cyclohexyloxycarbonyl; haloalkoxycarbonyl groups such as trichloroethoxycarbonyl; acyl groups such as phthaloyl and *o*-nitrophenoxycarbonyl; and other well-known blocking groups such as the *o*-nitrophenylthio group and the trityl group.

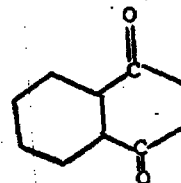
The acylating acid of formula II may be in its (+) or (-) isomeric form or a mixture of the two isomers (the d, l form), thus producing the corresponding compound of formula I in which the 1 - N - [ω - amino - α - hydroxyalkanoyl] group is in its (+) [or (R)] form or its (-) [or (S)] form, or a mixture thereof. Each such isomeric form, and the mixture thereof, is included within the scope of this invention. In one preferred embodiment, the acylating acid of formula II is in its (-) form. In another preferred embodiment the acylating acid of formula II is in its (+) form.

In one embodiment of the invention the starting material is polysilylated kanamycin A or B (and preferably polysilylated kanamycin A). In another embodiment the starting material is polysilylated kanamycin A or B (and preferably polysilylated kanamycin A) containing a blocking group other than silyl

on the 6'-amino moiety, said blocking group preferably being selected from those of the formulae



and



wherein R^1 and R^2 are alike or different and each is H, F, Cl, Br, NO_2 , OH, (lower)alkyl or (lower)alkoxy, and X is Cl, Br, F or I, and Y is H, Cl, Br, F or I. The most preferred blocking group is the carbobenzyloxy group. In the case of the last stated blocking group the B—HN grouping then becomes B—N to give phthalimido.

In a preferred embodiment of the invention the acylating derivative of the acid of Formula II is an active ester, and preferably its active ester with N-hydroxysuccinimide, N-hydroxy-5-norbornene-2,3-dicarboximide or N-hydroxyphthalimide. In another preferred embodiment the acylating derivative of the acid of Formula II is a mixed acid anhydride, and preferably its mixed acid anhydride with pivalic acid, benzoic acid, isobutylcarbonic acid or benzylcarbonic acid.

In a most preferred embodiment, this invention relates to the preparation of 1-N-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A or a nontoxic pharmaceutically acceptable acid addition salt thereof, which comprises acylating polysilylated kanamycin A with a mixed acid anhydride of L-(-)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid (and preferably its mixed acid anhydride with pivalic acid, benzoic acid, isobutylcarbonic acid or benzylcarbonic acid) in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

In another most preferred embodiment, this invention relates to the preparation of 1-N-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A or a nontoxic pharmaceutically acceptable acid addition salt thereof, which comprises acylating polysilylated kanamycin A containing a carbobenzyloxy group on the 6'-amino moiety with a mixed acid anhydride of L-(-)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid (and preferably its mixed acid anhydride with pivalic acid, benzoic acid, isobutylcarbonic acid or benzylcarbonic acid) in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

In another most preferred embodiment, this invention relates to the preparation of 1-N-[L-(-)- γ -amino- α -hydroxybutyryl]kanamycin A or a nontoxic pharmaceutically acceptable acid addition salt thereof, which comprises acylating polysilylated kanamycin A with an active ester of L-(-)- γ -

benzyloxycarbonylamino - α - hydroxybutyric acid (and preferably its active ester with N-hydroxysuccinimide, N-hydroxy-5-norbornene-2,3-dicarboximide or N-hydroxyphthalimide) in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

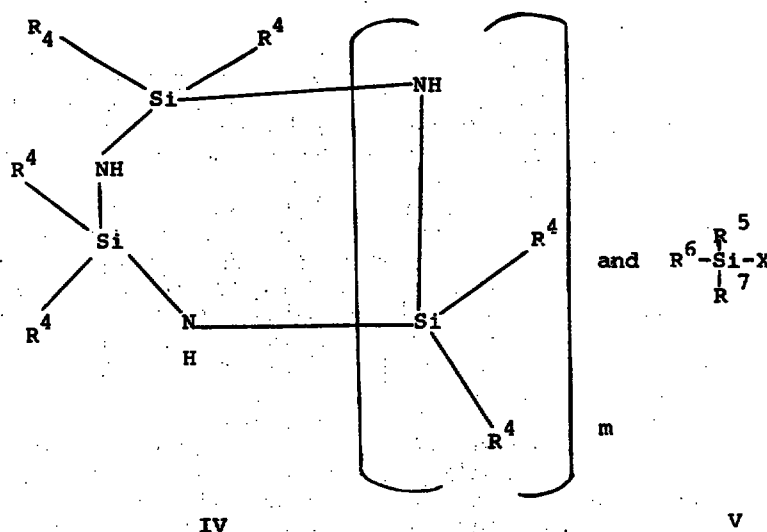
In another most preferred embodiment, this invention relates to the preparation of 1 - N - [L - (-) - γ - amino - α - hydroxybutyryl]kanamycin A or a nontoxic pharmaceutically acceptable acid addition salt thereof, which comprises acylating polysilylated kanamycin A containing a carbobenzyloxy group on the 6'-amino moiety with an active ester of L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid (and preferably its active ester with N-hydroxysuccinimide, N-hydroxy-5-norbornene-2,3-dicarboximide or N-hydroxyphthalimide) in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

In another aspect, the present invention provides polysilylated A or B containing a blocking group other than silyl on the 6'-amino moiety. In a preferred embodiment the material is polysilylated kanamycin A or B (and preferably polysilylated kanamycin A) containing an average number of silyl groups (and preferably trimethylsilyl groups (and preferably trimethylsilyl groups) per molecule of from 4 to 8. In another preferred embodiment the material is polysilylated kanamycin A or B (and preferably polysilylated kanamycin A) containing a blocking group other than silyl on the 6'-amino group and containing an average number of silyl groups (and preferably trimethylsilyl groups) per molecule of from 3 to 7.

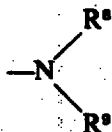
As used herein and in the claims, the term "nontoxic, pharmaceutically acceptable acid addition salt" of a compound of Formula I means a mono-, di-, tri- or tetrasalt formed by the interaction of one molecule of a compound of Formula I with 1-4 equivalents of a nontoxic, pharmaceutically acceptable acid. Included among these acids are acetic, hydrochloric, sulfuric, maleic, phosphoric, nitric, hydrobromic, ascorbic, malic and citric acid, and those other acids commonly used to make salts of amine-containing pharmaceuticals.

Acylation of the polysilylated kanamycin A or B starting material (with or without a blocking group other than silyl on the 6'-amino moiety) may, in general be conducted in an organic solvent in which the starting material has sufficient solubility. These starting materials are highly soluble in most common organic solvents. Suitable solvents include for example, acetone, diethyl ketone, methyl n-propyl ketone, methyl isobutyl ketone, methyl ethyl ketone, acetonitrile, glyme, diglyme, dioxane, toluene, tetrahydrofuran, cyclohexanone, methylene chloride, chloroform, carbon tetrachloride and mixtures of acetone/butanol or diethyl ketone/butanol. The choice of solvent is dependent on the particular starting materials employed. Ketones, generally, are the preferred solvents. The most advantageous solvent for the particular combination of reactants being utilized can readily be determined by routine experimentation.

Suitable silylating agents for use in preparing the polysilylated kanamycin starting materials utilized herein include those of the formula



wherein R^5 , R^6 and R^7 are selected from hydrogen, halogen, (lower)alkyl, halo(lower)alkyl and phenyl, at least one of the said R^5 , R^6 and R^7 groups being other than halogen or hydrogen; R^6 is (lower)alkyl, m is an integer of 1 to 2 and X is selected from halogen and



wherein R^5 is hydrogen or (lower)alkyl and R^6 is hydrogen, (lower)alkyl or



in which R^5 , R^6 and R^7 are as defined above.

Specific silyl compounds of Formulas IV and V are: trimethylchlorosilane, hexamethyldisilazane, triethylchlorosilane, methyltrichlorosilane, dimethyldichlorosilane, triethyl bromosilane, tri-n-propylchlorosilane, methyl-diethylchlorosilane, dimethylethylchlorosilane, dimethyl-t-butylchlorosilane, phenyldimethylbromosilane, benzylmethylethylchlorosilane, phenylethylmethylchlorosilane, triphenylchlorosilane, triphenylfluorosilane, tri-o-tolylchlorosilane, tri-p-dimethylaminophenylchlorosilane, N-ethyltriethylsilylamine, hexaethyldisilazane, triphenylsilylamine, tri-n-propylsilylamine, tetraethyldimethyldisilazane, hexaphenyldisilazane and hexa-p-tolyldisilazane. Also useful are hexaalkylcyclotrisilazanes and octa-alkylcyclotetrasilazanes. Other suitable silylating agents are silylamides (such as trialkylsilylacetamides and bis-trialkylsilylacetamides), silylureas (such as trimethylsilylurea) and silylureides. Trimethylsilylimidazole also may be utilized.

A preferred silyl group is the trimethylsilyl group and preferred silylating agents for introducing the trimethylsilyl group are hexamethyldisilazane, bis(trimethylsilyl)acetamide and trimethylsilylacetamide. Hexamethyldisilazane is most preferred.

When utilizing polysilylated kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety as a starting material, said starting material may be prepared either by polysilylating the desired 6'-N-blocked kanamycin A or B, or by introducing the desired 6'-N blocking group into polysilylated kanamycin A or B.

Methods for the introduction of silyl groups into organic compounds, including certain aminoglycosides, are known in the art. The polysilylated kanamycins (with or without a blocking group other than silyl on the 6'-amino moiety) may be prepared by methods which are known *per se*, or as described in this specification.

As used herein, the term polysilylated kanamycin A or B refers to kanamycin A or B containing from two to ten silyl groups in the molecule. Thus, the term polysilylated kanamycin A or B, does not include persilylated kanamycin A or B, which would contain eleven silyl groups in the molecule.

The precise number of silyl groups (or their location) present in the polysilylated kanamycin starting materials (with or without a blocking group other than silyl on the 6'-amino moiety) is not known. We have found that both undersilylation and oversilylation lower the yield of the desired product and increase the yield of other products. In the case of gross under- or oversilylation, little or none of the desired product may be formed. The degree of silylation which will give the greatest yield of desired product will depend on the particular reactants being used in the acylation step. The most advantageous degree of silylation using any combination of reactants can readily be determined by routine experimentation.

When preparing 1 - N - [L - (-) - γ - amino - α - hydroxybutyryl]kanamycin A by acylating polysilylated kanamycin A with the N-hydroxysuccinimide ester of L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid in acetone solution, we have found that good yields of the desired product are obtained by utilizing polysilylated kanamycin A which has been prepared by reacting from 4 to

5.5 moles of hexamethyldisilazane per mole of kanamycin A. Greater or lesser amounts of hexamethyldisilazane may be utilized, but the yield of desired product in the subsequent acylation step is lowered significantly. In the specific process set forth above we prefer to utilize from 4.5 to 5.0 moles of hexamethyldisilazane per mole of kanamycin in order to obtain maximum yield of product in the acylation step.

It will be appreciated that each mole of hexamethyldisilazane is capable of introducing two equivalents of the trimethylsilyl group into kanamycin A or B. Both kanamycin A and B have a total of eleven sites (NH₂ and OH groups) which might be silylated, while kanamycin A and B containing a blocking group other than silyl on the 6'-amino moiety have a total of 10 such sites. Thus, 5.5 moles of hexamethyldisilazane per mole of kanamycin A or B could theoretically completely silylate all OH and NH₂ moieties of the kanamycin, while 5.0 moles of hexamethyldisilazane could completely silylate one mole of kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety. However, we believe that such extensive silylation does not take place with these molar ratios during reasonable reaction time periods, although higher degrees of silylation are obtained in a given reaction time when a silylation catalyst is added.

Silylation catalysts greatly accelerate the rate of silylation. Suitable silylation catalysts are well known in the art and include *inter alia* amine sulfates (e.g. kanamycin sulfate), sulfamic acid, imidazole and trimethylchlorosilane. Silylation catalysts generally promote a higher degree of silylation than is required in the process of this invention. However, oversilylate kanamycin A or B can be used as starting material if it is first treated with a desilylating agent to reduce the degree of silylation before the acylation reaction is carried out.

Good yields of desired product are obtained when acylating polysilylated kanamycin A prepared using a 5.5:1 molar ratio of hexamethyldisilazane to kanamycin A. However, when kanamycin A silylated with a 7:1 molar ratio of hexamethyldisilazane (or with a 5.5:1 molar ratio in the presence of a silylation catalyst) was acylated in acetone with the N-hydroxysuccinimide ester of L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid, less than a 1% yield of the desired product was obtained. However, when this same "oversilylated" kanamycin A was acylated with the same acylating agent in acetone solution to which water [21 moles water per mole of kanamycin; 2.5% water (W/V)] had been added as a desilylating agent 1 hour before acylation, a yield of approximately 40% of the desired product was obtained. The same results are obtained if the water is replaced by methanol or other active hydrogen compound capable of effecting desilylation, e.g. ethanol, propanol, butanediol, methyl mercaptan, ethyl mercaptan or phenyl mercaptan.

Although it is usual to utilize dry solvents when working with silylated materials, we have surprisingly found that, even in the absence of "oversilylation", the addition of water to the reaction solvent prior to acylation often gives equally good yields, and sometimes gives better yields of desired product than in a dry solvent. In acylation reactions conducted in acetone at the usual concentrations of 10-20% (W/V) of polysilylated kanamycin A, we have found that excellent yields of L - (-) - γ - amino - α - hydroxybutyrylkanamycin A were obtained when adding up to 28 moles of water per mole of polysilylated kanamycin A; at 20% concentration, 28 moles per mole is approximately 8% water. With other combinations of reactants, even more water may be tolerated or be beneficial. The acylation reaction may be conducted in solvents containing up to about 40% water, although at such high water concentrations one must utilize short acylation times in order to avoid excessive desilylation of the polysilylated kanamycin A or B starting material. Accordingly, as used herein and in the claims, the term "substantially anhydrous organic solvent" is intended to include solvents containing up to about 25% water. A preferred range is up to about 20% water, a more preferred range is up to about 8% water, and a most preferred range is up to about 4% water.

As indicated above, the most desirable degree of silylation for any combination of acylation reactants may be readily determined by routine experimentation. It is believed that the preferred average number of silyl groups in the starting material will usually be between 4 and 8 for kanamycin A or B and between 3 and 7 for kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety, but this is only theory and is not considered an essential part of this invention.

The duration and temperature of the acylation reaction are not critical.

Temperatures in the range of -30°C to 100°C may be used for reaction times ranging from one hour up to a day or more. We have found that the reaction usually proceeds well at room temperature and, for convenience and economy, prefer to conduct the reaction at ambient temperature. However, for maximum yields and selective acylation, we prefer to conduct the acylation at from 0° to 5° .

Acylation of the 1-amino moiety of the polysilylated kanamycin A or B (with or without a blocking group other than silyl on the 6'-amino moiety) may be conducted with any acylating derivative of the acid of Formula II which is known in the art to be suitable for the acylation of a primary amino group. Examples of suitable acylating derivatives of the free acid include the corresponding acid anhydrides, mixed anhydrides, e.g. alkoxyformic anhydrides, acid halides, acid azides, active esters and active thioesters. The free acid may be coupled with the polysilylated kanamycin starting material after first reacting said free acid with N,N'-dimethylchloroformiminium chloride [cf. Great Britain 1,008,170 and Novak and Weichert, *Experientia* XXI, 6, 360 (1965)] or by the use of an N,N'-carbonyldiimidazole or an N,N'-carbonylditriazole [cf. South African Specification 63/2684] or a carbodiimide reagent [especially N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide or N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide: cf. Sheenan and Hess, *J.A.C.S.*, 77, 1967 (1955)], or of an alkynylamine reagent [cf. R. Buijle and H. G. Viehe, *Angew. Chem. International Edition*, 3, 582, (1964)] or of an isoxazolium salt reagent [cf. R. B. Woodward, R. A. Olofson and H. Mayer, *J. Amer. Chem. Soc.*, 83, 1010 (1961)], or of a ketenimine reagent [cf. C. L. Stevens and M. E. Munk, *J. Amer. Chem. Soc.*, 80, 4065 (1958)] or of hexachlorocyclotriphosphatriazine or hexabromocyclotriphosphatriazine (U.S. Pat. No. 3,651,050) or of diphenylphosphoryl azide [DDPA: *J. Amer. Chem. Soc.*, 94, 6203-6205 (1972)] or of diethylphosphoryl cyanide [DEPC: *Tetrahedron Letters* No. 18, pp. 1595-1598 (1973)] or of diphenyl phosphite [*Tetrahedron Letters* No. 49, pp. 5047-5050 (1972)]. Another equivalent of the acid is a corresponding azolide, i.e., an amide of the corresponding acid whose amide nitrogen is a member of a quasiaromatic five membered ring containing at least two nitrogen atoms, i.e., imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As will be appreciated by those skilled in the art, it sometimes may be desirable or necessary to protect the hydroxyl group of the acylating derivative of the acid of Formula II, e.g. when utilizing acylating derivatives such as an acid halide. Protection of the hydroxyl group may be accomplished by means known in the art, e.g. by use of a carbobenzyloxy group, by acetylation or by silylation.

After completion of the acylation reaction, all blocking groups are removed by methods known *per se* to yield the desired product of Formula I. The silyl groups may, for example, readily be removed by hydrolysis with water preferably at low pH. Blocking group B of the acylating derivative of the acid of Formula II, and the blocking group on the 6'-amino moiety of the polysilylated kanamycin starting material (if present) may also be removed by known methods. Thus, a t-butoxycarbonyl group may be removed by the use of formic acid, a carbobenzyloxy group by catalytic hydrogenation, a 2-hydroxy-1-naphthcarbonyl group by acid hydrolysis, a trichloroethoxycarbonyl group by treatment with zinc dust in glacial acetic acid, the phthaloyl group by treatment with hydrazine hydrate in ethanol under heating.

Yields of product were determined by various methods. After removal of all blocking groups and chromatography on a CG-50 (NH_4^+) column, the yield of amikacin could be determined by isolation of the crystalline solid from the appropriate fractions or by microbiological assay (turbidimetric or plate) of the appropriate fractions. Another technique which we utilized was high performance liquid chromatography of the unreduced acylation mixture, i.e. the aqueous solution obtained after hydrolysis of the silyl groups and removal of organic solvent but before hydrogenolysis to remove the remaining blocking group(s). This assay was not a direct assay for amikacin or BB-K29, but for the corresponding mono- or di-N-blocked compounds.

The instrument utilized was a Waters Associates ALC/GPC 244 high pressure liquid chromatograph with a Waters Associates Model 440 absorbance detector and a 30 cm $\times$ 3.9 mm i.d. μ -Bondpak C818 column, under the following conditions:

Mobile Phase: 25% 2-propanol
75% 0.01M sodium acetate pH 4.0
Flow Rate: 1 ml./minute

Detector: UV at 254 nm.
Sensitivity: 0.04 AUFS
Diluent: DMSO
Injected Amount: 5 μ l
concentration: 10 mg./ml.

Chart speed varied, but 2 minutes/inch was typical. The above conditions gave UV traces with peaks which were easy to measure quantitatively. The results of the above analyses are referred to in the specification as HPLC assays.

In order to avoid the repetition of complex chemical names, the following abbreviations are sometimes utilized in this specification.

AHBA	L-(-)- γ -amino- α -hydroxybutyric acid	
BHBA	N-Carbobenzyloxy derivative of AHBA	
HONB	N-hydroxy-5-norbornene-2,3-dicarboximide	
NAE	N-hydroxy-5-norbornene-2,3-dicarboximide	
(or BHBA—'ONB')	activated ester of BHBA	
HONS	N-hydroxysuccinimide	
SAE	N-hydroxysuccinimide activated ester of	
(or BHBA—'ONS')—	BHBA	
DCC	dicyclohexylcarbodiimide	
DCU	dicyclohexylurea	
HMDS	hexamethyldisilazane	
BSA	bis(trimethylsilyl)acetamide	
MSA	trimethylsilylacetamide	
TFA	trifluoroacetyl	
t-BOC	tert. butyloxycarbonyl	

"Dicalite" is a trademark of the Great Lakes Carbon Corporation for diatomaceous earth.

"Amberlite CG-50" is a Trademark of the Rohm & Haas Co. for the chromatographic grade of a weakly acid cationic exchange resin of the carboxylic-polymethacrylic type.

" μ -Bondapak" is a Trademark of Waters Associates for a series of high performance liquid chromatography columns.

All temperatures herein are given in degrees centigrade.

As used herein, the terms "(lower)alkyl" and "(lower)alkoxy" refer to alkyl or alkoxy groups containing from 1 to six carbon atoms.

Description of the Preferred Embodiments

Example 1

Preparation of 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A Amikacin by Selective Acylation of Poly(trimethylsilyl) 6'-N-Carbobenzyloxykanamycin A in Anhydrous Diethyl Ketone

6'-N-Carbobenzyloxykanamycin A (15 g., 24.24 m. moles) was slurried in 90 ml. of dry acetonitrile and heated to reflux under a nitrogen atmosphere. Hexamethyldisilazane (17.5 g., 108.48 m. moles) was added slowly over 30 minutes, and the resulting solution was refluxed for 24 hours. After removal of the solvent *in vacuo* (40°) and complete drying under vacuum (10 mm), 27.9 g. of a white, amorphous solid was obtained [90.71% calculated as 6'-N-Carbobenzyloxykanamycin A (Silyl)].

This solid was dissolved in 150 ml. of dry diethyl ketone at 23°. L-(-)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (NAE) (11.05 g., 26.67 m. moles) dissolved in 100 ml. of dry diethyl ketone at 23° was added slowly with good agitation over 1/2 hour. The solution was stirred at 23° for 78 hours. The yellow, clear solution (pH 7.0) was diluted with 100 ml. of water. The pH of the mixture was adjusted to 2.8 (3N HCl) and stirred vigorously at 23° for 15 minutes. The aqueous phase was separated, and the organic phase was extracted with 50 ml. of pH 2.8 water. The combined aqueous fractions were washed with 50 ml. of ethyl acetate. The solution was placed in a 500 ml. Parr bottle, together with 5 g. of 5% palladium on carbon catalyst (Engelhard) and reduced at 50 psi H₂ for 2 hours at 23°. The mixture was filtered through a pad of Dicalite which was then washed with an additional 30 ml. of water. The colorless filtrate was concentrated *in vacuo* (40—45°) to 50 ml. The solution was charged on a 5x100 cm CG-50 (NH₄+) ion exchange column. After

washing with 1000 ml. of water, unreacted kanamycin A, 3 - [L - (-) - γ - amino - α - hydroxybutyryl] - kanamycin A (BB-K29) and amikacin were eluted with 0.5N ammonium hydroxide. Polyacyl material was recovered with 3N ammonium hydroxide. Bioassay, thin layer chromatography and optical rotation were used to monitor the progress of elution. The volume and observed optical rotation of each fraction of eluate, as well as the weight and percent yield of solid isolated from each fraction by evaporation to dryness, are summarized below:

	Material	Volume (ml)	$\alpha_{D^{25}}$	Weight (gms.)	% Yield	
10	Kanamycin A	1000	+0.115	0.989	9.15	10
	BB-K29	1750	+0.24	4.37	32.0	
	Amikacin	2000	+0.31	6.20	47.4	
	Polyacyls	900	+0.032	0.288	2.0	

The spent diethyl ketone layer was shown by high performance liquid chromatography to contain an additional 3—5% amikacin.

The crude amikacin (6.20 gms.) was dissolved in 20 ml. of water and diluted with 20 ml. of methanol, and 20 ml. of isopropanol was added to induce crystallization. There was obtained 6.0 gms. (45.8% of crystalline amikacin.

Example 2

Preparation of 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A Amikacin by Selective Acylation of Poly(trimethylsilyl) 6'-N-Carbobenzyloxykanamycin A in Anhydrous Acetone

Poly(trimethylsilyl) 6'-N-carbobenzyloxy kana A prepared as in Example 1 (103 g., .081 moles, calculated as 6'-N-Carbobenzyloxykanamycin A (Silyl)₆) was dissolved in 100 ml. of dry acetone at 23°. L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid N - hydroxy - 5 - norbornene - 2,3 - dicarboximide ester (NAE) (35.24 g., .085 moles) dissolved in 180 ml. of dry acetone at 23° was added slowly with good agitation to the solution of poly(trimethylsilyl) 6'-N-Carbobenzyloxykanamycin A over a 15 minute period. the solution was stirred at 23° for 20 hours under a nitrogen atmosphere. The pale yellow, clear solution (pH 7.2) was diluted with 100 ml. of water. The pH of the mixture was adjusted to 2.5 (3N HCl) and stirring continued at 23° for 15 minutes. Acetone was removed using steam-ejector vacuum at about 35°. The solution was placed in a 500 ml. Parr bottle, together with 10 g. of 5% palladium on carbon catalyst (Engelhard) and reduced at 40 psi H₂ for 2 hours at 23°. The mixture was filtered through a pad of diatomaceous earth which was then washed with an additional 50 ml. of water. After concentration to approximately 1/3 volume, the solution (pH 6.9—7.2) was charged on a 6x110 cm. CG-50 (NH₄⁺) ion exchange column and eluted with a stepwise gradient from H₂O to 0.6 N ammonium hydroxide to recover amikacin. An automatic polarimeter was used to monitor the progress of elution. Combinations were made on the bases of thin layer chromatography evaluation. The combined amikacin fractions were concentrated to 25—30% solids. The solution was diluted with an equal volume of methanol, followed by two volumes of isopropanol to induce crystallization. There was recovered 18.2 g. (40%) of crystalline amikacin.

The recovery of 12% kanamycin A, 40% BB-K29 and 5% polyacylated kanamycin gave a material balance of 97%.

Example 3

Preparation of 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A Amikacin by Selective Acylation of Poly(trimethylsilyl) Kanamycin A, Using *In Situ* Blocking A. Poly(trimethylsilyl) Kanamycin A

Kanamycin A free base (18 g. activity, 37.15 m. moles) was slurried in 200 ml. of dry acetonitrile and heated to reflux. Hexamethyldisilazane (29.8 g., 184.6 m. moles) was added over 30 minutes and the mixture was stirred at reflux for 78 hours to give a light yellow clear solution. Removal of the solvent under vacuum left an amorphous solid residue (43 gm., 94%) [calculated as kanamycin A (silyl)₁₀].

B. 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A

p-(Benzyloxycarbonyloxy)benzoic acid (5.56 g., 20.43 m. moles) was slurried in 50 ml. of dry acetonitrile at 23°. N,O-bis-Trimethylsilyl acetamide (8.4 g., 41.37 m. mole) was added with good stirring. The solution was held for 30 minutes at 23°, and then added over 3 hours with vigorous stirring to a solution of

poly(trimethylsilyl)kanamycin A (21.5 g., 17.83 m. mole, calculated as the (silyl)₁₀ compound) in 75 ml. of dry acetonitrile at 23°. The mix was stirred for 4 hours, the solvent was removed *in vacuo* (40°), and the oily residue was dissolved in 50 ml. of dry acetone at 23°C.

L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid N - hydroxy - 5 - norbornene - 2,3 - dicarboximide ester (NAE) (8.55 g., 20.63 m. moles) in 30 ml. of acetone was added to the above solution over a period of 5 minutes. The mixture was held at 23°C for 78 hours. The solution was diluted with 100 ml. of water and the pH (7.0) lowered to 2.5 (6*N* HCl). The mixture was placed in a 500 ml. Parr bottle together with 3 g. of 5% palladium on carbon catalyst (Engelhard) and reduced at 40 psi H₂ for 2 hours at 23°. The mixture was filtered through a pad of diatomaceous earth which was then washed with 20 ml. of water. The combined filtrate and washings (168 ml.) were determined by microbiological assay against *E. coli* to contain approximately 11,400 mcg/ml. (19% yield) of amikacin.

Example 4

Preparation of 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A Amikacin by Selective Acylation of Poly(trimethylsilyl) Kanamycin A

A. Poly(trimethylsilyl) Kanamycin A

A suspension of 10 g. (20.6 m. moles) kanamycin A in 100 ml. of dry acetonitrile and 25 ml. (119 m. moles) 1,1,1,3,3,3-hexamethyldisilazane was refluxed for 72 hours. A clear light yellow solution resulted. The solution was stripped to dryness *in vacuo* at 30—40°C. There was obtained 21.3 g. of poly(trimethylsilyl) Kanamycin A as a light tan amorphous powder [85% yield calculated as kanamycin A (silyl)₁₀].

B. 1-N-[L-(-)- γ -Amino- α -hydroxybutyryl]kanamycin A

To a solution of 2.4 g. (2.0 m. moles) of poly(trimethylsilyl) Kanamycin A in 30 ml. of dry acetone was added slowly 2.0 m. moles of L - (-) - γ - benzyloxycarbonylamino - α - hydroxybutyric acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (NAE) in 10 ml. of dry acetone at 0—5°C. The reaction mixture was stirred at 23°C for a week and then stripped to dryness *in vacuo* at a bath temperature of 30—40°C. Water (60 ml.) was then added to the residue, followed by 70 ml. of methanol to obtain a solution. The solution was acidified with 3*N* HCl to pH 2.0 and then reduced at 50 psi H₂ for 2 hours, using 500 mg of 5% palladium on carbon catalyst. The material was filtered, and the combined filtrate and washings were determined by microbiological assay against *E. coli* to contain a 29.4% yield of amikacin.

Example 5

Preparation of Amikacin by Selective N-Acylation of Polytrimethylsilyl 6'-N-Carbobenzoyl Kanamycin A in Anhydrous Acetone

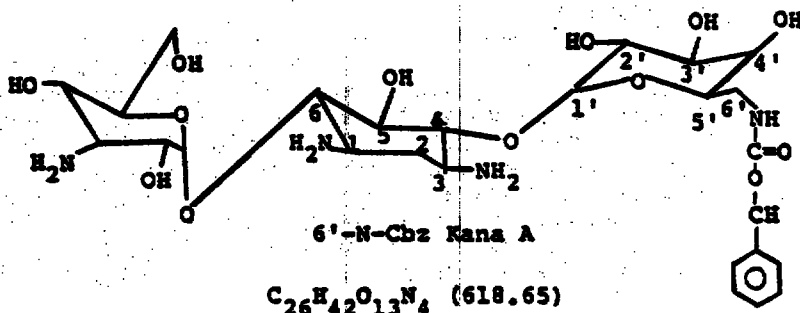
I. Summary

Silylation of 6'-N-carbobenzoyl Kana A in acetonitrile using hexamethyldisilazane (HMDS) affords the 6'-N-carbobenzoyl Kana A (silyl)₉ intermediate (I). This silylated Kana A is readily soluble in most organic solvents. Acylation with NAE in anhydrous acetone at 23° using a 4% molar excess of NAE relative to 6'-N-Cbz Kana A input afforded a mixture containing only Cbz derivatives of amikacin and BB-K29, some unreacted Kana A and some polyacyl material. No BB-K11 was detectable in any of these studies. Elution of an acetone acylation mix, after reduction and workup, from a CG-50 (NH₄⁺) column using an ammonium hydroxide gradient afforded isolated yields of pure amikacin in the 40% range.

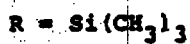
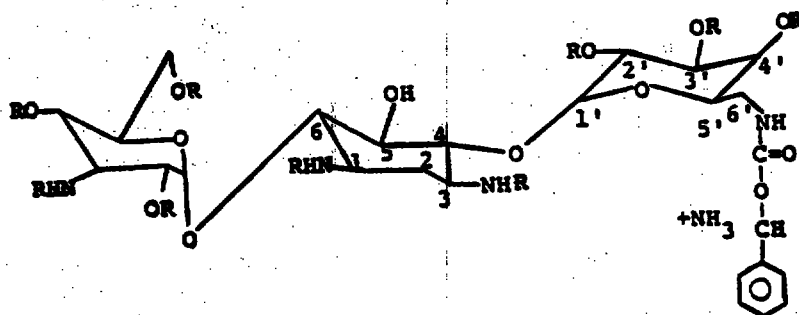
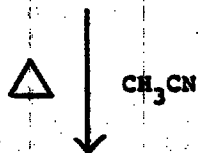
21

II. Equations

A.

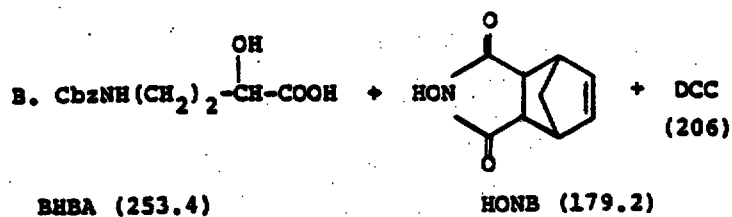


HMDS (161.4)

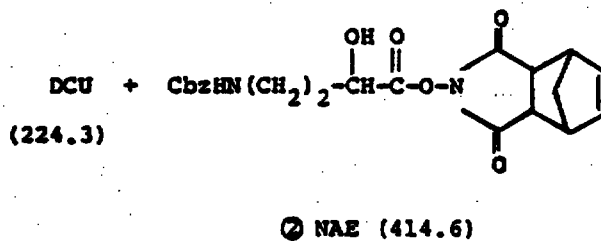


① 6'-N-Cbz Kana A (Silyl)₉

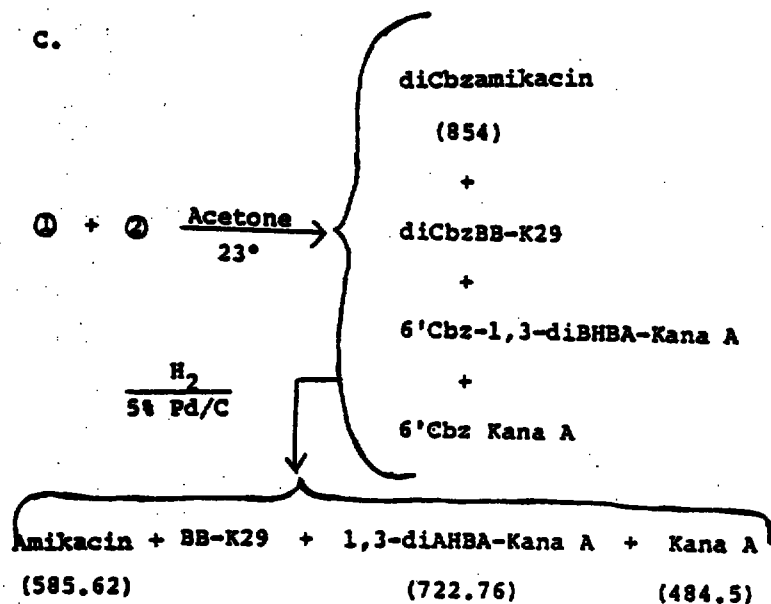
$C_{53}H_{114}O_{13}N_4Si_9$ (1268.3)



Acetone



C.



CG-50 (NH_4^+)

Amikacin

14		1,598,294	14
III. Materials			
		Wgt. g.	Vol. ml.
	6'-N-Cbz Kana A	50	Moles .081
	HMDS	58.9	.365
5	Acetonitrile		300
	BHBA	21.5	.085
	HONB	15.2	.085
	DCC	17.48	.085
10	Acetone		260
	CG-50 (NH ₄ ⁺)		3000
	Methanol		As required
	IPA		As required
IV. Safety			
15	6'-N-Cbz Kana A—	No direct information available.	15
	Acetonitrile—	Avoid dust contact. Treat as a cyanide. Avoid breathing vapors. May cause skin irritation.	
20	Hexamethyldisilazane— (HNDS)	Irritant, handle with care.	20
	6'-N-Cbz Kana A (Silyl) ₆ —	No direct information available, handle with care.	
	BHBA—	Toxicity is not established.	
25	HONB—	Avoid exposure to solids.	25
	DCC—	Toxicity unknown. Use precaution in handling.	
30	Acetone—	A severe skin and eye irritant. Avoid inhalation of mist or vapors. Toxic.	30
	NAE—	Flammable, Inhalation may produce headache, fatigue, excitement, bronchial irritation, and, in large amounts, narcosis.	
35	Methanol—	No direct information available; always handled directly as solution in acetone.	35
40	Isopropanol—	Flammable. Poisoning may occur from ingestion, inhalation or percutaneous absorption.	40
		Flammable. Ingestion or inhalation of large quantities of the vapor may cause headache, dizziness, mental depression, vomiting, narcosis.	
45	Ammonium hydroxide—	Toxic vapors. Wear mask, avoid contact with liquid.	45
	CG-50 (NH ₄ ⁺)—	No toxicity data available, handle with care.	
V. Procedure			
50	A. Preparation 6'-N-Carbobenzyloxykanamycin A (silyl) ₆ [6'-N-Cbz Kana A (Silyl) ₆]		50
	1. Slurry 50 g. of 6'-N-carbobenzyloxykanamycin A (KF <4%) in 300 ml. of acetonitrile (KF <0.01%). Bring to reflux (74°) maintaining a stream of dry nitrogen through the slurry.		
55	2. Add slowly over a 30 minute period 75.8 ml. hexamethyldisilazane (HMDS). Complete solution will occur with evolution of ammonia gas.		55
	3. Continue refluxing for 18—20 hours under a nitrogen purge.		
60	4. Concentrate the clear, light yellow solution under vacuum (bath temp. 40—50°) to a foamy solid. Yields, of the silyl ₆ compound 89—92 g. (90—94% Theory). NOTE: For future reference; in other solvent studies this solid is normally not isolated but used directly for the acylation.		60

B. Preparation of N-hydroxy-5-norbornene-2,3-dicarboximide ester of L-(-)- α -carbobenzyloxyamino- α -hydroxybutyric acid (NAE)

1. Dissolve 21.5 g of L-(-)- γ -carbobenzyloxyamino- α -hydroxybutyric acid (BHBA) in 100 ml. of dry acetone at 23° followed by 15.2 g. of N-hydroxy-5-norbornene-2,3-dicarboximide (HONB). A complete solution will result.

2. Over 30 minutes add a solution of 17.48 g. of dicyclohexylcarbodiimide (DCC) in 50 ml. of acetone with agitation. The temperature will rise to approximately 40° during the addition with precipitation of dicyclohexylurea (DCU).

3. Agitate the slurry for 3—4 hours allowing the temperature to equilibrate to 23—25°.

4. Remove the urea derivative by filtration; wash the cake with 30 ml. acetone. Save the filtrate plus washings for the acylation step below.

C. Acylation of 6'-N-Cbz Kana A (Silyl)₆

1. Dissolve the 6'-N-Cbz Kana A (silyl)₆ isolated in Part A, Step 4 in 100 ml. of dry acetone at 23—24°.

2. With good agitation slowly add the NAE solution prepared in Part B over a 15 minute period. The temperature will gradually rise to approximately 40°. Allow the solution to equilibrate to 23° and continue stirring for 18—20 hours under a nitrogen atmosphere.

3. Add 100 ml of water and lower the pH (6.9—7.2) to 2.2—2.5 with 6N hydrochloric acid. Agitate for 15 minutes at 23°.

(NOTE: A second layer may form—this does not present a problem in the workup).

4. Remove acetone under vacuum at a bath temperature of 30—35°. Transfer the concentrate to a suitable hydrogenation vessel (prepurged with nitrogen). Add 10 g. 5% palladium on carbon catalyst, and hydrogenate at 40 psi for 2—3 hours.

5. Filter the mixture through a Dicalite pad, washing the hydrogenation vessel and cake with an additional 50 ml. water.

6. Concentrate the filtrate plus wash to approximately 1/3 volume (50 ml.) under vacuum at 40—45°.

7. Check the pH. It should be in the range 6.9—7.2. If not, adjust with 1N ammonium hydroxide. Charge the mixture on a CG-50 (NH₄⁺) column (6×110 cm.).

8. Wash the column with 1000 ml. of deionized water. Then elute with 0.5—0.6N ammonium hydroxide using an automatic polarimeter to monitor the progress of elution. The order of elution is as follows:

Residual Kana A→BB-K29→Amikacin. No BB-K11 was detected in any of our acylation workups. Polyacyl material i.e. the 1,3-diAHBA analog of Kana A, is recovered by washing the column with 3N ammonium hydroxide.

9. Combine the amikacin fractions and concentrate to 25—30% solids. Dilute with 1 volume of methanol, and seed with amikacin crystals.

10. Add slowly over 2 hours 2 volumes of isopropanol (IPA) with good stirring, and crystallize at 23° for 6—8 hours.

11. Filter the solid, wash with 50 ml. of 1:1:2 water/methanol/IPA mixture, and finally with 25 ml. IPA.

12. Dry in a vacuum oven at 40° for 12—16 hours. Yield: 17.3—19.0 g. (38—42%) of amikacin having the following properties:

TLC

CHCl₃-methanol-NH₄OH-water (1:4:2:1), 5×20 cm. silica gel plates from Quantum Industries—one zone as detected with ninhydrin (RF~0.4).

Specific Rotation

23°	H ₂ O	0.1M NH ₄ OH	0.1M H ₂ SO ₄
[α]			
589	+101.6	+101.9	+103.5
C=1.0%			

13. The recovery of BB-K29 in this system was also 39—42%, residual Kana A 10—14% and 1,3-di AHBA-Kana A approximately 5% to give a material balance >95%.

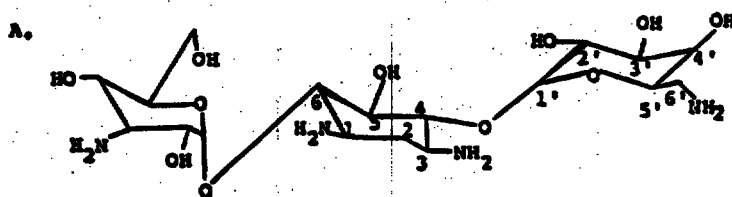
Example 6

Preparation of Amikacin by Selective N-Acylation of Polytrimethylsilyl Kana A in Anhydrous Acetone

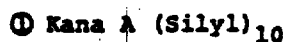
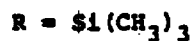
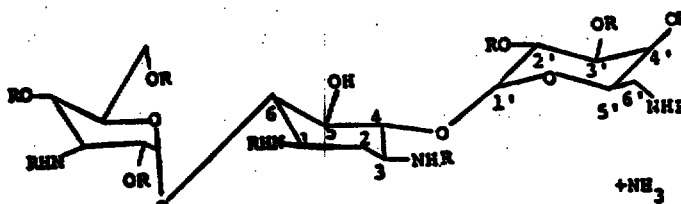
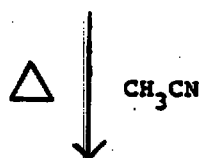
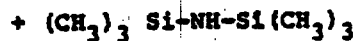
I. Summary

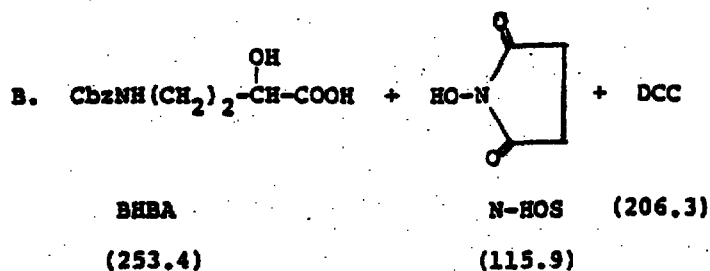
Silylation of Kana A 'base' in acetonitrile using hexamethyldisilazane (HMDS) yielded polytrimethylsilyl Kana A. The extent of silylation is as yet uncertain, but for the time being is assumed to be Kana A (Silyl)₁₀. Polysilylated Kana A is readily soluble in most organic solvents. Acylation with SAE in anhydrous acetone at 23° using a 1:1 molar ratio of SAE relative to Kana A input afforded a mixture containing Cbz derivatives of amikacin and BB-K29, usually in the ratio 2—3/1; BB-K6 (approximately 5—8%), unreacted Kana A (15—20%) and some polyacyl material (approximately 5—10%). Again, as was seen in our previous work on the acylation of polytrimethylsilyl 6'-N-Carbobenzoxy Kana A, no BB-K11 was detected in any of these experiments. Reduction and work-up of an acetone acylation mix, followed by chromatography on a CG-50-(NH₄⁺) column using 0.5N ammonium hydroxide, afforded isolated crystalline amikacin in the 34—39% range.

II. Equations

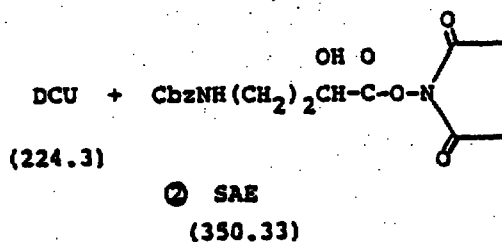


Kana A 'base'





EtOAc



C.

① + ②

Acetone
23°

Cbz Amikacin (720)
+
Cbz BB-K29
+
Cbz BB-K6
+
Kana A
+
Polyacyls (Primarily 1,3-diBHBA-Kana A)

H₂
5% Pd/C

Amikacin+BB-K29 + BB-K6 + 1,3-dihBA-Kana A + Kana A
(585.62) (722.76)

CG-50 (NH₄⁺)

Amikacin

III. Materials

		Wgt. g.	Vol., ml.	Moles	
5	Kana A 'base'	50		.103	5
	HMDS (Sp. gr. 0.774)	86.68	112	.537	
	Acetonitrile		600		
	SAE	35.03		.10	
	Acetone		850		
10	CG-50 (NH ₄ ⁺)		3000		10
	Methanol		As required		
	IPA		As required		

IV. Safety

Kana A 'base'—
Kana A (Silyl)₁₀—

Known drug—usual caution advised.

No direct information available,
handle with care.

Other materials—

See Example 5

V. Procedure

A. Preparation of Kana A (Silyl)₁₀

1. Slurry 50 g. of Kana A 'base' (KF 2.5—3.5%) in 500 ml. of acetonitrile (KF <0.01%). Bring to reflux (74°) maintaining a stream of dry nitrogen through the slurry.

2. Add slowly over a 30 minute period 112 ml. hexamethyldisilazane (HMDS). Complete solution will occur within 4—5 hours with evolution of ammonia gas.

3. Continue refluxing for 22—26 hours under a nitrogen purge.

4. Concentrate the clear faint yellow solution under vacuum (40°) to a syrupy residue. Flush with an additional 100 ml. acetonitrile, and dry completely under high vacuum for 3—6 hours. Yields of whitish amorphous solid are 109—115 g. (90—95% of theory, calculated as Kana A (Silyl)₁₀).

B. Preparation of n-Hydroxysuccinimide ester of L - (-) - α - carbobenzyloxyamino - α - hydroxybutyric acid (SAE)

1. Dissolve 100 g of L-(-)- α -benzyloxycarbonylamino- α -hydroxybutyric acid (BHBA) and 45.38 g. of N-hydroxysuccinimide (N-HOS) in 1300 ml. of ethyl acetate (KF <0.05%) with stirring at 23°C.

2. Dissolve 81.29 g. of dicyclohexylcarbodiimide (DCC) in 400 ml. of ethyl acetate (KF <0.05%) at 23°C. With good agitation add this solution over 30 minutes to step 1 solution. The temperature will rise to ~40—42°C with concurrent precipitation of dicyclohexylurea (DCU). Agitate the slurry 3—4 hours allowing the temperature to equilibrate to 23°C.

3. Filter the DCU; wash the cake with 250 ml. of ethyl acetate (KF <0.05%). Discard the DCU cake. Save the filtrate and washes.

4. Concentrate the filtrate plus washes to ~500 ml. (in vacuo at 30—35°C). Some product will crystallize out.

5. Transfer the concentrate to a suitable vessel and add with vigorous agitation 100 ml. of heptane. If necessary, add seed crystals of SAE. Crystallization will begin almost immediately. Agitate the slurry for 30 minutes at 23°C.

6. Add, over 30 minutes, 400 ml. of heptane and agitate the slurry 4—5 hours at 23°C.

7. Filter and wash the cake with 200 ml. of 3:1 heptane/ethyl acetate followed by 100 ml. of heptane.

8. Dry in a vacuum oven at 30—35°C for 18—20 hours.

Yield is 110.1—131.4 g. (80—95%).

MP—119—120° with softening at 114° (Corr.).

TLC—4 acetone:12 benzene:1 CH₃CO₂H— Detection 1% aqueous KMO₄.

Rf—0.7 for SAE; 0.2 BHBA on 2×10 cm prescored silica gel plates from Analtech Inc.

C. Acylation of Kana A (Silyl)₁₀

1. Dissolve the Kana A (Silyl)₁₀ isolated in Part A, Step 4 in 500 ml. dry acetone at 23°C.

2. With good agitation add rapidly the SAE prepared in Part B (35.03 g.) as a 10% solution in dry acetone over a 5—10 minute period. The temperature will rise approximately 5°. Allow the solution to equilibrate to 23°, and continue stirring for 18—20 hours.

3. The light orange, clear solution is diluted with 400 ml. of water, and the pH (7.0—7.5) lowered to 2.2—2.5 with 3N hydrochloric acid. The clear solution is now agitated at 23° for 15—30 minutes.

4. Acetone is removed under vacuum at a bath temperature of 30—35° (a small amount of material may separate at this point, but presents no problem). Transfer the concentrate to a suitable hydrogenation vessel. Add 10 g. 5% palladium on carbon catalyst, and hydrogenate at 50 psi for 2—3 hours.

5. Filter the mixture through a Dicalite pad, and wash the hydrogenation vessel and cake with an additional 2×50 ml. water.

6. Concentrate the filtrate plug washings to approximately 1/3 volume (150—165 ml.) under vacuum at 40—45°.

7. The pH at this point is in the range 6.0—7.0. The mixture is charged on a CG-50 (NH₄⁺) column (6x110 cm).

8. Wash the column with 1000 ml. of deionized water. Elute with 0.5N ammonium hydroxide using an automatic polarimeter to monitor the progress of elution. The order of elution is as follows:

Residual Kana A→BB-K6→BBK29→Amikacin. No. BB-K11 was detected in any of our experiments.

9. Combine the amikacin fractions and concentrate to 25—30% solids. Dilute with 1 volume methanol, and seed with amikacin crystals.

10. Add slowly over 2 hours 2 volumes of IPA with good stirring and crystallize at 23° for 6—8 hours.

11. Filter the solid, wash with 35 ml. of 1:1:2 water/methanol/IPA, and finally with 35 ml. IPA.

12. Dry in a vacuum over at 40° for 16—24 hours. Yield: 19.91—22.84 g. (34—39%) IR, PMR and CMR spectral data in addition to specific rotation were completely consistent for the desired structure.

TLC System

CHCl₃/methanol/NH₄OH/water (1:4:2:1) 5x20 cm. silica gel plates from Quantum Industries—1 Zone amikacin having R_f~0.4 (Ninhydrin Detection).

Example 7

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Tetrahydrofuran With the Mixed Acid Anhydride of Pivalic Acid and BHBA

A. Preparation of Mixed Anhydride

BHBA (5.066 gm., 20.0 m. moles), BSA (4.068 mg., 20.0 m. moles) and triethylamine (2.116 g., 22.0 m. moles) were dissolved in 200 ml. of sieve dried tetrahydrofuran. The solution was refluxed for 2 1/4 hours and then chilled to -10°C. Pivaloyl chloride (2.412 gm., 20.0 m. moles) was added over a period of 2—3 minutes, with stirring, and stirring was continued for 2 hours at -10°C. The temperature was then allowed to climb to 23°C.

B. Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A

Poly(trimethylsilyl) 6'-N-Cbz Kana A prepared as in Example 1 (5.454 gm., 4.97 m. moles, calculated as 6'-Cbz Kana A (silyl)₁₀) was dissolved in 50 ml. dry (molecular sieve) tetrahydrofuran at 23°C. One-half of the solution of mixed anhydride prepared in step A, above, (10.0 m. moles) was added over a period of twenty minutes, with stirring, and stirring was continued for 7 days.

Water (100 ml.) was then added to the reaction mixture, and the pH (5.4) was adjusted to 2.0 with 3M H₂SO₄. Stirring was continued for 1 hour and the solution was extracted with ethyl acetate. Polyacylated material began to crystallize, so the reaction mixture was filtered. After drying over P₂O₅, the recovered solids weighed 0.702 gms. The extraction of the reaction mixture was continued for a total of 4x75 ml. of ethyl acetate, after which the excess ethyl acetate was stripped from the aqueous layer. An aliquot of the aqueous solution was subjected to assay by HPLC. The resulting curve indicated a 26.4% yield of di-Cbz amikacin.

The aqueous layer was then hydrogenated in a Parr apparatus at 50 p.s.i. H₂ pressure for two hours, using 0.5 gm. 10% Pd on carbon catalyst. The material was filtered, and the combined filtrate and washings were determined against *E. coli* to contain a 31.2% yield of amikacin. Amikacin/BB-K29 ratio approximately 9—10/1; traces of polyacyl and unreacted Kana A present.

Example 8

Effect of Water on the Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kana A in Acetone Solution at 23°C.

A. Anhydrous Solvent

Poly(trimethylsilyl) Kana A prepared as in Example 3 (2.40 gm., 2.0 m. moles, calculated as Kana A (silyl)₁₀) was dissolved in 20 ml. of acetone which had been dried with a molecular sieve. The solution was stirred at 23°C and a solution of SAE (0.701 gm., 2.0 m. moles) in 10 ml. of sieve dried acetone was added over a period of 10 seconds. Stirring was continued at 23°C for 22 hours. Water (50 ml.) was added and the pH (7.5) was adjusted to 2.5. The acetone was stripped *in vacuo* at 40°C and the aqueous solution was then reduced at 51 p.s.i. H₂ pressure at 23°C

for two hours, utilizing 1.0 gm. of 10% Pd on carbon as catalyst. Microbiological assay showed a 31.24% yield of amikacin.

B. Water Added to Solvent

Step A, above, was repeated, except that 1.0 ml. (56 m. moles) of water was added to the poly(trimethylsilyl) Kana A solution, and stirred for 15 minutes, prior to acylation with SAE. Microbiological assay showed a 33.80% yield of amikacin.

Example 9

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Acetone with the Mixed Anhydride of BHBA and Isobutylcarbonic Acid

A. Preparation of Mixed Anhydride

BHBA (1,267 gm., 5.0 m. moles) and N-trimethylsilylacetamide (MSA) (1,313 gm., 10.0 m. moles) in 20 ml. of sieve dried acetone was stirred at 23°C, and triethylamine (TEA) (0.70 ml., 5.0 m. moles) were added. The mixture was refluxed under a N₂ atmosphere for 2 1/2 hours. The mixture was cooled to -20°C and isobutylchloroformate (0.751 gm., 0.713 ml., 5.50 m. moles) was added. Triethylamine hydrochloride immediately began to separate. The mixture was stirred for 1 hour at -20°C.

B. Acylation

Poly(trimethylsilyl) 6'-N-Cbz Kana A prepared as in Example 1 (6.215 gm., 4.9 m. moles, calculated as the (silyl)₆ compound) was dissolved in 20 ml. of sieve dried acetone, with stirring, at 23°C. The solution was cooled to -20°C and the cold mixed anhydride solution from step A was slowly added over a period of 30 minutes. The reaction mixture was stirred for an additional 1 1/2 hours at -20°C and then for 17 hours at 23°C. The reaction mixture was then poured into 150 ml. of water at 23°C with stirring, the pH (7.75) was adjusted to 2.5 with 3N HCl, and stirring was continued for 15 minutes. Acetone was then stripped *in vacuo* at 40°C. An aliquot of the resulting aqueous solution was subjected to assay by HPLC. The resulting curve indicated a 34.33% yield of di-Cbz amikacin.

The main portion of the aqueous solution was reduced at 50 p.s.i. H₂ pressure at 23°C for 3 1/4 hours, utilizing 2.0 gms of Pd/C catalyst. The catalyst was removed by filtration and the combined filtrate and washings were determined by microbiological assay against *E. coli* to contain a 35% yield of amikacin.

Example 10

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in 3-Pentanone

Poly(trimethylsilyl) 6'-N-Cbz Kana prepared as in Example 1 (30 gm., 23.65 m. moles, calculated as 6'-N-Cbz Kana A (silyl)₆) dissolved in 100 ml. sieve dried 3-pentanone was stirred at 23°C, and NAE (26.02 m. moles, 10% excess) was added over a period of 40 minutes. Stirring was continued for 113 hours at 23°C and the mixture was then added to 250 ml. water with vigorous stirring. The pH (7.3) was adjusted to 2.5 with 3N HCl, the mixture was stirred for an additional 30 minutes, and the 3-pentanone was stripped *in vacuo* at 40°C. The aqueous solution was extracted with 4x100 ml. of ethyl acetate. An aliquot of the aqueous solution was then subjected to assay by HPLC. The resulting curve indicated a 46.12% yield of di-Cbz amikacin.

The main portion of the aqueous reaction mixture was reduced at 51.0 p.s.i. H₂ pressure at 23°C for 2 1/2 hours, utilizing 3.0 gms. of 10% Pd/C catalyst. Microbiological assay of an aliquot of the combined filtrate and washings indicated a 40.24% yield of amikacin. The main portion of the reduced aqueous reaction mixture was then concentrated *in vacuo* at 40°C to approximately 100 ml. and fractionated on a CG-50 (NH₄⁺) ion exchange column (4 inchesx4 feet, containing approximately 10 liters of resin). The aqueous solution was charged on the column, the column was washed with 5 liters of water, and the material was eluted with 0.5N NH₄OH (followed by 3N NH₄OH to elute polyacetylated products). Polarimetry of the fractions showed the presence of a 42.7% yield of amikacin, a 12.0% yield of unreacted kanamycin A, a 12.4% yield of polyacylated material and a 23.2% yield of BB-K29.

Example 11

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Anhydrous Cyclohexanone For Varying Times

A. Poly(trimethylsilyl) 6'-N-Cbz Kana A prepared as in Example 1 (2.537 gm.,

2.0 m. moles, calculated as 6'-N-Cbz Kana A (silyl)₆) in 300 ml. dry cyclohexanone was acylated for 20 hours at 23°C with an NAE solution in dry cyclohexanone (10.8 ml. of 0.1944 m. mole/ml. solution, 2.10 m. mole). The reaction mixture was then added to 150 ml. of water, with stirring, and the pH (5.6) was adjusted to 2.5 with 3N HCl. The cyclohexanone was stripped *in vacuo* at 40°C and an aliquot of the remaining aqueous phase was taken for assay by HPLC. The main portion of the aqueous phase was reduced under 50 p.s.i. H₂ pressure for 3 hours at 23°C, using 1.0 gm. of 10% Pd/C catalyst. The catalyst was removed by filtration and the combined filtrate and washings were assayed microbiologically for amikacin.

B. Reaction A, above, was repeated, except that the acylation was continued for 115 hours instead of 20 hours.

		Yields			
		HPLC Assay (di-Cbz Amikacin)	Microbiological Assay Amikacin Turbidimetric	Plate	
15	Reaction A	49.18%	42.87%	39.16%	15
	Reaction B	56.17%	55.39%	38.45%	

Example 12

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Anhydrous Tetrahydrofuran For Varying Times

20 A. Example 11 A was repeated except that dry tetrahydrofuran was utilized as solvent instead of dry cyclohexanone.

B. Example 11 B was repeated except that dry tetrahydrofuran was utilized as solvent instead of dry cyclohexanone.

		Yields			
		HPLC Assay (di-Cbz Amikacin)	Microbiological Assay Amikacin Turbidimetric	Plate	
25	Reaction A	29.27%	28.34%	28.18%	25
	Reaction B	33.39%	21.52%	28.63%	

Example 13

30 Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Anhydrous Dioxane For Varying Times

A. Example 11 A was repeated except that the acylation was continued for 44 hours utilizing dry dioxane as the solvent.

35 B. Example 11 B was repeated except that the acylation was continued for 18 1/2 hours utilizing dry dioxane as the solvent.

		Yields			
		HPLC Assay (di-Cbz Amikacin)	Microbiological Assay Amikacin Turbidimetric	Plate	
40	Reaction A	39.18%	43.27%	33.36%	40
	Reaction B	42.82%	22.55%	33.37%	

Example 14

Preparation of Amikacin by Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A in Anhydrous Diethyl ketone at 75°C

45 To a stirred solution of poly(trimethylsilyl) 6'-N-Cbz Kana A prepared as in Example 1 (2.537 gm., 2.0 m. moles, calculated as 6'-N-Cbz Kana A (silyl)₆) in 32 ml. sieve dried diethyl ketone at 75°C was added a solution of NAE (10.8 ml. of 0.1944 m. moles/ml. of diethyl ketone, 2.10 m. moles) over a period of 15 minutes. Stirring was continued at 75°C for an additional 3 hours after which the mixture was poured into 150 ml. of water. The pH was adjusted to 2.8 with 3N HCl and the diethyl ketone was stripped *in vacuo* at 40°C. HPLC assay of an aliquot of the aqueous phase indicated a 39.18% yield of di-Cbz amikacin.

50 The main portion of the aqueous phase was reduced under 49.8 p.s.i. H₂ pressure for 3 1/4 hours at 23°C, using 1.0 gram of Pd/C catalyst. The catalyst was removed by filtration and the combined filtrate and washings were assayed microbiologically for amikacin. Turbidometric assay showed 27.84% yield and Plate assay showed 28.6% yield.

55

Example 15

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kana A with NAE at 0—5° After Back Hydrolysis With Water

A. Silylation of Kanamycin A Using HMDS With TMCS as Catalyst

Kanamycin A (10 gm of 97.6% purity, 20.14 m. moles) in 100 ml. of sieve-dried acetonitrile was brought to reflux under a nitrogen atmosphere. A mixture of HMDS (22.76 gm., 141 m. moles, 7 moles per mole of Kanamycin A) and TMCS (1 ml., 0.856 gm., 7.88 m. moles) was added to the refluxing reaction mixture over a period of 10 minutes. Reflux was continued for 4-3/4 hours and the mixture was then cooled, concentrated *in vacuo* to a yellow viscous syrup and dried under high vacuum for 2 hours. The yield of product was 23.8 gms. (97.9%, calculated as Kanamycin A (silyl)₁₀).

B. Acylation

Poly(trimethylsilyl) kanamycin A (23.8 gms., 20.14 m. moles) prepared in step A above was dissolved in 250 ml. of sieve-dried acetone at 23° and then cooled to 0—5° Water (3.63 ml, 201.4 m. moles, 10 moles per mole of polysilylated kanamycin A) was added, with stirring, and the mixture was allowed to stand under moderate vacuum for 30 minutes. NAE (19.133 m. moles, 0.95 moles per mole of polysilylated kanamycin A) in 108.3 ml of acetone was then added over a period of <1 minute. The mixture was stirred at 0—5° for 1 hour, diluted with water, the pH adjusted to 2.5, and the acetone was then removed *in vacuo*. The aqueous solution was then reduced at 50 p.s.i. H₂ pressure at 23° for 2-1/2 hours using 2.0 gms of 10% Pd on carbon as a catalyst. The reduced reaction mixture was filtered through Dicalite, concentrated to ca. 100 ml. *in vacuo* at 40° and then charged on CG-50(NH₄⁺) column (6 liters resin, 5×100 cm.). It was washed with water and then eluted with 0.6N—1.0N—3N NH₄OH. There was obtained 60.25% amikacin, 4.37% BB-K6, 4.35% BB-K29, 26.47% kanamycin A and 2.18% polyacyls.

Example 16

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) 6'-N-Cbz Kana A with SAE at 0—5° After Back Methanolysis

A. Silylation of 6'-N-Cbz Kanamycin A

6'-N-Cbz kanamycin A (20.0 gm., 32.4 m. moles) in 200 ml. of sieve-dried acetonitrile was brought to reflux under a nitrogen atmosphere. HMDS (47.3 ml., 226.8 m. moles, 7 moles per mole of 6'-N-Cbz kana A) was added over a 10 minute period and reflux was continued for 20 hours. The mixture was then cooled, concentrated *in vacuo*, and dried under high vacuum for 2 hours to give 39.1 gms. of white amorphous solid (95.4% yield, calculated as 6'-N-Cbz kana A (silyl)₉).

B. Acylation

Poly(trimethylsilyl) 6'-N-Cbz kana A (39.1 gm., 32.4 m. moles) prepared in step A above was dissolved in 400 ml. of dry acetone, with stirring, at 23°. Methanol (6.6 ml., 162 m. moles, 5 moles per mole of polysilylated 6'-N-Cbz kana A) was added and the mixture was stirred at 23° for 1 hour under a strong nitrogen purge. The mixture was cooled to 0—5° and a solution of SAE (11.35 gm., 32.4 m. moles) in 120 ml. of pre-cooled, dry acetone was added. The mixture was stirred for an additional 3 hours at 0—5° and then placed in a 4° cold room for 1 week. Water (300 ml) was added, the pH was adjusted to 2.0, the mixture was stirred for 1 hour, and the acetone was then stripped *in vacuo*. The resultant aqueous solution was reduced at 54.0 p.s.i. H₂ pressure for 17 hours at 23° utilizing 3.0 gm. of 10% Pd on carbon as catalyst. It was then filtered through Dicalite, concentrated *in vacuo* to 75—100 ml., charge on a CG-50(NH₄⁺) column and eluted with water and 0.6N NH₄OH. There was obtained 52.52% amikacin, 14.5% BB-K29, 19.6% kanamycin A and 1.71% polyacyls.

Example 17

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kana A With SAE at 0—5° After Back Hydrolysis With Water

A. Silylation of Kanamycin A with TMCS in Acetonitrile Using Tetramethylguanidine as Acid Acceptor

Kanamycin A (4.88 gm., 10.07 m. mole) was suspended in 100 ml. of sieve-dried acetonitrile with stirring at 23°. To the stirred suspension was added tetramethylguanidine (TMG) (16.234 gm., 140.98 m. moles, 14 moles per mole of kanamycin A). The mixture was heated to reflux and TMCS (15.32 gm., 140.98 m.

(32)

moles, 14 moles per mole of kanamycin A) was added over a 15 minute period. A white precipitate of TMG · HCl formed after about one-half of the TMCS had been added. The mixture was cooled to room temperature, concentrated to a tacky residue and dried under high vacuum for 2 hours. The solid was triturated with dry THF (100 ml.), and the insoluble TMG · HCl was filtered off and washed with 5×20 ml. portions of THF. The combined filtrate and washings were concentrated *in vacuo* at 40° to a tacky residue and dried under high vacuum for 2 hours. There was obtained 10.64 gms. of a light cream tacky residue (87.6% yield, calculated as kanamycin A (silyl)₁₀).

10 B. Acylation

Poly(trimethylsilyl) kanamycin A (10.64 gm., 10.07 m. moles) prepared in step A above was dissolved in 110 ml. of sieve-dried acetone, with stirring, at 23° and the solution was cooled to 0–5°. Water (1.81 ml., 100.7 m. moles, 10 moles per mole of polysilylated kana A) was added and the solution was stirred for 30 minutes under moderate vacuum. SAE (3.70 gm., 10.57 m. moles, 5% excess) in 40 ml. of pre-cooled dry acetone was added over a period of <1 minute, and the mixture was stirred for one hour. The mixture was worked up by the general procedure in Example 16B to give ca. 50% amikacin, ca. 10% BB-K29, 5–8% BB-K6, ca. 20% kanamycin A and 5–8 polyacyls.

20 Example 18

Preparation of Poly(trimethylsilyl) Kanamycin A in Pyridine Using HMDS

Kanamycin A (10.0 gms., 20.64 m. moles) was suspended in 100 ml. sieve-dried freshly distilled pyridine at 23°. A nitrogen purge was started and the suspension was brought to reflux. HMDS (17.33 gms., 107.32 m. moles, 5.2 moles per mole of kanamycin A) was added over a period of 10 minutes and the mixture was refluxed for 19 hours. It was then cooled to room temperature, concentrated *in vacuo* to a light yellow-gold syrup, and dried under high vacuum to a white amorphous solid. There was obtained 22.1 gms. (92.6% yield, calculated as kanamycin A (silyl)₁₀).

30 Example 19

Preparation of Poly(triethylsilyl) Kanamycin A Using Triethylchlorosilane With Triethylamine as Acid Acceptor

Kanamycin A (5.0 gms. of 97.6% purity, 10.07 m. moles) was suspended in 100 ml. of sieve-dried acetonitrile at 23°. Triethylamine (TEA) (33.8 ml., 24.5 gm., 241.7 m. moles) was added and the suspension was brought to reflux. A solution of trichloroethylsilane (23.7 ml., 21.3 gm., 140.98 m. moles) in 25 ml. dry acetonitrile was added over a 20 minute period. Reflux was continued for an additional 7 hours and the mixture was cooled to room temperature, whereupon long fine needles of TEA · HCl separated out. The mixture was allowed to stand at room temperature for ca. 16 hours, concentrated *in vacuo* at 40° to a tacky solid and dried for 2 hours under high vacuum to a deep orange tacky solid. The solid was triturated with 100 ml dry THF at 23° and the insoluble TEA · HCl was filtered off, washed with 5×20 ml of THF, and dried to give 16.0 gms of TEA · HCl. The combined filtrate and washings were concentrated *in vacuo* to a solid and dried under high vacuum for 2 hours. There was obtained 19.3 gms of poly(triethylsilyl) kanamycin A as a deep orange viscous syrup.

45 Example 20

Preparation of Poly(trimethylsilyl) Kanamycin A Using bis-Trimethylsilylurea

Kanamycin A (10.0 gm. of 99.7% purity, 20.58 m. moles) was suspended in 200 ml. of sieve-dried acetonitrile, with stirring, at 23°. To the suspension was added bis-trimethylsilylurea (BSU) (29.45 gms., 144.01 m. moles, 7 moles per mole of kanamycin), and the mixture was brought to reflux under a nitrogen atmosphere. Reflux was continued for 17 hours and the reaction mixture was then cooled to room temperature. A small amount of insoluble material present was removed by filtration, washed with 3×10 ml. portions of acetonitrile and dried (1.1381 gms.). Infrared showed this to be BSU plus a small amount of unreacted kanamycin A. The combined filtrate and washings were cooled at 4° for 16 hours. Additional solid separated, was recovered as above, (7.8 gms.) and was shown by infrared to be BSU plus urea. The light yellow filtrate and washings were concentrated *in vacuo* at 40° and dried under high vacuum to give 27.0 gm. of a white solid which was partly tacky and partially fine needle-like crystals. The solid was treated with 150 ml. of heptane at 23°, the insoluble portion was removed by filtration, washed with 2×50 ml. portions of heptane and dried, to give 6.0 gms. of white needles (shown by

infrared to be BSU plus urea). The combined filtrate and washings were concentrated *in vacuo* at 40° and dried under high vacuum for 2 hours to give 20.4 gms. of white needles, the infrared spectrum of which was typical for polysilylated kanamycin A. Calculations showed the product to contain an average of 7.22 trimethylsilyl groups.

Example 21

Preparation of Amikacin by the Acylation of Per(trimethylsilyl) Kanamycin A After Partial Desilylation With 1,3-Butanediol

A. Preparation of Per(trimethylsilyl) kanamycin A

Kanamycin A (10.0 gm., 20.639 m. moles) was suspended in 100 ml. of sieve-dried acetonitrile, with stirring, at 23°. The suspension was brought to reflux under a nitrogen purge and HMDS (23.322 gms., 144.5 m. moles, 7 moles per mole of kanamycin A) was added over a period of ten minutes. Reflux was continued for 16 hours and the mixture was then cooled to room temperature, concentrated *in vacuo* and dried for 2 hours under high vacuum. There was obtained 24.3 gm. of a white, tacky residue (92.1% yield, calculated as kanamycin A (silyl)₁₁).

B. Acylation

Per(trimethylsilyl) kanamycin A (24.3 gm.) prepared in step A above was dissolved in 240 ml. of sieve-dried acetone, with stirring, at 23°. To this solution was added 1,3-butanediol (9.25 ml., 103.2 m. mole), 5 moles per mole of per(trimethylsilyl) kanamycin A. The mixture was stirred at 23° for 2 hours under a nitrogen purge and then cooled at 0—5°. SAE (7.23 gm., 20.64 m. moles) in 70 ml. of pre-cooled acetone was added over a period of about 1 minute. The mixture was stirred at 0—5° for 3 hours and then allowed to stand in a 4° cold room for ca. 16 hours. Water (200 ml.) was added, the pH was adjusted to 2.5 and the clear yellow solution was stirred at 23° for 30 minutes. The acetone was stripped *in vacuo* and the aqueous solution was reduced at 55.0 p.s.i. H₂ pressure at 23° for 2 hours using 3.0 gm. of 10% Pd on carbon as catalyst. The reduced solution was filtered through Dicalite and chromatographed as in Example 16B to give 47.50% amikacin, 5.87% BB-K29, 7.32% BB-K6, 24.26% kanamycin A and 7.41% polyacyls.

Example 22

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kanamycin A Prepared in THF Using SAE With Sulfamic Acid Catalyst

To a refluxing mixture of kanamycin A (5.0 gm., 10.32 m. moles) in 50 ml. of sieve-dried tetrahydrofuran (THF) were added sulfamic acid (100 mg.) and HMDS (12.32 gm., 76.33 m. moles). The mixture was refluxed for 18 hours, with complete solution occurring after 6 hours. The solution was cooled to 23°, treated with 0.1 ml. of water and held at 23° for 30 minutes. A solution of SAE (3.61 gm., 10.3 m. moles) in 36 ml. of THF was added over a period of 30 minutes. After stirring for 3 hours, the mixture was diluted with 100 ml. of water and the pH was adjusted to 2.2 with 10% H₂SO₄. It was stirred for 30 minutes at 23° and then concentrated *in vacuo* to remove THF. The resulting aqueous solution was reduced at 50 p.s.i. H₂ pressure for 2 hours at 23° using 10% Pd on carbon as a catalyst. The reduced solution was filtered through Dicalite and the solids were washed with water. The combined filtrate and washings (150 ml.) were determined by microbiological assay against *E. coli* to contain 1225 mcg./ml. (31.5% activity yield) of amikacin.

Example 23

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kanamycin A with the N-Hydroxysuccinimide Ester of Di-Carbobenzyloxy AHBA

A. Preparation of Dicarbobenzyloxy L-(—)- α -Amino- α -hydroxybutyric Acid N-Hydroxysuccinimide Ester

Dicarbobenzyloxy L-(—)- α -amino- α -hydroxybutyric acid (8 gm., 20.65 m. moles) and N-hydroxysuccinimide (2.37 gm., 20.65 m. moles) were dissolved in 50 ml. of dry acetone at 23°. Dicyclohexylcarbodiimide (4.25 gm., 20.65 m. moles) dissolved in 20 ml. of dry acetone was added and the total was agitated at 23° for 2 hours. Dicyclohexylurea was filtered off, the filter cake was washed with 10 ml. of dry acetone, and the filtrate and washings were combined.

B. Acylation

Poly(trimethylsilyl) kanamycin A, prepared according to the general procedure of Example 21 from 10.0 gms. (20.639 m. moles) of kanamycin A, was

dissolved in 100 ml. of dry acetone. The solution was cooled to 0—5°, 3.7 ml. of deionized water was added, and the solution was stirred at 0—5° for 30 minutes under moderate vacuum.

5 To this solution was added the solution of the di-Cbz-blocked acylating agent prepared in step A, and the mixture was stirred at 0—5° for 30 minutes. The mixture was diluted with water, the pH was adjusted to 2.2 and the acetone was removed *in vacuo*. The aqueous solution was reduced by the general procedure of Example 22 and then filtered through Dicalite. Chromatography showed 40—45% amikacin, ca. 10% BB-K29, a trace of BB-K6, ca. 30% kanamycin A and a small amount of polyaclys.

Example 24

Preparation of Poly(trimethylsilyl) Kanamycin A Using HMDS with Imidazole as Catalyst

15 Kanamycin A (11 gm., 22.7 m. moles) and 100 mg of imidazole were heated to reflux in 100 ml. of sieve-dried acetonitrile, under a nitrogen purge HMDS (18.48 gm., 114.5 m. moles, 5 moles per mole of kanamycin A) was added over a period of 30 minutes and the mixture was refluxed for 20 hours. Complete solution occurred in ca. 2-1/2 hours. The solution was cooled to 23° and the solvent was removed *in vacuo* to leave 21.6 gms. of poly(trimethylsilyl) kanamycin A as a foamy residue (93.1% yield, calculated as kanamycin(silyl)₁₁).

Example 25

Preparation of 1-N-[L-(—)- γ -Amino- α -hydroxybutyryl]kanamycin B (BB-K26) by the Acylation of Poly(trimethylsilyl) Kanamycin B With SAE

25 A. Preparation of Poly(trimethylsilyl) Kanamycin B Using HMDS With TMCS Catalyst

25 Kanamycin B (25 gm., 51.7 m. moles) in 250 ml. of sieve-dried acetonitrile was heated to reflux under a stream of nitrogen. HMDS (62.3 gm., 385.81 m. moles, 7.5 moles per mole of kanamycin B) was added over a period of 30 minutes followed by 1 ml. of TMCS as catalyst. The mixture was refluxed for 21 hours with complete solution after 1 hour. The solvent was then removed *in vacuo* at 60° and the oily residue was held at 60° under high vacuum for 3 hours. There was obtained 53.0 gm. of poly(trimethylsilyl) kanamycin B (85.2% yield, calculated as kanamycin B (silyl)₁₀).

35 B. Acylation

35 The poly(trimethylsilyl) kanamycin B prepared in step A above (53.0 gm.) was dissolved in 500 ml. of dry acetone at 0—5°, methanol (20.9 ml.) was added, and the mixture was stirred *in vacuo* for 30 minutes at 0—5°. A solution of SAE (18.1 gm., 51.67 m. moles) in 200 ml. of pre-cooled dry acetone was added over a period of less than 1 minute and the mixture was stirred for 30 minutes at 0—5°. The mixture was worked up according to the general procedure of Example 22 and then loaded on a column of CG-50 (NH₄⁺) (8×120 cm.). It was eluted with an NH₄OH gradient from 0.6N to 3N. There was obtained 38% of BB-K26, 5% of the corresponding 6'-N-acylated kanamycin B (BB-K22), 10% of the corresponding 3-N-acylated kanamycin B (BB-K46) 14.63% kanamycin B and a small amount of polyacylated kanamycin B.

Example 26

Preparation of Poly(trimethylsilyl) kanamycin A Using HMDS With Kanamycin A Sulfate as Catalyst

50 Kanamycin A (19.5 gm., 40.246 m. moles) and kanamycin A sulfate (0.5 gm., 0.858 m. mole) [total=20.0 gm., 41.0 m. moles] in 200 ml. of sieve-dried acetonitrile was brought to reflux. HMDS (60.3 ml., 287.7 m. moles, 7 moles per mole of kanamycin A) was slowly added and the mixture was refluxed for 28 hours. It was then stripped to dryness on a rotary evaporator and dried under steam injector vacuum. There was obtained 47.5 gms. of poly(trimethylsilyl) kanamycin A as a pale yellow oil (95.82% yield, calculated as kanamycin A (silyl)₁₀).

Example 27

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kanamycin A With N-Trifluoroacetyl Blocked AHBA N-Hydroxysuccinimide Ester

60 A. Preparation of N-Trifluoroacetyl AHBA and Conversion to its N-Hydroxysuccinimide Ester

To a suspension of AHBA (5.0 gm., 42 m. moles) in 100 ml. THF was added trifluoroacetic anhydride (40 gm., 191 m. moles), with stirring, over a 10 minute

period. The solution was stirred for 18 hours at 23° and then concentrated to dryness *in vacuo* at 50°. The residue was dissolved in 100 ml. of aqueous methanol (1:1) and stirred for 1 hour. It was then concentrated to dryness *in vacuo* and redissolved in 50 ml. H₂O. The aqueous solution was extracted with 3×50 ml. portions of MIBK and, after drying over Na₂SO₄, the extract was concentrated to an oil. Traces of solvent were removed by adding and distilling off 4 ml. of water. On standing the oil changed to a waxy, crystalline solid (2.5 gm., 28% yield).

The N-trifluoroacetyl AHBA (2.4 gm., 11.3 m. moles) was dissolved in 50 ml. dry acetone and N-hydroxysuccinimide (1.30 gm., 11.31 m. moles) was added to the solution. A solution of dicyclohexylcarbodiimide (2.33 gm.) in 20 ml. of dry acetone was slowly added. The reaction mixture was stirred for 2 hours at 23° and the precipitated dicyclohexylurea was removed by filtration and washed with a small amount of acetone. The combined filtrate and washings (a solution of the N-hydroxysuccinimide ester of N-trifluoroacetyl AHBA) was utilized in the next step without isolation.

B. Acylation

To a solution of poly(trimethylsilyl) kanamycin A prepared as in Example 26 (11.31 m. moles) in 54 ml. of acetone was added 2.0 ml. (113.4 m. moles) of water, and the mixture was stirred *in vacuo* at 0–5° for 30 minutes. The N-hydroxysuccinimide ester of N-trifluoroacetyl AHBA prepared in step A above (11.31 m moles) was added to the mixture and it was then held at 5° for 1 hour. The pH was then adjusted to ca. 2.0 with 20% H₂SO₄; the mixture was stirred for 30 minutes and the pH was then raised to ca. 6.0 with NH₄OH. The mixture was then stripped to dryness in a rotary evaporator to give 14.4 gm. of a tacky off-white solid. The solid was dissolved in 100 ml. of water the pH was raised from 5.5 to 11.0 with 10 N NH₄OH and the solution was heated in an oil bath at 70° for 1 hour. The pH (9.5) was then lowered to 7.0 with HCl, the solution was polish filtered to remove a small amount of insolubles and the filter was washed with water. The combined filtrate and washings (188 ml.) was applied to a CG-50 (NH₄⁺) column (8×90 cm.), washed with 2 liters of water and eluted with a NH₄OH gradient (0.6N–1.0N-concentrated). There was obtained 28.9% amikacin, 5.0% BB-K6, 5.7% BB-K29, 43.8% kanamycin A, 3.25% polyacetyls plus 14.3% of an unknown material which was in the first fraction off the column.

Example 28

Preparation of Amikacin by the Acylation of Poly(trimethylsilyl) Kanamycin A With *t*-Butyloxycarbonyl Blocked AHBA N-Hydroxysuccinimide Ester

A. Preparation of *t*-BOC AHBA and Conversion to its N-Hydroxysuccinimide Ester

A solution of AHBA (5.0 gm., 42 m. moles) in 100 ml. of water and 20 ml. of acetone was adjusted to pH 10 with 10N NaOH. Over a period of 3–4 minutes was added 11.6 gm. (53 m. moles) of di-*t*-butyl dicarbonate, and the solution was stirred for 35 minutes while maintaining the pH at 10 by the periodic addition of 10N NaOH. The acetone was removed *in vacuo* and the aqueous phase was washed with 40 ml. of ethyl acetate. The pH of the aqueous solution was lowered to 2.0 with 3N HCl and it was then extracted with 3×30 ml. of MIBK. The combined MIBK extracts were dried over Na₂SO₄ and concentrated to a clear oily residue (8.2 gm., 89%).

The *t*-BOC-AHBA (4.25 gm., 19.4 m. moles) was dissolved in 50 ml. of acetone and N-hydroxysuccinimide (2.23 gm., 19.4 m. moles) was added. A solution of dicyclohexylcarbodiimide (4.00 gm., 19.4 m. moles) in 20 ml. of acetone was slowly added and the mixture was stirred for 2 hours at 23°. The precipitated dicyclohexylurea was removed by filtration and was washed with a small amount of acetone. The combined filtrate and washings (a solution of the N-hydroxysuccinimide ester of *t*-BOC-AHBA) was utilized in the next step without isolation.

B. Acylation

To a solution of poly(trimethylsilyl) kanamycin A prepared as in Example 26 (41.28 m. moles) in 94 ml. of acetone was added 3.5 ml. (194 m. moles) of water, and the mixture was stirred *in vacuo* at 0–5° for 30 minutes. The N-hydroxysuccinimide ester of *t*-BOC-AHBA prepared in step A above (19.4 m. moles) was added and the mixture was allowed to stand at 5° for 1 hour. Water (200 ml.) was added and the pH (7.0) was lowered to 2.0 with 20% H₂SO₄. After 30 minutes stirring the pH was

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wherein R is OH or NH₂ and n is 0 or an integer of 1 or 2, or a nontoxic pharmaceutically acceptable acid addition salt thereof, which process comprises acylating polysilylated kanamycin A or B or polysilylated kanamycin A or B



raised to ca. 6.0 with NH_4OH and the mixture was stripped to dryness *in vacuo* to give 36.3 gms. of a golden oil. The oil was dissolved in 200 ml. of trifluoroacetic acid, allowed to stand for 15 minutes and stripped to dryness in a rotary evaporator. The oil was washed with water and the water was flashed off. Concentrated NH_4OH was added to pH 6.0 and was flashed off. The resulting solid was dissolved in water, filtered, and the filter washed with water. The combined filtrate and washings (259 ml.) were loaded on a CG-50 (NH_4^+) column (8×92 cm.), washed with 4 liters of water and eluted with an NH_4OH gradient (0.6N—1.0N-concentrated). There was obtained 40.32% amikacin, 4.58% BB-K6, 8.32% BB-K29, 30.50% kanamycin A and 7.43% polyaclys.

Example 29

The general procedure of Example 1 is repeated, except that the 6'-N-carbobenzyloxykanamycin A used therein is replaced by an equimolar weight of 6'-N-carbobenzyloxykanamycin B, and there is thereby produced 1-N-[L-(−)- γ -amino- α -hydroxybutyryl]-kanamycin B.

Example 30

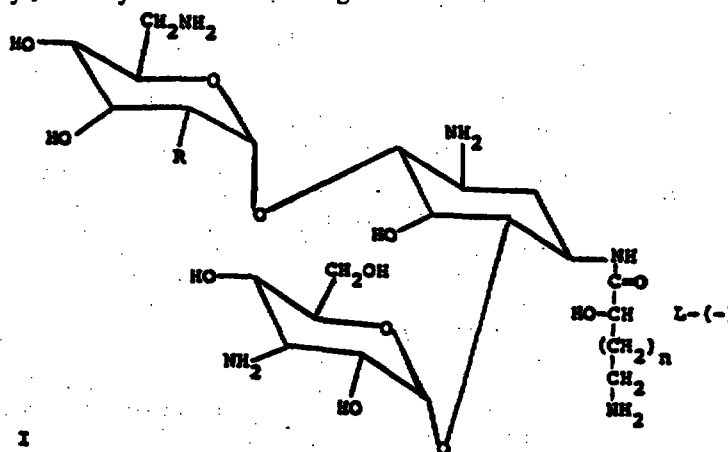
The general procedure of Example 1 is repeated except that the L-(−)- γ -benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxy-5-norbornene-2,3-dicarboximide ester used therein is replaced by L-(−)- β -benzyloxycarbonylamino- α -hydroxypropionic acid N-hydroxy-5-norbornene-2,3-dicarboximide ester and L-(−)- β -benzyloxycarbonyl amino- α -hydroxyvaleric acid N-hydroxy-5-norbornene-2,3-dicarboximide ester, respectively, and there is thereby produced 1-N-[L-(−)- β -amino- α -hydroxypropionyl]kanamycin A and 1-N-[L-(−)- δ -amino- α -hydroxyvaleryl]kanamycin A, respectively.

Example 31

The general procedure of Example 25 is repeated except that the L-(−)- γ -benzyloxycarbonyl amino- α -hydroxybutyric acid N-hydroxy-ester used therein is replaced by L-(−)- β -benzyloxycarbonylamino- α -hydroxy propionic acid N-hydroxysuccinimide ester and L-(−)- δ -benzyloxycarbonylamino- α -hydroxyvaleric acid N-hydroxysuccinimide ester, respectively, and there is thereby produced 1-N-[L-(−)- β -amino- α -hydroxypropionyl]kanamycin B and 1-N-[L-(−)- δ -amino- α -hydroxyvaleryl]kanamycin B, respectively.

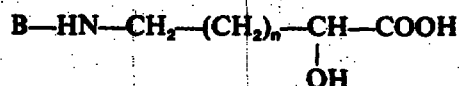
WHAT WE CLAIM IS:—

1. A process for the preparation of a 1-N-[ω -amino- α -hydroxyalkanoyl]kanamycin A or B having the formula



wherein R is OH or NH_2 , and n is 0 or an integer of 1 or 2, or a nontoxic pharmaceutically acceptable acid addition salt thereof, which process comprises acylating polysilylated kanamycin A or B or polysilylated kanamycin A or B

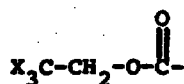
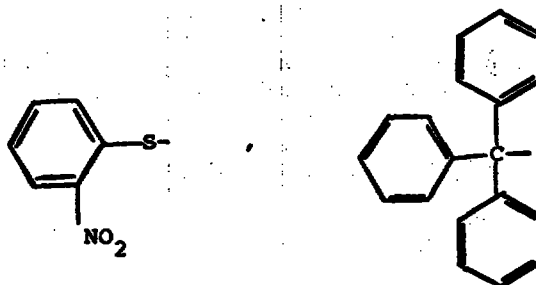
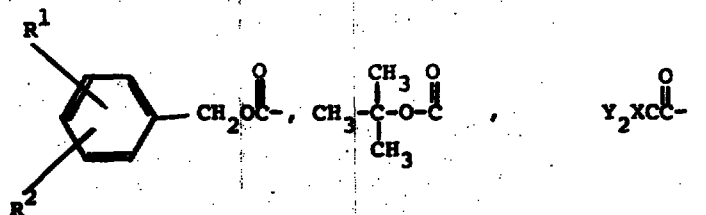
containing a blocking group other than silyl on the 6'-amino moiety with an acylating derivative of the acid of the formula



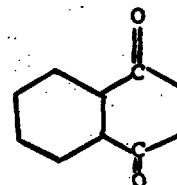
in which n is 0 or an integer of 1 or 2 and B is an amino-blocking group, in a substantially anhydrous organic solvent, and subsequently removing all blocking groups.

2. A process as claimed in claim 1 wherein the acylating derivative of the acid is an active ester of a mixed acid anhydride.

3. A process as claimed in claim 1 or claim 2 wherein the amino-blocking group B of the acylating derivative of the acid is



and



wherein R^1 and R^2 are the same or different and each is H, F, Cl, Br, NO_2 , OH, (lower)alkyl or (lower)alkoxy, and X is Cl, Br, F or I and Y is H, Cl, Br, F or I, and in the case of the last stated blocking group the B—HN grouping then becomes B—N to give rise to phthalimido.

4. A process as claimed in claim 2 or claim 3 wherein the acylating derivative of the acid is its active ester with N-hydroxysuccinimide, N-hydroxy-5-norbornene-2, 3-dicarboximide or N-hydroxyphthalimide.

5. A process as claimed in claim 2 or claim 3 wherein the acylating derivative of the acid is its mixed acid anhydride with pivalic acid, benzoic acid, isobutylcarbonic acid or benzylcarbonic acid.

6. A process as claimed in any one of the preceding claims wherein the polysilylated kanamycin A or B contains a carbobenzyloxy group on the 6'-amino moiety.

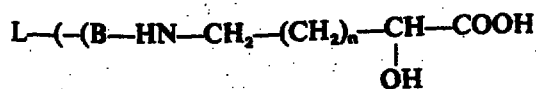
7. A process as claimed in any one of the preceding claims wherein the polysilylated kanamycin A or B starting material contains an average number of silyl groups per molecule of from 4 to 8.

8. A process as claimed in any one of claims 1 to 6 wherein the polysilylated kanamycin A or B starting material contains a blocking group other than silyl on the 6'-amino moiety and also contains an average number of silyl groups per molecule of from 3 to 7.



9. A process as claimed in any of the preceding claims wherein the silyl groups are trimethylsilyl.

10. A process for the preparation of a 1-N(L-(-)- ω -amino- α -hydroxyalkanoyl)kanamycin A as claimed in any one of the preceding claims wherein the acylating derivative of the acid is of the formula



11. A process as claimed in any one of the preceding claims wherein the reaction is conducted in a solution containing 10 to 20% polysilylated kanamycin starting material.

12. A process as claimed in any one of the preceding claims wherein the desired 1-N-acylated product is separated from the reaction mixture by chromatography.

13. A process as claimed in claim 1 substantially as hereinbefore described with reference to any one of the Examples.

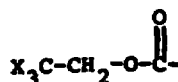
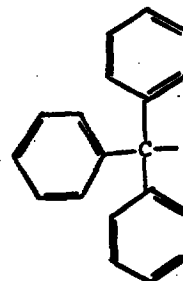
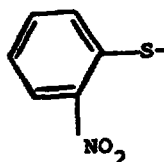
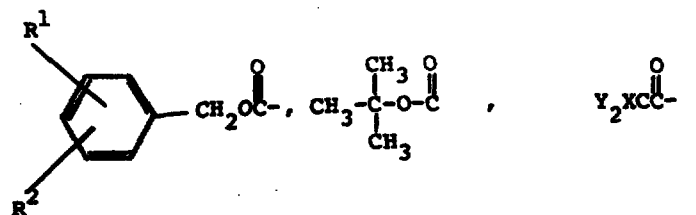
14. Polysilylated kanamycin A or B, or polysilylated kanamycin A or B containing a blocking group other than silyl on the 6'-amino moiety.

15. A polysilylated kanamycin as claimed in claim 14 in which the silyl groups are trimethylsilyl.

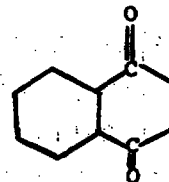
16. Polysilylated kanamycin A as claimed in claim 14 or claim 15 containing an average number of silyl groups per molecule of from 4 to 8.

17. Polysilylated kanamycin A as claimed in claim 14 or claim 15 having a blocking group other than silyl on the 6'-amino moiety and containing an average number of silyl groups per molecule of from 3 to 7.

18. A polysilylated kanamycin A as claimed in any one of claims 14 to 17 in which the blocking group on the 6'-amino moiety is



and



wherein R^1 and R^2 are the same or different and each is H, F, Cl, Br, NO_2 , OH, (lower)alkyl or (lower)alkoxy, and X is Cl, Br, F or I and Y is H, Cl, Br, F or I.

19. A polysilylated kanamycin A as claimed in any one of claims 14, 15, 17 or 18, in which the blocking group on the 6'-amino moiety is the carbobenzyloxy group.

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20. A 1-N-[ω -amino- α -hydroxyalkanoyl]kanamycin A or B whenever prepared by a process as claimed in any one of claims 1 to 13.

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