

Aminocyclitol Antibiotics

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

The aminocyclitol class of compounds includes some of our longest and best known antibiotics, streptomycin and neomycin. It also includes some of the most clinically useful antibiotics, such as gentamicin. Because of their clinical utility, and in spite of their long familiarity, interest in the class remains high and, indeed, seems to be increasing. This volume deals with the current status of structural, synthetic, and biosynthetic studies.

The aminocyclitol class and its members are defined and described in Chapter 1, an introduction complete with figures and references to recent review articles. The longest section of the book follows, devoted to the very extensive recent synthetic efforts directed toward aminocyclitol antibiotics. Sumio Umezawa discusses his recent preparation of neomycin B, the latest in a series of total syntheses that have developed routes useful for preparing modified aminocyclitol antibiotics with improved properties. In Chapter 3, Tetsuo Suami discusses an alternative route to new aminocyclitol antibiotics, that involving modification of an existing antibiotic, describing his own syntheses based on the pseudo-disaccharide neamine as a starting material. In Chapter 4 Juji Yoshimura describes approaches to branched-chain aminocyclitols, employing both Diels–Alder reactions and nitrosugar cyclizations. Other chapters on synthetic research also deal with branched-chain aminocyclitols (Kiely and Riordan, Chapter 5) or neamine and its modification (Magerlein, Chapter 10; Ku et al., Chapter 12). The latter chapter also treats modified kanamycins, as do chapters by Verheyden et al. (Chapter 13) and Cron et al. (Chapter 14), while Chapter 11 by McAlpine et al. treats modified gentamicins and seldomycins. Finally, there are four chapters on spectinomycin—one (by White et al., Chapter 6) describing the first total synthesis of spectinomycin, and three (by Thomas et al.; Rosenbrook and Carney; Foley and Weigele; Chapters 7–9, respectively) dealing with modification of that antibiotic.

The second major section of the book deals with structural studies of aminocyclitol antibiotics. In Chapter 15 Takayuki Naito provides and analyzes a very extensive set of ^{13}C spectra, the most useful spectroscopic data for aminocyclitols' structural assignments. The two other chapters in this section (McAlpine et al., Chapter 16 and Shirahata et al., Chapter 17) assign structures to new fortimicins, aminocyclitols with a 1,4-diamino substitution pattern.

The final section of the book deals with biochemical studies of the aminocyclitols. In Chapter 22 Bernard Davis discusses the multiple mechanisms of action of this class of antibiotics, while Julian Davies (Chapter 18) discusses the other side of the picture, the various mechanisms by which the target bacteria enzymatically inactivate the aminocyclitol antibiotics. In Chapter 19 Kenneth Rinehart discusses the biosynthesis of aminocyclitols and describes a technique based on biosynthetic studies that uses mutants to prepare new antibiotics—a technique that is also the subject of the paper by Cléophax et al. (Chapter 21). Finally, the role of mutasynthetic and chemical techniques in preparing improved clinical candidates in the gentamicin class is assessed by Peter Daniels in Chapter 20.

Overall, then, the present book and the symposium on which it was based point to the vigor of current research on aminocyclitol antibiotics, with both elegant and useful organic chemical syntheses being carried out, and antibiotics of novel structure being discovered. New insights into the mechanisms of action and inactivation of aminocyclitols are emerging and their biosynthesis is not only being better understood, but is leading to new, less toxic compounds.

We would like to thank the authors for their continued patience and cooperation, and Lois Shield and Julia Zvilius for their assistance in preparing this book.

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February 5, 1980

Aminocyclitol Antibiotics: An Introduction

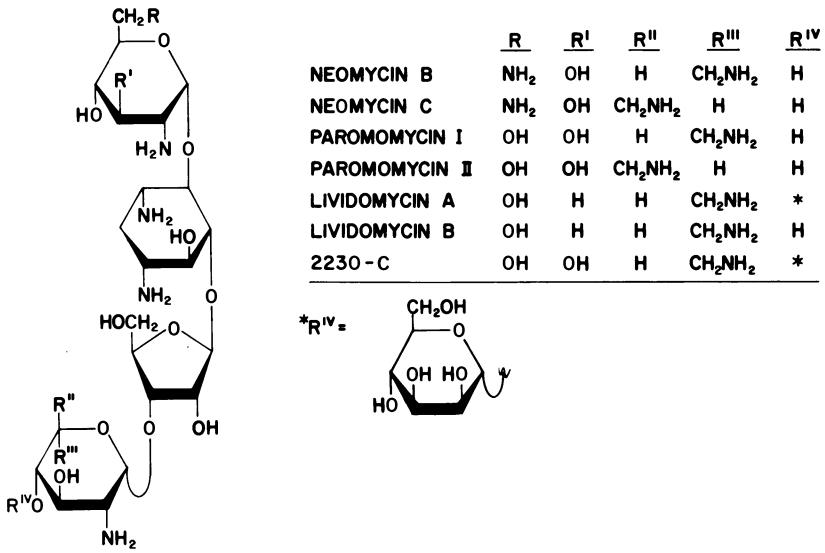
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Aminocyclitol antibiotics constitute an important class of clinically useful therapeutic agents, whose discovery dates from that of streptomycin in 1944. These compounds, which are less precisely, though widely, also referred to as aminoglycoside antibiotics, include some of the most successful commercial antibiotics and new members of the class are being added yearly. Numerous reviews of aminocyclitols are available (1-8), providing references to the individual antibiotics. Thus, the present introductory section will simply indicate the breadth and diversity of the class, providing specific references only to the newer members.

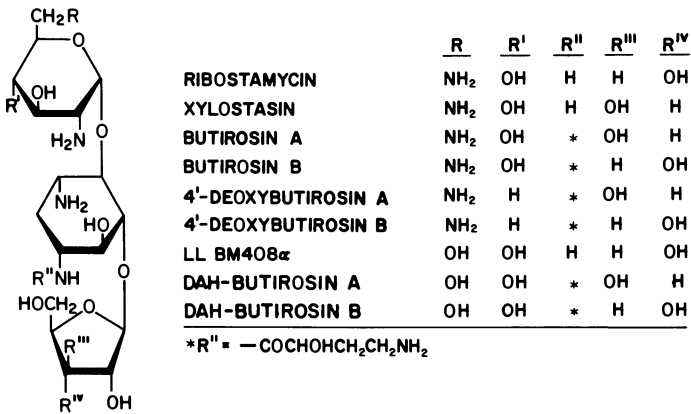
Aminocyclitols can be divided into a very large group which contain 2-deoxystreptamine (a 1,2,3-trideoxy-1,3-diamino analog of scyllo-inositol) and a somewhat smaller group containing other aminocyclitols. The deoxystreptamine antibiotics can be further grouped according to the number and location of substituents attached to the deoxystreptamine unit. 4,5-Disubstituted deoxystreptamines include neomycins, paromomycins, lividomycins and 2230-C (Figure 1). These pseudotetra- and pseudopentasaccharides all contain one or more diaminohexoses. Another group of 4,5-disubstituted deoxystreptamine antibiotics consists of pseudotrisaccharides, including ribostamycin, xylostasin, the butirosins and the compounds now referred to (9) as 6'-deamino-6'-hydroxybutirosins (formerly BU-1709 E₁ and E₂), and LL BM408 α (10) (Figure 2). These compounds are characterized by a di- or mono-aminohexose and a ribose or xylose substituent and in some members of the class the 1-amino group of deoxystreptamine is substituted by an α -hydroxy- γ -aminobutyryl group.

The 4,6-disubstituted deoxystreptamine antibiotics are characterized by the presence at C-4 of a 2-aminohexose (usually modified), together with a second amino (usually a 3-amino) sugar at C-6. These antibiotics include the kanamycins, tobramycin and the nebramycins (Figure 3), the seldomycins (11,12) (Figure 4), as well as the very large group of gentamicins and related compounds (Figure 5), and the dehydro analogs of the gentamicins



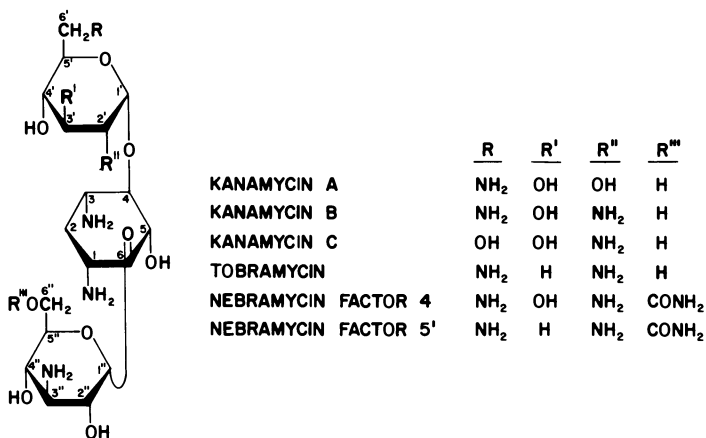
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Figure 1. Neomycin group of 4,5-disubstituted deoxystreptamine antibiotics (1)



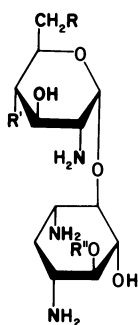
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Figure 2. Ribostamycin group of 4,5-disubstituted deoxystreptamine antibiotics (1)

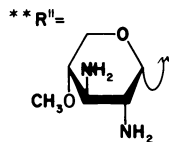
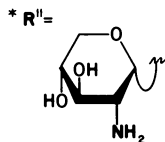


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Figure 3. Kanamycin group of 4,6-disubstituted deoxystreptamine antibiotics (1)

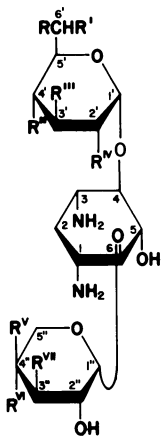


	<u>R</u>	<u>R'</u>	<u>R''</u>
SELDOMYCIN FACTOR 1	OH	OH	*
" " 2	NH ₂	H	H
" " 3	NH ₂	OH	*
" " 5	NH ₂	H	**



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Figure 4. Seldomycin group of 4,6-disubstituted deoxystreptamine antibiotics (1)



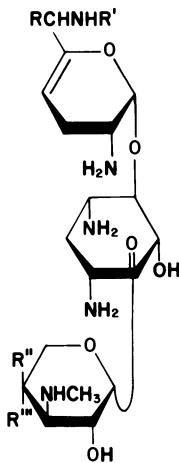
	<u>R</u>	<u>R'</u>	<u>R''</u>	<u>R'''</u>	<u>R^{IV}</u>	<u>R^V</u>	<u>R^{VI}</u>	<u>R^{VII}</u>
GENTAMICIN A	H	OH	OH	OH	NH ₂	H	OH	NHCH ₃
A₁	H	OH	OH	OH	NH ₂	OH	H	NHCH ₃
A₂	H	OH	OH	OH	NH ₂	H	OH	OH
A₃	H	NH ₂	OH	OH	OH	OH	H	NHCH ₃
A₄	H	OH	OH	OH	NH ₂	H	OH	N(CHO)CH ₃
X₂	H	OH	OH	OH	NH ₂	OH	CH ₃	NHCH ₃
B	H	NH ₂	OH	OH	OH	OH	CH ₃	NHCH ₃
B₁	CH ₃	NH ₂	OH	OH	NH ₂	OH	CH ₃	NHCH ₃
C₁	CH ₃	NHCH ₃	H	H	NH ₂	OH	CH ₃	NHCH ₃
C_{1a}	H	NH ₂	H	H	NH ₂	OH	CH ₃	NHCH ₃
C₂	CH ₃	NH ₂	H	H	NH ₂	OH	CH ₃	NHCH ₃
C_{2a}*	CH ₃	NH ₂	H	H	NH ₂	OH	CH ₃	NHCH ₃
C_{2b}† ("C_{2a}")	H	NHCH ₃	H	H	NH ₂	OH	CH ₃	NHCH ₃
G-418	CH ₃	OH	OH	OH	NH ₂	OH	CH ₃	NHCH ₃
JI-20A (GENTAMICIN X_g)	H	NH ₂	OH	OH	NH ₂	OH	CH ₃	NHCH ₃
JI-20B	CH ₃	NH ₂	OH	OH	NH ₂	OH	CH ₃	NHCH ₃

*STEREISOMER OF C₂ at C-6'

†SAGAMICIN

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Figure 5. Gentamicin group of 4,6-disubstituted deoxystreptamine antibiotics (1)



	<u>R</u>	<u>R'</u>	<u>R''</u>	<u>R'''</u>
SISOMICIN	H	H	OH	CH ₃
VERDAMICIN	CH ₃	H	OH	CH ₃
G-52 (Sch I7726)	H	CH ₃	OH	CH ₃
66-40B	H	H	H	OH
66-40D	H	H	OH	H

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Figure 6. Sisomicin group of 4,6-disubstituted deoxystreptamine antibiotics (1)

(Figure 6), including sisomicin. At the present time the gentamicins are the most commercially important of the aminocyclitol antibiotics.

The mono-substituted deoxystreptamines are a considerably smaller and less important group of antibiotics, characterized by a somewhat greater diversity of chemical structure, of which apramycin (13) (Figure 7), with its diaminoctose, is perhaps the strangest. The destomycins, hygromycin B, and the SS-56 components (Figure 8) also belong to this class, most of the class containing the highly unusual destomic acid (aminoheptonic acid) unit. The latter group also provides a transition in that the deoxystreptamine unit is sometimes N-methylated or hydroxyl-substituted. Thus, the destomycin-SS-56 antibiotics have some relationship to spectinomycin (Figure 9), in which the aminocyclitol (actinamine) is fully substituted and the amino groups are methylated.

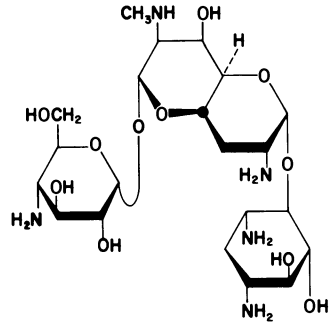
The final examples of diaminocyclitol antibiotics containing amino groups (substituted) in the 1- and 3-positions are the streptomycins (Figure 10), including bluensomycin, which contains a substituted monoaminocyclitol. These are characterized by guanidines, with carbamimidoyl groups attached to the amino groups in the aminocyclitol ring, as well as by a branched chain pentose, streptose or dihydrostreptose, and N-methyl-L-glucosamine.

A recently discovered group of diaminocyclitols contains the fortimicins (14) and the sporaricins (15) (Figure 11), compounds containing substituted 1,4-diaminocyclitols, again usually N-methylated and sometimes substituted further by an acyl group. The fortimicins are presently undergoing clinical trial.

Finally, a very few structurally dissimilar aminocyclitol antibiotics contain a monoaminocyclitol (Figure 12). The first of these reported was hygromycin A; other representatives are minosaminomycin and the validamycins, which contain a single amino group linked to two separate cyclitol rings, each with a branching hydroxymethyl substituent.

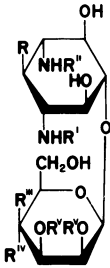
The present volume covers a number of aspects of aminocyclitol chemistry and biology and an attempt has been made in the ensuing pages to group papers according to their content. Thus, several papers deal with synthesis and modification of aminocyclitol antibiotics, some deal with structure-activity relationships, others with structural assignments. Still others deal with biological aspects, such as mode of action, mode of inactivation, and biosynthesis of these important compounds.

Acknowledgment. We appreciate greatly the permission received from the Journal of Antibiotics to use Figures 1-10 and 12, which have been adapted from those appearing in J. Antibiot., 29, 319-353 (1976).



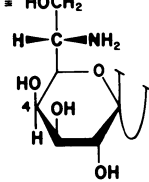
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Figure 7. Apramycin, a monosubstituted deoxystreptamine antibiotic (1)



	R	R'	R''	R'''	R''''	R'''''
DESTOMYCIN A	H	CH ₃	H	OH	H	*
DESTOMYCIN B	H	CH ₃	CH ₃	H	OH	†
DESTOMYCIN C	H	CH ₃	CH ₃	OH	H	*
HYGROMYCIN B	H	H	CH ₃	OH	H	*
SS-56 A	H	H	H	H	OH	H
SS-56 B	H	H	H	OH	H	H
SS-56 C	OH	H	H	OH	H	*
A-396-I (SS-56D)	H	H	H	OH	H	*

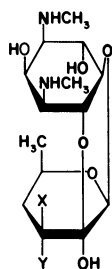
*R'''' = DESTOMIC ACID (ORTHO ESTER) = HOCH₂



†R'''' = 4-EPIDESTOMIC ACID (ORTHO ESTER)

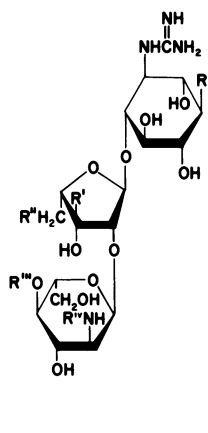
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Figure 8. Destomycin group of monosubstituted deoxystreptamine antibiotics (1)

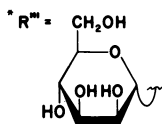


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Figure 9. Aminocyclitol antibiotics containing actinamine (1). Spectinomycin (hydrate form): X = Y = OH; dihydro-spectinomycin: X = H, Y = OH



	R	R ^I	R ^{II}	R ^{III}	R ^{IV}
STREPTOMYCIN	-NHC(=NH)NH ₂	CHO	H	H	CH ₃
N-DEMETHYLSTREPTOMYCIN	-NHC(=NH)NH ₂	CHO	H	H	H
HYDROXYSTREPTOMYCIN	-NHC(=NH)NH ₂	CHO	OH	H	CH ₃
MANNOSIDOSTREPTOMYCIN	-NHC(=NH)NH ₂	CHO	H	*	CH ₃
MANNOSIDOHYDROXYSTREPTOMYCIN	-NHC(=NH)NH ₂	CHO	OH	*	CH ₃
DIHYDROSTREPTOMYCIN	-NHC(=NH)NH ₂	CH ₂ OH	H	H	CH ₃
BLUENSOMYCIN	-OCONH ₂	CH ₂ OH	H	H	CH ₃



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Figure 10. Aminocyclitol antibiotics containing streptidine or bluensidine (1)

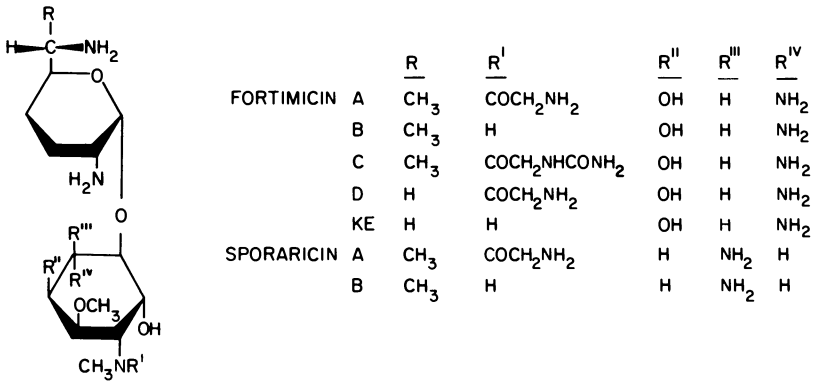
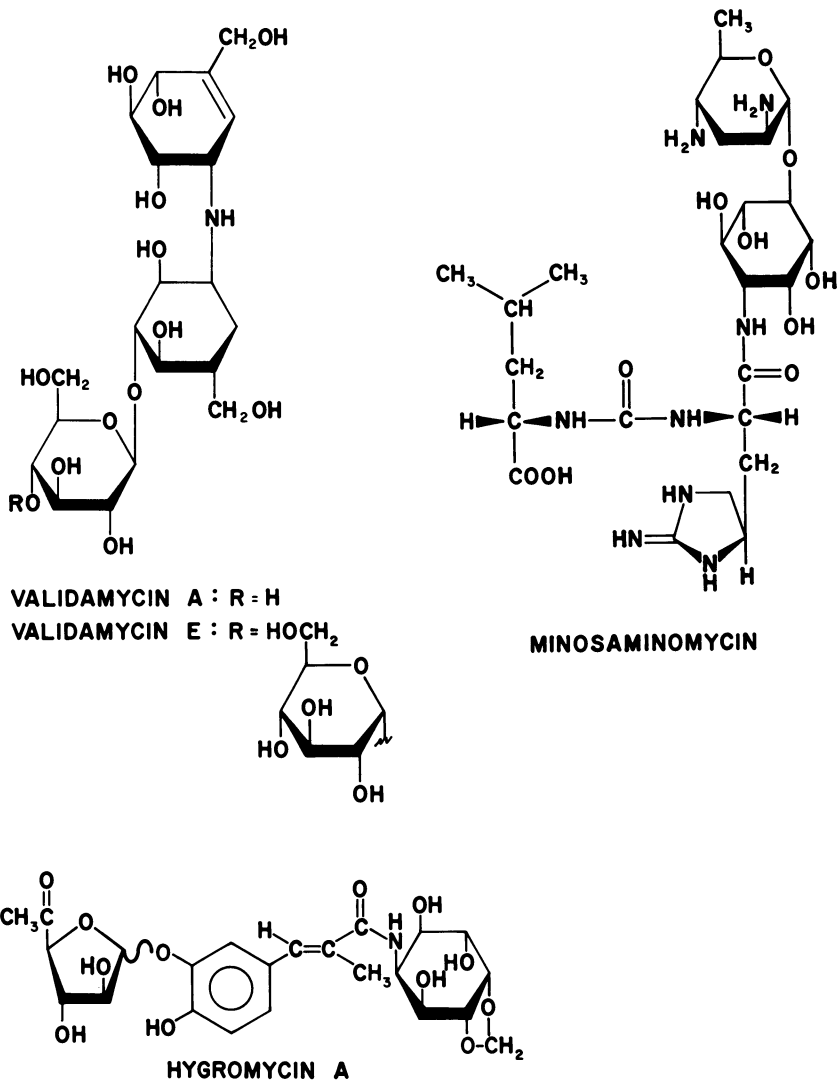


Figure 11. Fortimicins and sporaricins, 1,4-diaminocyclitol antibiotics



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Figure 12. Monoaminocyclitol antibiotics (1)

Literature Cited

1. Rinehart, K. L., Jr.; Stroshane, R. M.: "Biosynthesis of Aminocyclitol Antibiotics." J. Antibiot., 1976, 29, 319-353.
2. Rinehart, K. L., Jr.: "Mutasyntesis of New Antibiotics." Pure and Appl. Chem., 1977, 49, 1361-1384.
3. Rinehart, K. L., Jr.: "The Neomycins and Related Antibiotics." John Wiley and Sons, Inc., New York, 1964.
4. Daniels, P. J. L.: "Aminoglycosides." Kirk-Othmer Encycl. Chem. Technol., 3rd Ed., 1978, 2, 819-852.
5. Umezawa, S.: "Structures and Syntheses of Aminoglycoside Antibiotics." Adv. Carbohydr. Chem. Biochem., 1974, 30, 111-182.
6. Umezawa, S.: "The Chemistry and Conformation of Aminoglycoside Antibiotics." In Mitsuhashi, S., Ed., "Drug Action and Drug Resistance in Bacteria. Vol. 2, Aminoglycoside Antibiotics." University Park Press, Tokyo, 1975; pp. 3-43.
7. Price, K. E.; Godfrey, J. C.; Kawaguchi, H.: "Effect of Structural Modifications on the Biological Properties of Aminoglycoside Antibiotics Containing 2-Deoxystreptamine." Adv. Appl. Microbiol., 1974, 18, 191-307.
8. Tanaka, N.: "Aminoglycoside Antibiotics." In Corcoran, J. W., and Hahn, F. E., Ed., "Antibiotics. III. Mechanism of Action of Antimicrobial and Antitumor Agents." Springer-Verlag, Berlin, 1975; pp. 340-364.
9. Takeda, K.; Aihara, K.; Furumai, T.; Ito, Y.: "Biosynthesis of Butirosins. I. Biosynthetic Pathways of Butirosins and Related Antibiotics." J. Antibiot., 1979, 32, 18-28.
10. Kirby, J. P.; Borders, D. B.; Korshalla, J. H.: "Antibiotic BM408 α ." U.S. Patent 3,928,317, Dec. 23, 1975; Chem. Abstr., 1976, 84, 149200g.
11. Egan, R. S.; Sinclair, A. C.; De Vault, R. L.; McAlpine, J. B.; Mueller, S. L.; Goodley, P. C.; Stanaszek, R. S.; Cirovic, M.; Mauritz, R. J.; Mitscher, L. A.; Shirahata, K.; Sato, S.; Iida, T.: "A New Aminoglycoside Antibiotic Complex--The Seldomycins. III. The Structures of Seldomycin Factors 1 and 2." J. Antibiot., 1977, 30, 31-38.

12. McAlpine, J. B.; Sinclair, A. C.; Egan, R. S.; De Vault, R. L.; Stanaszek, R. S.; Cirovic, M.; Mueller, S. L.; Goodley, P. C.; Mauritz, R. J.; Wideburg, N. E.; Mitscher, L. A.; Shirahata, K.; Matsushima, H.; Sato, S.; Iida, T.: "A New Aminoglycoside Antibiotic Complex--The Seldomycins. IV. The Structure of Seldomycin Factor 5." J. Antibiot., 1977, 30, 39-49.
13. O'Connor, S.; Lam, L. K. T.; Jones, N. D.; Chaney, M. O.: "Apramycin, a Unique Aminocyclitol Antibiotic." J. Org. Chem., 1976, 41, 2087-2092.
14. Egan, R. S.; Stanaszek, R. S.; Cirovic, M.; Mueller, S. L.; Tadanier, J.; Martin, J. R.; Collum, P.; Goldstein, A. W.; De Vault, R. L.; Sinclair, A. C.; Fager, E. E.; Mitscher, L. A.: "Fortimicins A and B, New Aminoglycoside Antibiotics. III. Structural Identification." J. Antibiot., 1977, 30, 552-563.
15. Deushi, T.; Nakayama, M.; Watanabe, I.; Mori, T.; Naganawa, H.; Umezawa, H.: "A New Broad-Spectrum Aminoglycoside Antibiotic Complex, Sporaricin. III. The Structures of Sporaricins A and B." J. Antibiot., 1979, 32, 187-192.

RECEIVED November 30, 1979.

Synthesis of Aminocyclitol Antibiotics

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The first aminoglycoside antibiotic described was streptomycin discovered by Waksman and coworkers in 1944, and, since then, remarkable development has been made in this field of antibiotics. The number of structurally elucidated aminocyclitol antibiotics of microbial origin is now about one hundred. Various antibiotics among them have been utilized for chemotherapy, being especially useful for treatment of serious Gram-negative infections.

The study of aminocyclitol antibiotics and other sugar-containing antibiotics has provided some fascinating and challenging problems in the field of carbohydrate chemistry. Structural, stereochemical and conformational studies of this group of antibiotics have been expedited particularly by means of proton n.m.r. spectroscopic techniques. For instance, the glycosidic linkage between streptose and streptidine was determined to be α -L in 1965 by Rinehart and McGilveray (1) on the basis of n.m.r. spectral studies. Recently, ^{13}C -n.m.r. spectroscopy and mass spectroscopy have greatly contributed to the advances in this field.

In addition, it should be noted that Reeves method (2,3) for determination of the conformations of pyranosides has greatly contributed to the advances in this field. Reeves method is concerned with the stereochemical relationship between adjacent hydroxyl groups on pyranosides and based on the difference in optical rotations between solutions of sugar derivatives in cuprammonium solution and water. For instance, the absolute configuration of unsymmetrically substituted 2-deoxystreptamine portion of deoxystreptamine-containing antibiotics was first established by application of this method, as reported by Rinehart and Hichens (4) in 1963. We successfully modified this method by use of a solution of tetraamminecopper(II) sulfate instead of the cuprammonium solution (5). With this reagent Cu(II)-complexing occurs between vicinal trans-equatorial amino and hydroxyl groups, but not between vicinal trans-equatorial hydroxyl groups. This affords useful information concerning the

stereochemical relationship between adjacent amino and hydroxyl groups. This reagent called "TACu" has widely been used for the conformational studies of amino sugars and aminocyclitol antibiotics.

During the past ten years, remarkable progress has been made in the chemistry and biochemistry of aminocyclitol antibiotics. The present paper is concerned with synthetic aspects, and I would like to present a contribution to the present problems of developing synthesis of aminocyclitol antibiotics.

With the culmination of several decades of work on the determination of structures of naturally occurring aminocyclitol antibiotics (6), it was inevitable that organic chemists would turn their attention toward total synthesis.

My interest in this field of antibiotics dates back to the structural studies on kanamycin in 1958. Since then, my associates have been interested in the synthesis of aminocyclitol antibiotics in order to learn more about the structure-activity relationships. Initially, we prepared a β,β -glycoside, an analogue of kanamycin (7), from 6-amino-6-deoxy-D-glucose and 2-deoxystreptamine via the usual Koenigs-Knorr condensation. However, this compound showed no antibacterial activity. This result suggested that the presence of the α -glycoside linkages in kanamycin might be essential for antibacterial activity. Several years later, we synthesized the corresponding α,α -glycoside (8), which has fairly strong antibacterial activity.

The most conspicuous difficulty associated with the synthesis of aminocyclitol antibiotics was the formation of the α -glycosidic linkage in a stereoselective manner. Furthermore, this synthesis necessitates extensive use of selective blocking groups.

Our first targets were paromamine (A) (9,10) and neamine (B) (11) which are pseudodisaccharides. (Figure 1). Paromamine is a constituent of kanamycin C, paromomycins, and others. Neamine is a constituent of neomycins, kanamycin B, ribostamycin, butirosins, and others. Both antibiotics were isolated from *Streptomyces* cultures. These syntheses of pseudodisaccharides provided useful intermediates for further syntheses of more complex aminocyclitol antibiotics, and, we synthesized kanamycin A (12,13), B (14,15) and C (16,17), butirosin B (18), and tobramycin (19).

Parenthetically, I should like to make mention of the formation of cyclic acetal and cyclic carbamate groups which offer useful routes to aminoglycosides.

The value of cyclic acetal for the protection of hydroxyl groups in carbohydrate chemistry is well known. Among acetalation reactions, the ketal exchange reaction reported by Evans, Parrish, and Long (20) in 1967 was extremely useful in our synthesis. The method involving the use of cyclohexanone dimethyl ketal or 2,2-dimethoxypropane in N,N-dimethylformamide (DMF) in the presence of a catalytic amount of p-toluenesulfonic acid

made it possible to form a ketal ring between adjacent trans-hydroxyls, often affording key intermediates. Furthermore, our synthesis required extensive studies on the stability of the acetals toward acids. Thus, we found that the stabilities of cyclic acetals could be practically differentiated, even if they are similarly located. For instance, the transketalization of tetra-N-benzoyloxycarbonyl derivative of neamine (C) with cyclohexanone dimethylketal gave a mixture of mono- and di-cyclohexylidene derivatives which contain very similar acetals attached to vicinal secondary hydroxyl groups on sugar and aminocyclitol portions. However, we could differentiate the two acetals. The 3',4'-O-cyclohexylidene group on the sugar ring is less stable than the 5,6-O-cyclohexylidene group on the deoxystreptamine portion, and, the former can be cleaved by addition of a proper amount of methanol or water to the reaction mixture to give the desired mono-acetal (D). (Figure 2).

As an unusual example, the acetal protection of dihydrostreptomycin was of structural interest. During the course of synthetic studies on dihydrostreptomycin and related compounds, we undertook to protect the hydroxyl groups of the antibiotic by conversion into its acetals. When 2''-N-benzoyloxycarbonyl-dihydrostreptomycin was treated with cyclohexanone dimethyl ketal in DMF in the presence of acid catalyst, we obtained a tetracyclohexylidene derivative (E), which was found to contain no O-(1-methoxycyclohexyl) group as judged by its p.m.r. spectrum. This fact was unusual, since the starting material has only three sets of hydroxyl groups for the ketal exchange. The fourth cyclohexylidene group was presumed to be formed between the guanidino and hydroxyl groups on the streptidine moiety. In order to clarify the structure of the protected derivative, we studied acetalation of trans-2-guanidino-cyclohexanol (F,G) as a model compound (21). (Figure 3).

Since the guanidino-cyclohexanol is scarcely soluble in common organic solvents, it was converted into p-toluenesulfonic acid salt, and treated with cyclohexanone dimethyl ketal in DMF in the presence of p-toluenesulfonic acid. The product (H) obtained in good yield was confirmed to contain a cyclohexylidene group by elemental analysis and p.m.r. spectral studies. Ir spectral studies of further acetylated derivative showed the absence of O-acetyl group, indicating that the cyclohexylidene group is bifunctionally coupled to the hydroxyl group and an amino or imino group of the guanidine group. Isopropylideneation of the guanidino-cyclohexanol also gave analogous results, giving (I).

On the other hand, spin decoupling studies of the p-methylbenzylidene derivative (J) showed coupling between the methine proton of the methylbenzylidene group and one of the NH protons of the guanidine group, indicating that the methylbenzylidene group is attached to a nitrogen bearing hydrogen. Furthermore, nuclear Overhauser effect was observed between the benzylidene

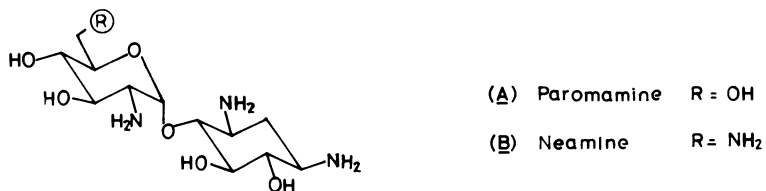


Figure 1.

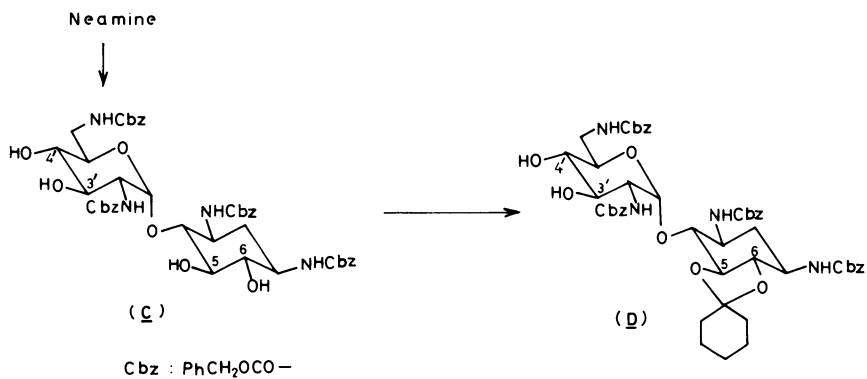
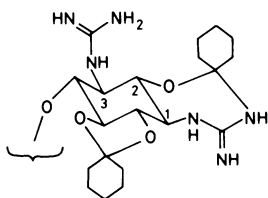
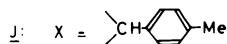
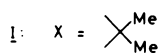
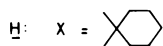
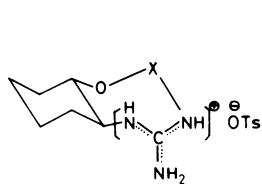
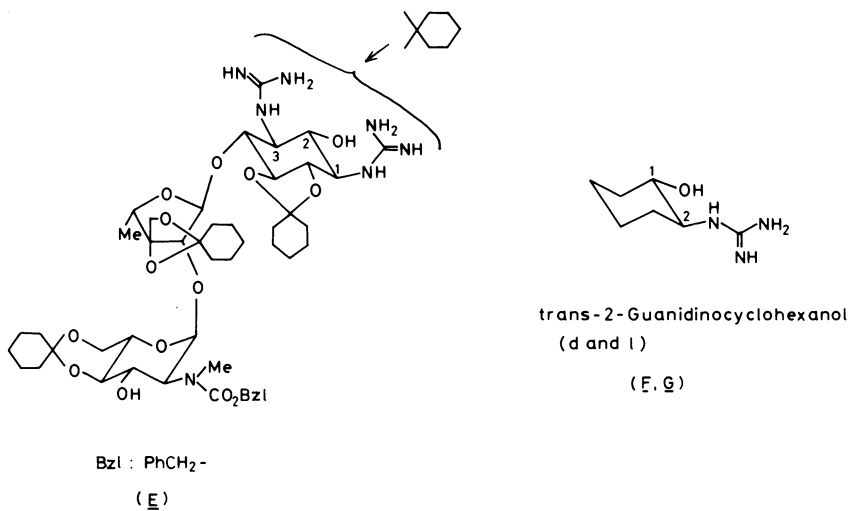


Figure 2.



2''-N-Benzoyloxycarbonyl-
tetracyclohexylidene-
dihydrostreptomycin
(K)

Figure 3.

methine proton and one of the methine protons of cyclohexane ring. These results suggested the seven-membered structure (J) for the p-methylbenzylidene derivative. The seven-membered structure was further confirmed by an X-ray crystallographic analysis of the p-toluenesulfonic acid salt of the cyclohexylidene derivative (H).

Thus, the tetracyclohexylidenated product of 2''-N-benzoyloxycarbonyl-dihydrostreptomycin is presumed to be a mixture of two positional isomers having the seven-membered ring between C-1 and 2 and between C-2 and 3 on the streptidine portion, as depicted by the formula (K).

On the other hand, it is obvious that the simultaneous protection of vicinal trans-equatorial amino and hydroxyl group has advantages in aminoglycoside syntheses. For this purpose, we found a facile procedure (22,23). (Figure 4).

Treatment of a benzoyloxycarbonylamino derivative of pyranosides or cyclohexane having vicinal trans-equatorial amino and hydroxyl groups with sodium hydride or potassium butoxide in dimethylformamide at room temperature led to the formation of a cyclic carbamate derivative, which is readily hydrolyzed by mild alkali such as dilute barium hydroxide. As an alternative method, treatment of an amino sugar or aminocyclitol with p-nitrophenoxycarbonyl chloride in the presence of Dowex 1x2 (OH⁻) or alkali in aqueous media gives a cyclic carbamate derivative. This reaction has been useful for a number of syntheses including syntheses of butirosin B, 3'-deoxybutirosins and dihydrostreptomycin.

An interesting example mentioned below involves the cyclic carbamate formation. (Figure 5). Acetylation of the aforementioned protected neamine (D) gave L, which, on decyclohexylideneation with aqueous acetic acid, gave M. Treatment of this compound with sodium hydride in dimethylformamide provided a cyclic carbamate (N), which has a single free hydroxyl group at C-5 of the deoxystreptamine portion. This intermediate is useful for the regioselective synthesis of 5-O-glycosides, and ribostamycin, butirosin B, and neomycin C have been synthesized by use of this intermediate in our laboratory (24). In regard to the protection of neamine, Kumar and Remers (25) have recently reported other derivatives useful for regioselective syntheses of ribostamycin and butirosin B.

Now, I would like to discuss results of some of our on-going studies in this area, including synthetic studies on neomycin group and streptomycin group.

We have recently reported the total synthesis of neomycin C (P), that represented the first synthesis of an antibiotic of pseudotetrasaccharides. Neomycin was independently discovered by Umezawa's group in 1948 and Waksman's group in 1949. The neomycin complex is widely used for tropical infections. Complete structures of neomycins were elucidated by Rinehart and coworkers (26) in 1963. Our synthesis (27) of neomycin C recen-

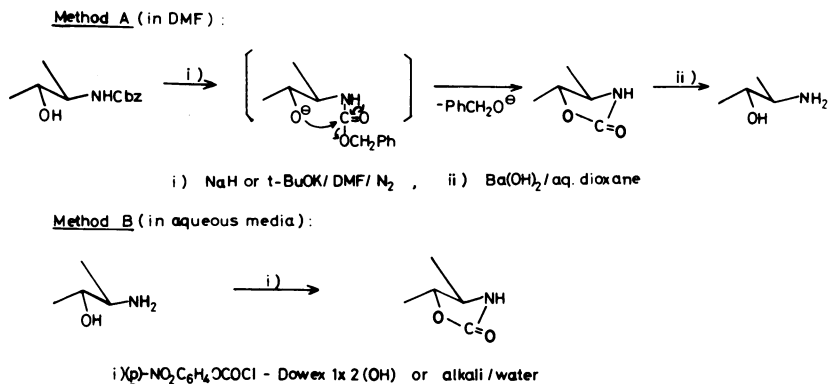


Figure 4. Protection of vicinal *trans*-equatorial NH₂ and OH

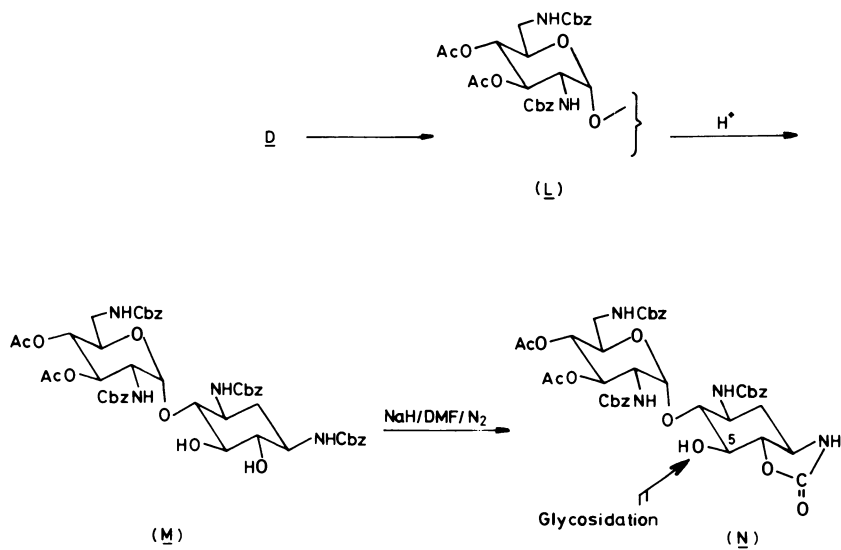


Figure 5.

tly reported involved pertinent protection of neosamine C and ribostamycin and α -glycosidation between them (Figure 6).

In this paper, I would like to discuss an approach to the total synthesis of neomycin B (Q). The key aspect of synthesis of neomycin B comprises the synthesis of a glycosyl halide derivative of neosamine B. Neosamine B is 2,6-diamino-2,6-dideoxy-L-idose, and, the synthesis of its derivative pertinent to 1,2-cis-glycosidation is more complicated than that of neosamine C. We have synthesized for this purpose 4-acetyl-6-azido-3-O-benzyl-2,6-dideoxy-2-N-(2,4-dinitrophenyl)-L-idopyranosyl bromide (Q). The 2-N-(dinitrophenyl) function is non-participating and it can be expected to form both α -L and β -L glycosidic linkages, though this function is not an effectual determinant in the formation of β -L glycoside. (Figure 7).

The starting material, 5,6-isopropylidene 1,2-oxazoline glucufuranose (R) reported by Konstas, Photaki, and Zervas (28) (1959) was treated with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF to give the 3-O-benzyl derivative (S). Treatment of S with methanolic hydrogen chloride at room temperature cleaved the isopropylidene and oxazoline rings, giving the N-benzoyl methyl glucoside (T), and, this was selectively benzoylated at C-6 with benzoyl chloride and pyridine to produce U. Tosylation then gave V. Reaction of this compound (V) with excess of sodium methoxide in methanol-dioxane at room temperature successfully provided 5,6-anhydro-2-benzamido-3-O-benzyl- α -L-idofuranoside (W) in a crystalline form by inversion of the configuration at C-5, in 84% yield. Treatment of the epoxide with sodium azide in DMF gave crystalline azide (X), which was transformed into the corresponding L-pyranose (Y) by the action of hydrochloric acid in methanol at 80°C. The crude pyranose was converted into its 2,4-dinitrophenyl derivative (Z) by treatment with 2,4-dinitrofluorobenzene and sodium bicarbonate in 72% yield from X. Acetylation of Z with acetic anhydride and pyridine gave the 1,4-O-diacetyl derivative (AA), which was syrupy, but analytically and chromatographically pure. Examination of the n.m.r. spectrum of this derivative showed that it does not exist in a classical chair conformation. Treatment of the 1-O-acetyl derivative (AA) with hydrogen bromide in tetrachloroethane at room temperature gave the desired glycosyl bromide (Q) which was an uncrystallizable syrup and could not be purified by chromatography.

On the other hand, a protected derivative of ribostamycin was prepared. Treatment of tetra-N-(benzyloxycarbonyl)-ribostamycin with benzenboronic acid in pyridine gave the boronate (BB). Acetylation then gave CC and the boronate ester group was removed by treatment with propane-1,3-diol as reported by Ferrier and Prasad (29) to give DD. The glycosidation reaction between Q and DD was effected with mercuric cyanide and Drierite in dichloromethane. Conventional processing afforded a mixture of glycosides in a 48% condensation yield. Separation by column

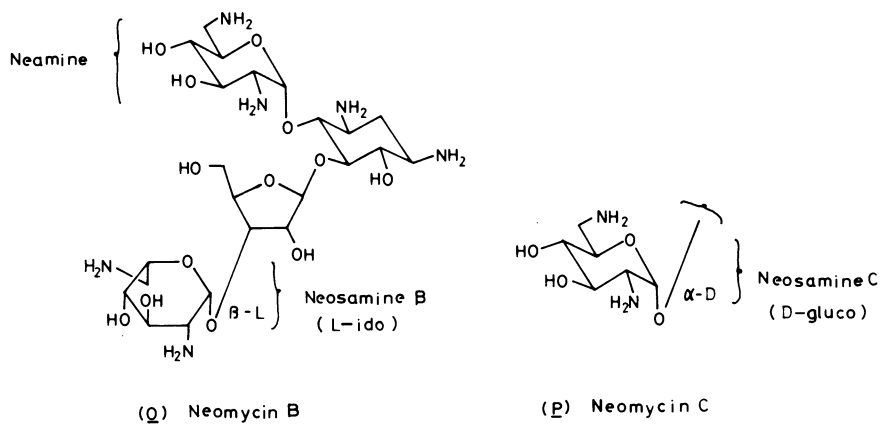


Figure 6.

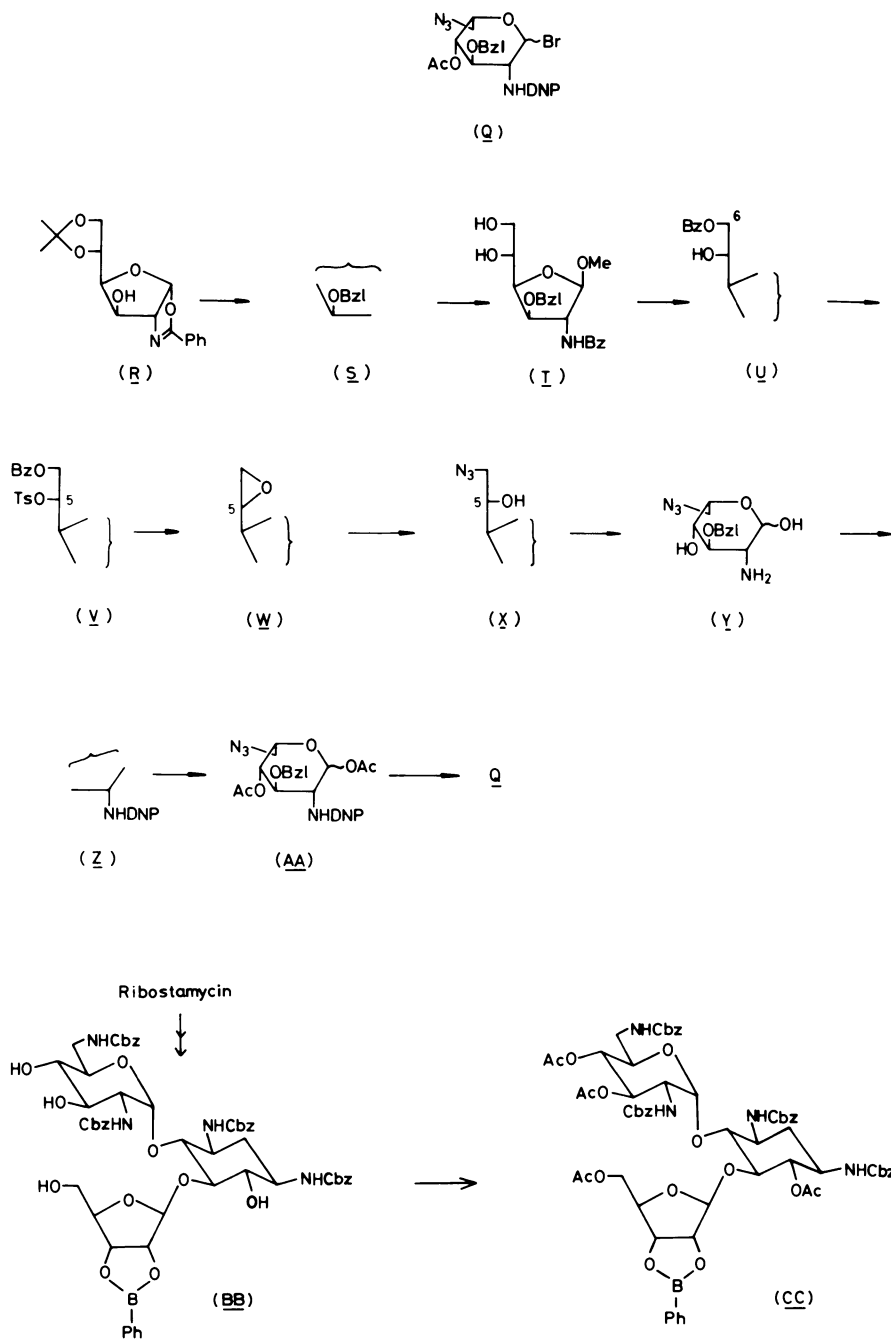


Figure 7.

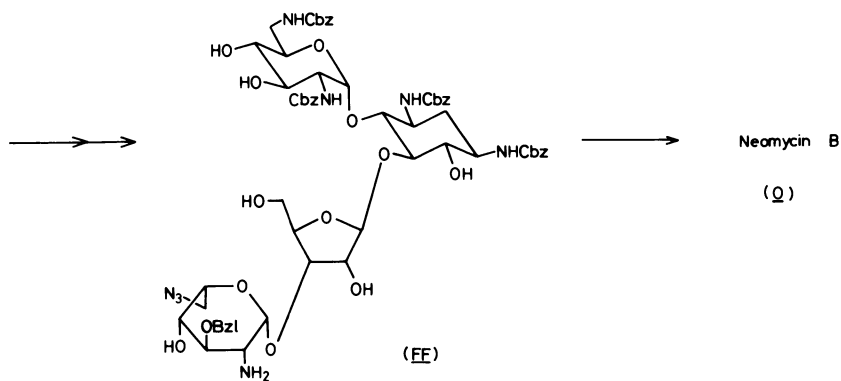
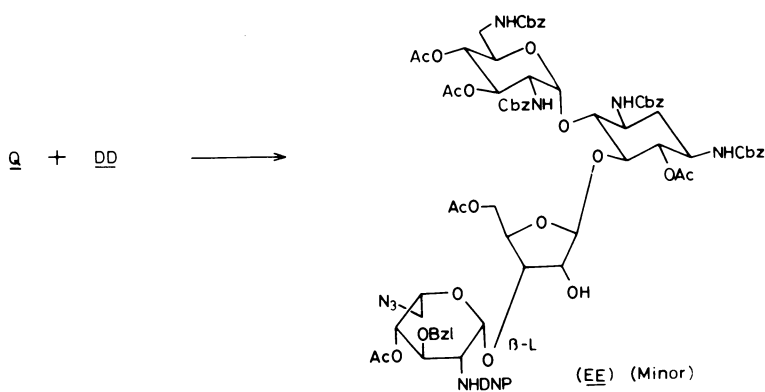
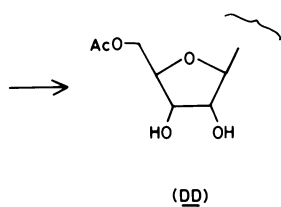


Figure 7. Continued

chromatography with silica gel gave three fractions and one of them contained the desired β -L-glycoside. However, further separation from another product, which may be α -L-glycoside, was very troublesome, giving a very poor yield of the desired pure glycoside (EE). Deacetylation of EE with methanolic ammonia followed by treatment with Dowex 1x2 (OH⁻ form) in aqueous acetone at room temperature for 2 hours gave FF. It should be noted that, when aqueous dioxane was used instead of aqueous acetone, the dinitrophenyl group could not be removed. Finally, catalytic hydrogenation of (FF) with palladium black in the presence of acetic acid in aqueous dioxane reduced the azide group and simultaneously removed the benzyloxycarbonyl and benzyl groups to give neomycin B, which was identical with that obtained from natural source by t.l.c. behavior and antibacterial activity on bioautograph. However, identification by comparison of their p.m.r. and ¹³C-n.m.r. spectra could not be done. Further device to make the β -L-glycoside of the L-amino-*idose* in good yield is under way.

Let us now turn to a discussion of the syntheses of aminocyclitol antibiotics which are effective against resistant bacteria. Drug resistance is a serious concern in present chemotherapy. Resistance mechanisms to aminocyclitol antibiotics have recently been revealed, and the research now covers a wide range (30). We will not refer the resistance mechanisms in any detail here, however, Figure 8 provides a brief summary of the inactivation mechanisms for kanamycin A so far reported.

Most of the clinically isolated strains of bacteria which are resistant to aminocyclitol antibiotics have been shown to possess enzymes that modify the structures by phosphorylation, acetylation, or adenylation to produce inactive derivatives. The N-acetylation and O-adenylation mechanisms are not very common, however, the 3'-O-phosphorylation is most commonly encountered, and, all aminocyclitol antibiotics possessing a hydroxyl group at position 3' are substrates for the phosphotransferase enzymes. A research group at our Institute of Microbial Chemistry elucidated the structure of inactivated kanamycin by enzymatic phosphorylation in 1967, and, their further studies on the substrate specificity of the enzyme with a variety of aminocyclitol antibiotics showed that the whole kanamycin structure is not required for the enzymatic action, but that only the 4-O-aminoglycosyl deoxystreptamine portion is necessary; paromamine and neamine are also phosphorylated at the 3'-hydroxyl group by the enzyme. The understanding of the biochemical nature of resistance has allowed the rational modification of many aminocyclitol antibiotics to improve antibacterial activity against resistant bacteria. We were interested in these tasks since these syntheses entail the regioselective modification of hydroxyl and amino groups on intricate structures.

There are two approaches to new derivatives, namely, total syntheses or modification of natural antibiotics. Our initial

approach involved the total syntheses of 3'-O-methyl and 3'-deoxy derivatives of kanamycin A because it was possible for us to secure these compounds by extension of the total syntheses of kanamycins. Thus, we found that deoxygenation at the 3'-position has a remarkable effect on antibacterial activity. The 3'-deoxykanamycin A (GG) (31,32) was as active as the parent antibiotic, and, moreover, it was active against resistant bacteria including *Escherichia coli* carrying R factor and resistant *Pseudomonas*, whereas the 3'-O-methylkanamycin A (HH) (33) showed substantial loss of antibacterial activity, suggesting that, although the 3'-hydroxyl group does not play an important role in the mechanism of antibacterial action, its masking may cause hindrance of binding of the antibiotic with the bacterial ribosome. (Figure 9). These results suggested that the principle of 3'-deoxygenation may be applied to make many aminocyclitol antibiotics active against resistant bacteria. The deoxygenation of aminoglycosides is of current interest, and, furthermore, several new deoxygenation reactions have recently been developed. Since the abovementioned synthesis is not useful for industrial purposes, we initiated another investigation to transform natural kanamycins into their deoxy derivatives.

During the course of our synthetic studies directed toward deoxy derivatives of aminosugars, a desirable deoxy derivative was prepared from 2,6-diamino-2,6-dideoxy-D-glucose which is a constituent of kanamycin B and other aminocyclitol antibiotics. The synthesis (34) is outlined in the Figure 10. The two amino groups of the glycoside are protected with methoxycarbonyl, ethoxycarbonyl, or benzyloxycarbonyl, and the two hydroxyl groups are mesylated or benzyloxysulfonated to give 3,4-sulfonate (II). Treatment of the disulfonate with sodium iodide and an excess of zinc dust in hot DMF affords the 3,4-unsaturated sugar (JJ) in excellent yield. The sodium iodide-zinc dust procedure was first introduced by Tipson and Cohen (35) in 1965 and subsequently used by Horton and coworkers (36) for introduction of 2,3-unsaturation into glucose. Catalytic hydrogenation and deprotection then gave 3,4-dideoxy aminoglycoside (LL). This procedure was successfully applied to the transformation of neamine, kanamycin B, ribostamycin, and butirosin B into their 3',4'-dideoxy derivatives (37,38,39,40), which were improved in antibacterial spectra.

Among these semi-synthetic antibiotics, the 3',4'-dideoxykanamycin B (MM) has recently been commercialized after clinical studies, as a drug for resistant infections, being assigned the generic name dibekacin; resistant bacteria including various strains of *E. coli* carrying R factors and *Pseudomonas aeruginosa* are remarkably sensitive to this drug. (Figure 11).

Consequently, we have studied improvement of the synthesis. One of the improved syntheses (41) is outlined in Figure 12. The characteristic feature of this synthesis is that the 3',4'-unsaturation was effected with sodium iodide in DMF in the absence

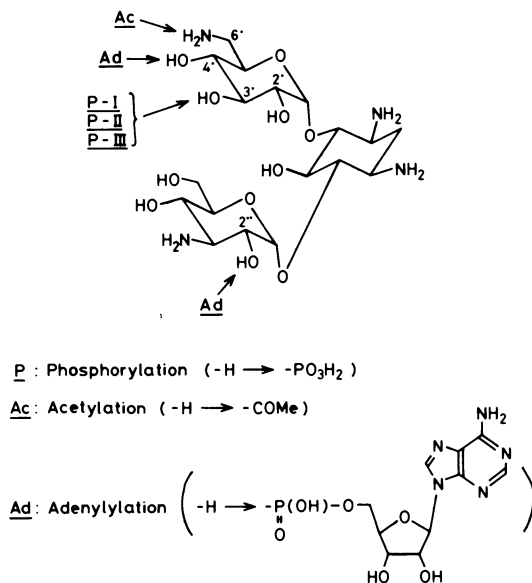
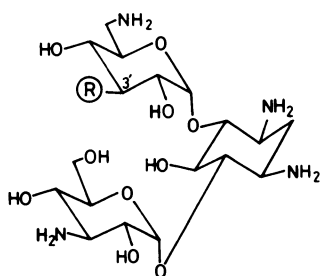
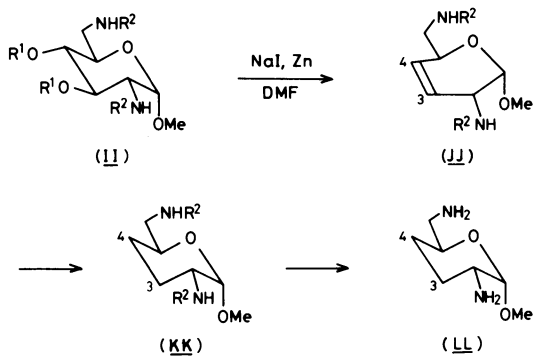


Figure 8. Inactivation of kanamycin A



- (GG) 3'-Deoxykanamycin A : R = H
 (HH) 3'-O-Methylkanamycin A : R = OMe

Figure 9.



$R^1 = \text{SO}_2\text{Me}$ or $\text{SO}_2\text{CH}_2\text{Ph}$

$R^2 = \text{CO}_2\text{Me}$, CO_2Et , $\text{CO}_2\text{CH}_2\text{Ph}$, or $\text{SO}_2\text{C}_6\text{H}_4\text{Me}(p)$

Figure 10. *Synthesis of a 3,4-dideoxy sugar*

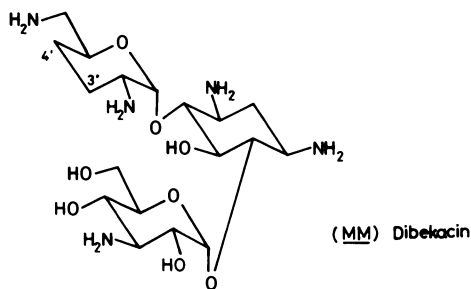


Figure 11.

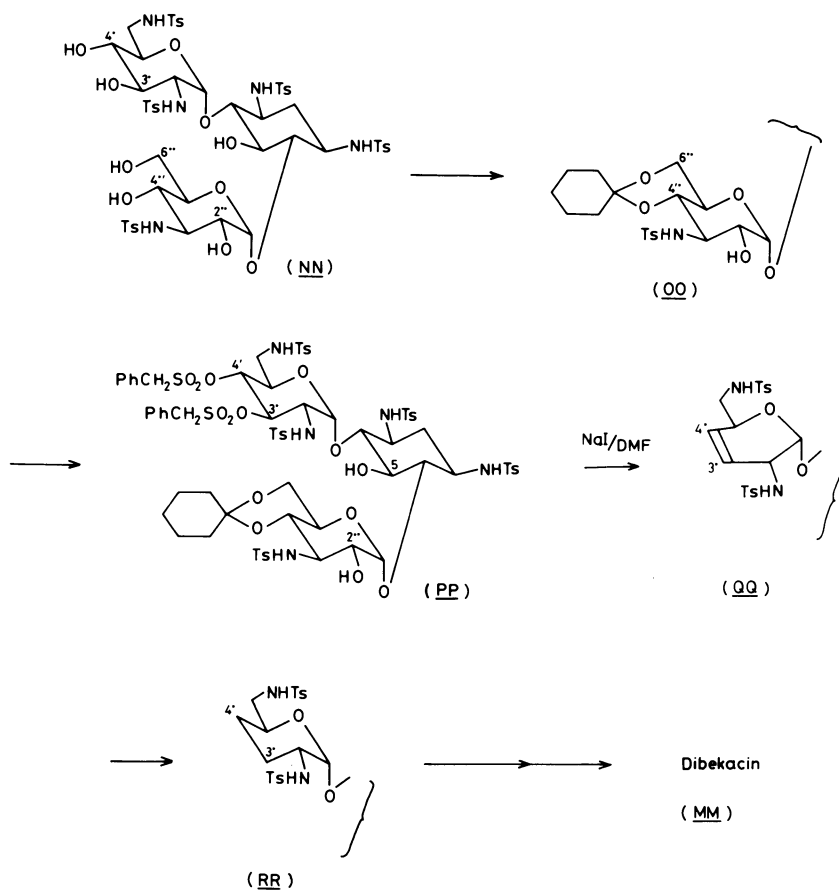


Figure 12.

of zinc dust. The synthesis began with the protection of the amino groups of kanamycin B with tosyl groups. Treatment of the penta-N-tosyl kanamycin B (NN) with cyclohexanone dimethyl ketal by the ketal-exchange procedure gave a mixture of 4'',6''-O- and 3',4',4'',6''-di-O-cyclohexylidene derivatives, however, addition of about one half equivalent of water as a 10% solution in DMF to the reaction mixture selectively removed the 3',4'-cyclohexylidene group to give the 4'',6''-O-cyclohexylidene derivative (OO) in 99% yield. Treatment of OO with benzylsulfonyl chloride in pyridine gave mainly the 3',4'-di-O-sulfonyl derivative (PP). It should be noted that the 2''-hydroxyl group is less active to benzylsulfonylation and that the 5-hydroxyl group on the deoxystreptamine portion is in a sterically hindered position. When the di-O-benzylsulfonyl derivative (PP) was treated with sodium iodide in hot DMF without zinc dust, it gave the 3',4'-unsaturated derivative (QQ) in 92% yield. Hydrogenation with platinum oxide then gave the 3',4'-dideoxy derivative (RR), from which the cyclohexylidene group was removed with aqueous acetic acid, and, finally, the remaining N-tosyl groups were removed by treatment with sodium in a mixture of liquid ammonia and ethylamine to afford dibekacin (MM).

Mesylation of the abovementioned 3',4'-diol (OO) was not selective, giving 3',4',2''-tri-O-mesyl derivative (SS), and, treatment of this compound with sodium iodide and zinc dust in hot DMF resulted in the formation of 2'',3''-aziridine derivative (TT). However, when the tri-O-mesyl derivative (SS) was treated with sodium iodide in hot DMF without zinc dust, it afforded the desirable 3',4'-unsaturated derivative (UU) in 92% yield, which was also led to dibekacin (MM) by a similar sequence of reactions in a good yield. The role of zinc dust as a determinant of aziridine formation is interesting. (See Figure 13).

Another important modification of aminocyclitol antibiotics was suggested by the presence of a peculiar aminoacyl residue in the structures of butirosins (VV) reported by the Parke-Davis researchers (42) in 1971, because comparison of butirosins with ribostamycin indicated a remarkable effect of the L-4-amino-2-hydroxybutyryl side chain (called HABA for short), on the antibacterial spectra, inhibiting kanamycin-resistant and -sensitive bacteria over a wide range. Kanamycin A modified in this way was reported by the Bristol-Banyu researchers (43) in 1972 and amikacin (WW) has recently been commercialized. (Figure 14).

We have also synthesized a number of 1-N-HABA derivatives of aminocyclitol antibiotics. Among them, I should like to mention briefly on an almost ideal modification of kanamycin B. 1-N-(L-4-amino-4-hydroxybutyryl)-6'-N-methyl-dibekacin (XX) was synthesized from dibekacin by a research group of our Institute (44). In this derivative, the 3'- and 4'-hydroxyl groups are deoxygenated, the 1-amino group is acylated with HABA, and furthermore 6'-amino group is methylated in order to prevent 6'-N-acetylation by acetyltransferase enzymes. This compound

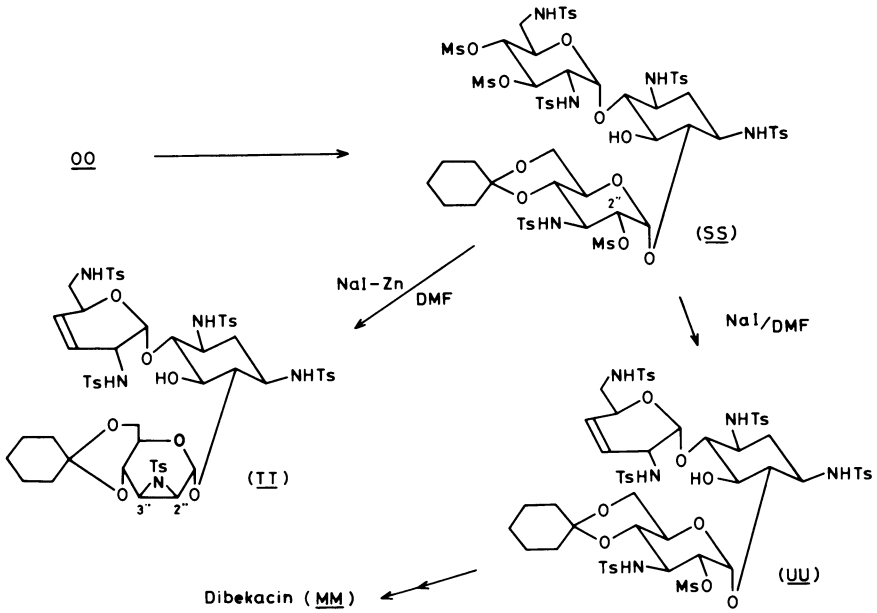


Figure 13.

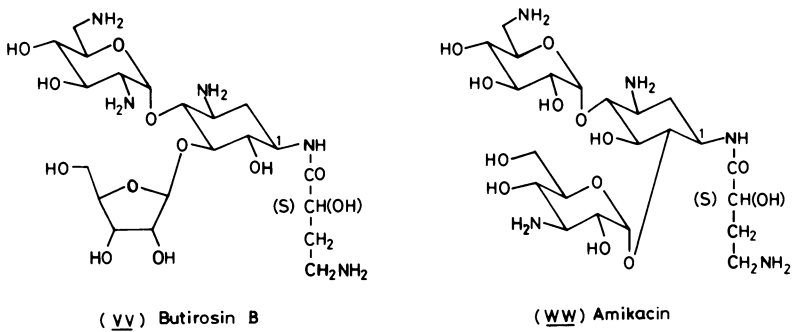


Figure 14.

inhibits almost all inactivations by phosphotransferases, adenylyltransferases, and acetyltransferases. Thus, a combination of deoxygenation, 1-N-acylation, and 6'-N-methylation provides significant activity against various resistant organisms. However, this compound is not commercialized because the cost is too high. (Figure 15).

We were also interested in the modification of streptomycin. In 1968, H. Umezawa and coworkers (45) and J. Davies and coworkers (46) revealed that the inactivated product is 3''-O-adenylyl derivative. Subsequently, in 1969, J. Davies and coworkers (47) reported inactivation by phosphorylation of the 3''-hydroxyl group by a phosphotransferase, and, in 1974, Mitsuhashi and coworkers (48) reported other inactivations by phosphorylation and adenylylation of the 6-hydroxyl group on streptidine portion. Among these inactivating enzymes, the 3''-O-phosphotransferase is frequently found in clinical isolates. (Figure 16).

The total syntheses of streptomycin (49) and dihydrostreptomycin (50,51) were achieved in my laboratory in 1974, and the synthesis was subsequently extended to the synthesis of the 3''-deoxydihydrostreptomycin (MMM) (52,53,54). This is the first successful modification in the streptomycin series, and, the product shows remarkable activities against strains producing the phosphotransferase or adenylyltransferase as well as against normal strains and some resistant bacteria of unknown mechanism, although it does not show improved activity against *Pseudomonas* strains.

With the activity of this deoxy-compound against resistant organisms confirmed, we turned to the transformation of dihydrostreptomycin itself into its 3''-deoxy derivative, and, very recently completed the transformation, which will be mentioned below. Needless to say, the transformation required a complicated protection of many functional groups, however, and this presented an unusual problem. When we aim at regioselective 3''-deoxygenation, the presence of guanidino groups complicated the situation to prepare a masked dihydrostreptomycin whose 3''-hydroxyl group is the only unprotected function, and, in addition, the masked entity has to be adaptable for deoxygenation reaction. I should say at this point that this transformation took a great deal of work to prepare a suitably masked entity and, in addition, to find out a suitable deoxygenation reaction.

Parenthetically here, I will mention a new deoxygenation reaction which was applicable to the 3''-deoxygenation of dihydrostreptomycin. In α -D-glucopyranosides, a difficulty lies in the deoxygenation of secondary hydroxyl groups attached to carbon atoms at which S_N2 process is hindered. As an approach to this problem, several radical-type deoxygenation reactions have recently been developed and successfully applied to the position unsusceptible to the S_N2 reactions. We recently reported a new radical-type 3-deoxygenation of α -D-glucopyranosides (55), which involves treatment of their 3-O-(N,N-dimethylsulfamoyl) deriva-

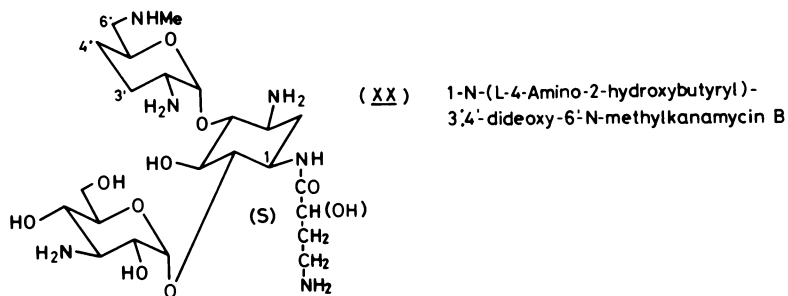


Figure 15.

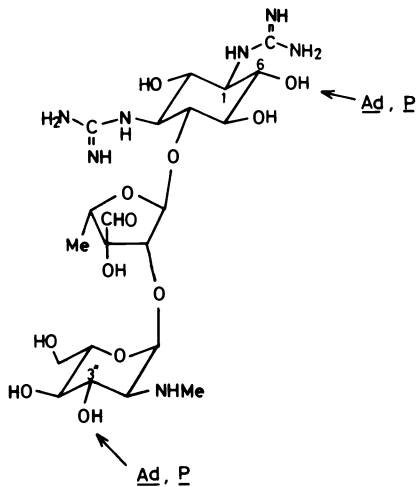


Figure 16. Inactivation of streptomycin

tives with sodium metal in liquid ammonia. (Figure 17).

The starting *N,N*-dimethylsulfamoyl derivatives were prepared from the corresponding glucopyranosides by reaction with sodium hydride and *N,N*-dimethylsulfamoyl chloride or with sulfuryl chloride, pyridine, and dimethylamine. The latter reagent is useful when a strongly basic condition should be avoided. The 3-*O*-(dimethylsulfamoyl) derivative were then treated with sodium metal in liquid ammonia or in a mixture of liquid ammonia and tetrahydrofuran at about -40°C for 1 hour to yield the corresponding 3-deoxy derivatives in high yields. For example, treatment of methyl 4,6-*O*-cyclohexylidene-2-deoxy-2-methoxycarbonylamino- α -D-glucopyranoside (YY) with sodium hydride and *N,N*-dimethylsulfamoyl chloride gave the 3-*O*-sulfamoyl derivative (ZZ), which, by reaction with sodium metal in liquid ammonia, led to the 3-deoxy derivative (AAA) in 83% yield. Another example (BBB) which has a benzyloxycarbonylmethylamino group similarly led to the 3-deoxy derivative (CCC), the benzyloxycarbonyl group being simultaneously removed. Later, you will see a stage very similar to the latter example in the regioselective 3''-deoxygenation of dihydrostreptomycin. The transformation of dihydrostreptomycin into its 3''-deoxy derivative is shown in Figure 18. When dihydrostreptomycin trihydrochloride was treated with equimolecular quantities of phenoxy carbonyl chloride and sodium carbonate in aqueous acetone with cooling about 2 hours, phenoxy carbonylation selectively occurred at the *N*-methyl group of the *L*-glucosamine portion, yielding DDD almost quantitatively. We have previously reported a similar selectivity in the case of benzyloxycarbonylation of dihydrostreptomycin (49). Treatment of DDD with tolualdehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid in DMF at 50°C under reduced pressure gave the acetal (EEE), two *p*-methylbenzylidene groups being selectively introduced, as judged from the methyl protons of the *p*-methylbenzylidene groups in the n.m.r. spectrum. It may be noted that, on prolonged treatment, further acetalation occurs at the streptidine portion. Reaction of (EEE) with potassium *t*-butoxide in DMF at 0°C for 1 hour formed a cyclic carbamate at C-2'',3'' in a good yield. We have usually used sodium hydride for the cyclization, however, in this case, we used potassium *t*-butoxide on trial and obtained higher yield. The cyclic carbamate derivative (FFF) was converted to its *p*-toluenesulfonate salt and treated with dihydropyran in the presence of *p*-toluenesulfonic acid in the usual way to give a mixture of per-tetrahydropyranyl derivatives (GGG) in which tetrahydropyranyl groups are introduced to the hydroxyl and guanidino groups on the streptidine portion. However, by treatment of the product with a mixture of 1*N* ammonia and methanol at 60°C for 2 hours, the tetrahydropyranyl groups attached to the guanidino groups were selectively removed to give HHH in 70% yield from FFF. Then, tosyl groups were introduced to the guanidino groups by treatment with excesses of sodium hydride and *p*-toluenesulfonyl

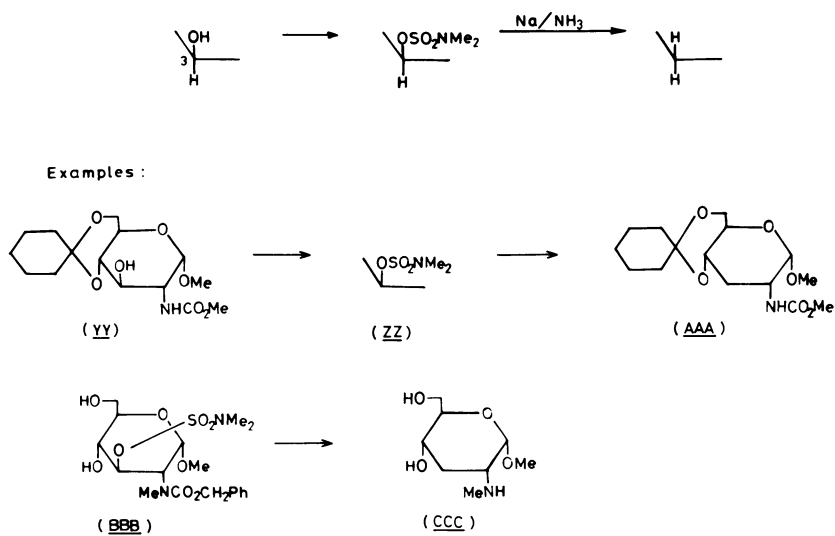


Figure 17. 3-Deoxygenation of α -D-glycopyranosides

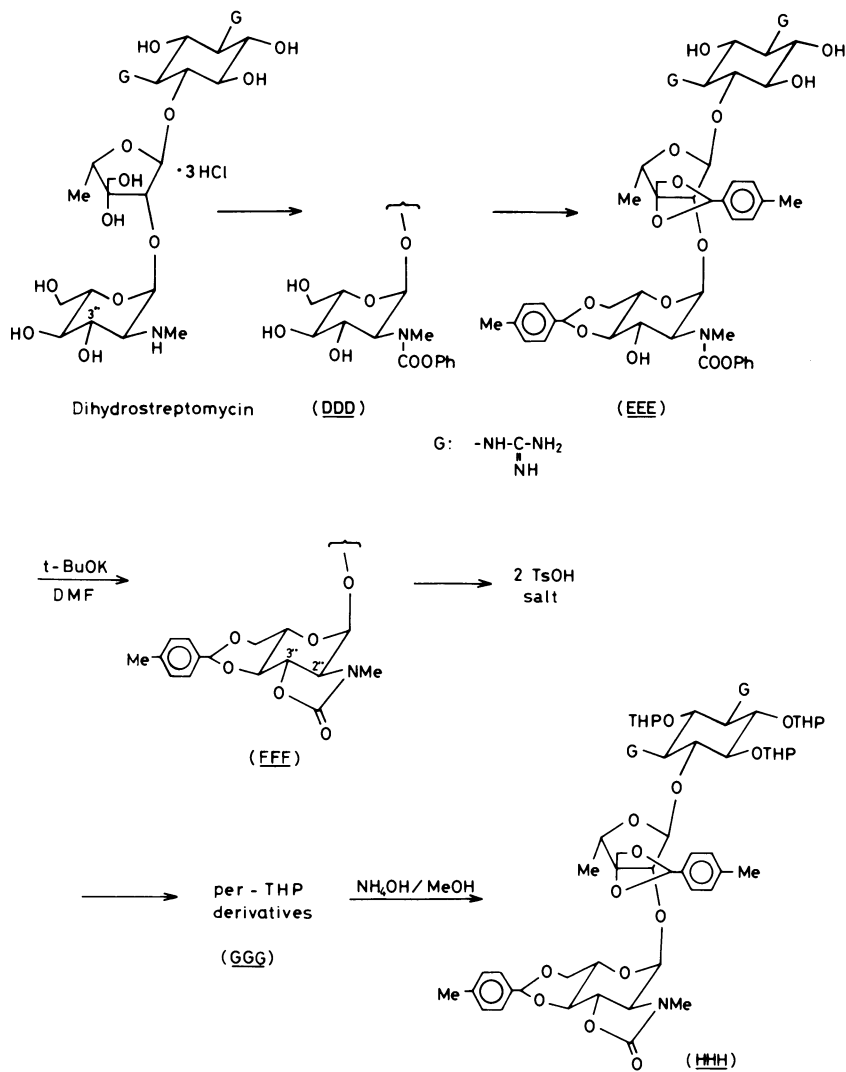


Figure 18.

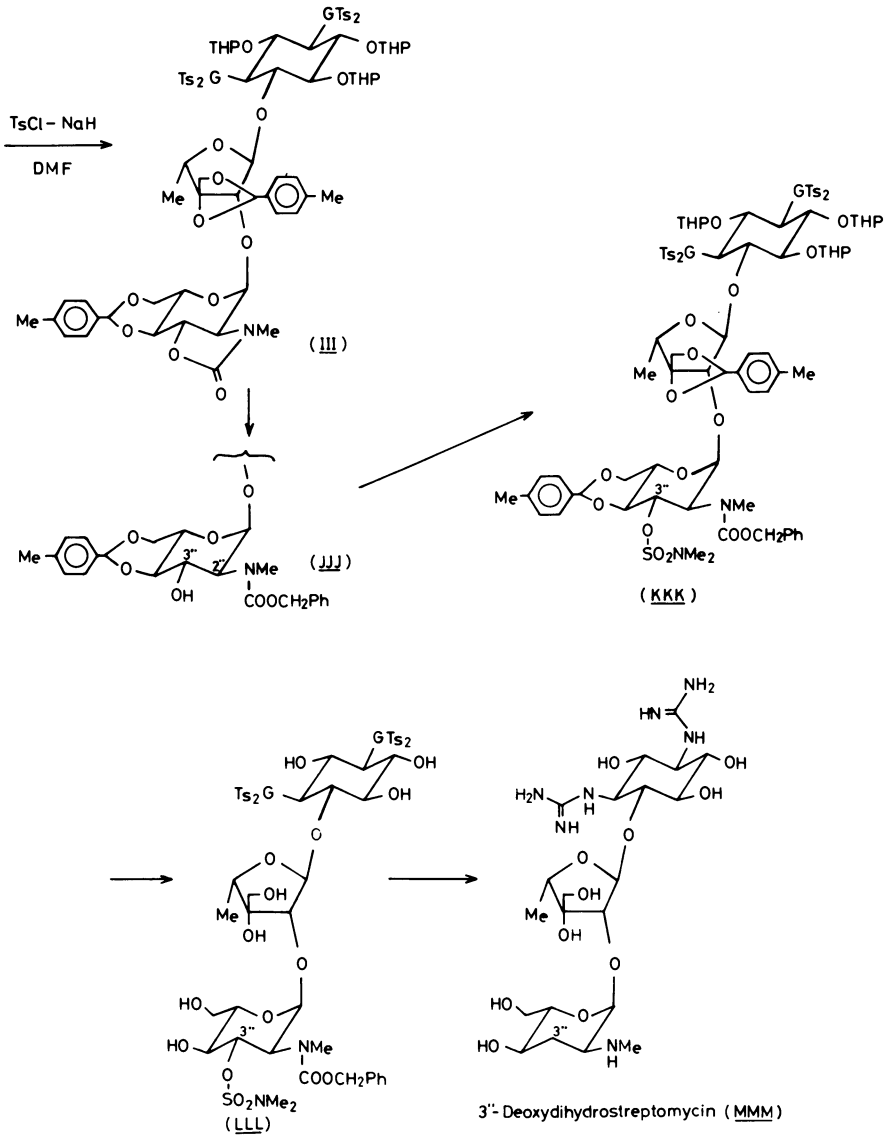


Figure 18. Continued

chloride in DMF at 0°C to give the tetra-tosyl derivative (III). In this derivative, it may be noted that the cyclic carbamate group is the only sensitive function to alkaline treatment. Thus, the cyclic carbamate group was cleaved by treatment with excess of sodium benzyolate in dioxane at room temperature to give the 2''-N-benzyloxycarbonyl derivative (JJJ), which has the only free function at C-3''.

Now we could apply the aforementioned deoxygenation procedure to the derivative (JJJ). Reaction of JJJ with sodium hydride and N,N-dimethylsulfamoyl chloride in DMF gave the 3''-O-sulfamoyl derivative (KKK). The p-methylbenzylidene and THP groups were removed by hydrolysis with aqueous acetic acid to give LLL. Finally, treatment of LLL with sodium metal in liquid ammonia simultaneously removed the dimethylsulfamoyloxy group and the tosyl and benzyloxycarbonyl groups, giving the 3''-deoxydihydrostreptomycin (MMM), which was identical with the authentic specimen obtained by a total synthesis in all respects.

In conclusion, the synthetic studies directed towards aminocyclitol antibiotics now cover a wide area, and we have a great number of references, which, includes, for instance, extensive studies on gentamicins by Daniels and associates of the Schering Corporation. On the other hand, we have learned from Nature the various structures of aminocyclitol antibiotics such as spectinomycin, kasugamycin, sisomicin, validamycins, apramycin, fortimicins, and others. In view of probable discoveries of new aminoglycosides, growing knowledge of mechanisms of antibiotic action and resistance, and development in synthetic chemistry, further advances in useful aminocyclitol antibiotics may be expected.

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Literature Cited

1. McGilveray, I. J. and Rinehart, K. L. Jr., J. Amer. Chem. Soc., (1965), 87, 4002.
2. Reeves, R. E., Advan. Carbohyd. Chem., (1951), 6, 107.
3. Barlow, C. B. and Guthrie, R. D., J. Chem. Soc. (C), (1967), 1194.
4. Hichens, M. and Rinehart, K. L. Jr., J. Amer. Chem. Soc., (1963), 85, 1547.
5. Umezawa, S., Tsuchiya, T., and Tatsuta, K., Bull. Chem. Soc. Jpn., (1966), 39, 1235.
6. Umezawa, S., Advan. Carbohyd. Chem. Biochem., (1974), 30, 111 - 182.

7. Koto, S., Ito, Y. and Umezawa, S., Bull. Chem. Soc. Jpn., (1965), 38, 1447.
8. Nishimura, Y., Tsuchiya, T. and Umezawa, S., Bull. Chem. Soc. Jpn., (1971), 44, 2521.
9. Umezawa, S. and Koto, S., Bull. Chem. Soc. Jpn., (1966), 39, 2014.
10. Umezawa, S., Miyazawa, T. and Tsuchiya, T., J. Antibiot. (Tokyo), (1972), 25, 530.
11. Umezawa, S., Tatsuta, K., Tsuchiya, T. and Kitazawa, E., J. Antibiot. (Tokyo), (1967), A20, 53.
12. Umezawa, S., Tatsuta, K. and Koto, S., J. Antibiot. (Tokyo), (1968), 21, 367.
13. Umezawa, S., Tatsuta, K. and Koto, S., Bull. Chem. Soc. Jpn., (1969), 42, 533.
14. Umezawa, S., Koto, S., Tatsuta, K., Hineno, H., Nishimura, Y. and Tsumura, T., J. Antibiot. (Tokyo), (1968), 21, 424.
15. Umezawa, S., Koto, S., Tatsuta, K., Hineno, H., Nishimura, Y. and Tsumura, T., Bull. Chem. Soc. Jpn., (1969), 42, 537.
16. Umezawa, S., Koto, S., Tatsuta, K. and Tsumura, T., J. Antibiot. (Tokyo), (1968), 21, 162.
17. Umezawa, S., Koto, S., Tatsuta, K. and Tsumura, T., Bull. Chem. Soc. Jpn., (1969), 42, 529.
18. Ikeda, D., Tsuchiya, T., Umezawa, S. and Umezawa, H., J. Antibiot. (Tokyo), (1972), 25, 741.
19. Takagi, Y., Miyake, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., J. Antibiot. (Tokyo), (1973), 26, 403.
20. Evans, M. E., Parrish, F. W. and Long, L. Jr., Carbohyd. Res., (1967), 3, 453.
21. Takagi, Y., Kawashima, O., Tsuchiya, T., Sano, H. and Umezawa, S., Bull. Chem. Soc. Jpn., (1976), 49, 3108.
22. Ikeda, D., Tsuchiya, T., Umezawa, S. and Umezawa, H., J. Antibiot. (Tokyo), (1972), 25, 741.
23. Umezawa, S., Takagi, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn., (1971), 44, 1411.
24. Umezawa, S., Pure & Appl. Chem., (1978), 50, No.11/12, 1453 - 1476.
25. Kumar, V. and Remers, W. A., J. Org. Chem., (1978), 43, 3327.
26. Rinehart, K. L. Jr. and Hichens, M., J. Amer. Chem. Soc., (1963), 85, 1547.
27. Umezawa, S. and Nishimura, Y., J. Antibiot. (Tokyo), (1977), 30, 189.
28. Konstas, S., Phostaki, I. and Zervas, L., Chem. Ber. (1959), 92, 1288.
29. Ferrier, R. J. and Prasad, D., J. Chem. Soc., (1965), 7429.
30. Umezawa, H., Advan. Carbohyd. Chem. Biochem., (1974), 30, 183 - 225.
31. Umezawa, S., Tsuchiya, T., Muto, R. and Umezawa, H., J. Antibiot. (Tokyo), (1971), 24, 274.

32. Umezawa, S., Nishimura, Y., Hineno, H., Watanabe, K., Koike, S., Tsuchiya, T. and Umezawa, H., Bull. Chem. Soc. Jpn., (1972), 45, 2847.
33. Umezawa, H., Tsuchiya, T., Muto, R. and Umezawa, S., Bull. Chem. Soc. Jpn., (1972), 45, 2842.
34. Umezawa, S., Okazaki, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn., (1972), 45, 3619.
35. Tipson, R. S. and Cohen, A., Carbohyd. Res., (1965), 1, 338.
36. Albano, E., Horton, D. and Tsuchiya, T., Carbohyd. Res., (1966), 2, 349.
37. Jikihara, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn., (1973), 46, 3507.
38. Umezawa, S., Umezawa, H., Okazaki, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn., (1972), 45, 3624.
39. Ikeda, D., Suzuki, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn., (1973), 46, 3210.
40. Ikeda, D., Tsuchiya, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn., (1974), 47, 3136.
41. Miyake, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Carbohyd. Res., (1976), 49, 141.
42. Woo, P. W. K., Dion, H. W. and Bartz, Q. R., Tetrahedron Lett., (1971), 2625.
43. Kawaguchi, H., Naito, T., Nakagawa, S. and Fujisawa, K., J. Antibiot. (Tokyo), (1972), 25, 695.
44. Umezawa, H., Linuma, K., Kondo, S., Hamada, M. and Maeda, K., J. Antibiot. (Tokyo), (1975), 28, 340.
45. Takasawa, S., Utahara, R., Okanishi, M., Maeda, K. and Umezawa, H., J. Antibiot. (Tokyo), (1968), 21, 477.
46. Yamada, T., Tipper, D. and Davies, J., Nature, (1968), 219, 288.
47. Ozzanne, B., Benveniste, R., Tipper, D. and Davies, J., J. Bacteriol., (1969), 100, 1144.
48. Suzuki, I., Takahashi, N., Shirato, S., Kawabe, H. and Mitsuhashi, S., International Symposium on Bacterial Resistance, Tokyo, October 1974.
49. Umezawa, S., Takahashi, Y., Usui, T. and Tsuchiya, T., J. Antibiot. (Tokyo), (1974), 27, 997.
50. Umezawa, S., Tsuchiya, T., Yamazaki, T., Sano, H. and Takahashi, Y., J. Amer. Chem. Soc., (1974), 96, 920.
51. Umezawa, S., Yamazaki, T., Kubota, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn., (1975), 48, 563.
52. Sano, H., Tsuchiya, T., Kobayashi, S., Hamada, M., Umezawa, S. and Umezawa, H., J. Antibiot. (Tokyo), (1976), 29, 978.
53. Sano, H., Tsuchiya, T., Kobayashi, S., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn., (1977), 50, 975.
54. Yamasaki, T., Tsuchiya, T. and Umezawa, S., J. Antibiot. (Tokyo), (1978), 31, 1233.
55. Tsuchiya, T., Watanabe, I., Yoshida, M., Nakamura, F., Usui, T., Kitamura, M. and Umezawa, S., Tetrahedron Lett., (1978), 3365.

Modification of Aminocyclitol Antibiotics

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Chemical and biological modifications of aminocyclitol antibiotics have been extensively studied to elucidate a structure-antimicrobial activity relationship. Most of the works done so far concerns modifications of an amino sugar moiety of an antibiotic.

Before 1969, few studies had been done on the dependence of antimicrobial activity on an aminocyclitol moiety structure. In 1969, Rinehart and his coworkers first prepared modified antibiotics named "Hybrimycins" by a newly devised technique of bioconversion (1,2,3). The hybrimycins are neomycin analogs in which an aminocyclitol moiety of neomycin, 2-deoxystreptamine, has been replaced by streptamine (*scyllo*-inosadamine-1,3) or 2-epistreptamine (*myo*-inosadamine-1,3). This bioconversion is now called "mutasynthesis" and is used widely as a powerful tool for a preparation of new antibiotics.

The investigations on hybrimycins stimulated us to initiate a study on a preparation of modified antibiotics that have other aminocyclitols rather than 2-deoxystreptamine in a 2-deoxystreptamine containing antibiotics.

Besides mutasynthesis, there are two other approaches to establish the relationship between a variation in the structure of aminocyclitol and antimicrobial activity of the corresponding aminocyclitol antibiotic. The second approach is a total synthesis of an antibiotic that contains an aminocyclitol of known structure. And the third one is a chemical modification of an aminocyclitol moiety of a naturally occurring antibiotic.

1. Mutasynthesis
2. Total Synthesis
3. Chemical Modification

In the second approach, we have attempted to synthesize

5-deoxyparomamine (A) and 5-deoxykanamine (B) (Fig. 1) by condensing a 2,5-dideoxystreptamine derivative (C) with the respective sugar derivative.

In the case of 5-deoxykanamine, B, bis-N,N'-(ethoxycarbonyl)-2,5-dideoxystreptamine, C was condensed with 6-azido-2,3,4-tri-O-benzyl-6-deoxy- α -D-glucopyranosyl chloride (D) in a mixture of benzene and dioxane in the presence of mercuric cyanide. The product mixture was fractionated by silica gel column chromatography. A pseudodisaccharide derivative (E) in which the azido-sugar was attached to 4-O of the 2,5-dideoxystreptamine in α -D-glycosidic linkage was obtained in 19% yield as a syrup and the corresponding 6-O derivative (F) was obtained in 17% yield, together with β -D-glycoside in a yield of 5%.

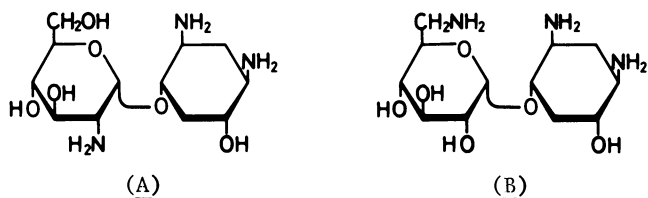
Catalytic reduction of the azido group of E in the presence of Raney nickel, followed by a treatment with ethyl chloroformate gave crystalline 4-O-(2',3',4'-tri-O-benzyl-6'-deoxy-6'-ethoxycarbonylamino- α -D-glucopyranosyl)-bis-N,N'-(ethoxycarbonyl)-2,5-dideoxystreptamine. Hydrogenolysis of the compound with palladium black and subsequent hydrolysis in aqueous barium hydroxide solution afforded 5-deoxykanamine, B in a yield of 4% (Fig. 2) (4).

When a D-glucosamine derivative was used, instead of the 6-azido sugar, 5-deoxyparomamine, A was obtained in an analogous reaction process (4). 5-Deoxyparomamine, A and 5-deoxykanamine, B were submitted to a determination of antimicrobial activity. 5-Deoxykanamine, B showed similar activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherlichia coli* and *Mycobacterium smegmatis*, compared to neamine (G). It is notable that 5-deoxykanamine, B showed considerable activity against a kanamycin resistant strain of *Klebsiella pneumoniae*, while neamine, G is not active against the resistant strain of microorganism (4) (Table 1).

This result encouraged us to modify aminocyclitols in naturally occurring antibiotics. We have been working mainly on a modification of neamine, G, since this is one of the most simple aminocyclitol antibiotics and is readily accessible as a by-product in the large scale production of antibiotics, such as kanamycins (Fig. 3).

Neamine, G has two hydroxyl groups on the C-5 and 6 positions of the 2-deoxystreptamine moiety. We have attempted to modify each one of these two hydroxyl groups selectively by deoxygenation, epimerization of the configuration, substitution with an amino group and glycoside formation.

Prior to the present study, each one of these two hydroxyl groups has had to be protected selectively with an appropriate protective group. When 3',4'-di-O-acetyl-1,3,2',6'-tetrakis-N-(ethoxycarbonyl)neamine (H) was treated with triethyl orthoacetate (1,1,1-triethoxyethane) in DMF in the presence of *p*-toluenesulfonic acid, 5,6-O-ethoxyethylidene derivative (I) was obtained. The ethoxyethylidene group attached to a trans vicinal diol is rather susceptible to an ordinary acid hydrolysis. Therefore, diluted hydrochloric acid or concentrated formic acid cleaved the acetal linkage of compound, I and only the starting material, H was recovered.



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Figure 1. 5-Deoxyparomamine (A) and 5-deoxykanamine (B)

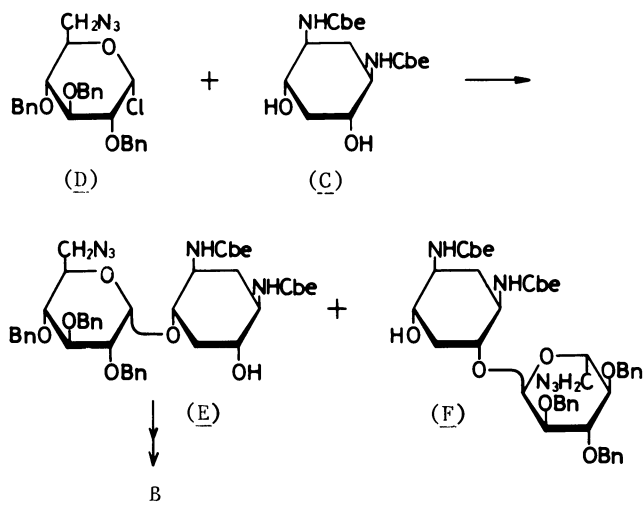


Figure 2. Synthesis of 5-deoxykanamine (B)

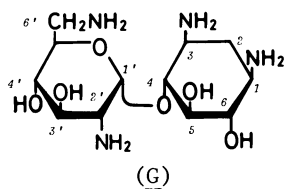


Figure 3. Neamine (G)

Table 1. Antimicrobial activity determined by paper disk method^{a)}

Compound (1 mg/ml)	Diameter (mm) of inhibition zone			
	<i>B. subtilis</i> 6633	<i>S. aureus</i> 6538P	<i>E. coli</i> K-12	<i>Myc. smeg.</i> 607
Neamine	31	24	35	30
5-Deoxyparomamine	27	15	28	-
5-Deoxykanamine	30	22	35	37

a) Determined by the Hole method in a concentration of 1 mg/ml. * Kanamycin resistant strain.

B. subtilis 6633 = *Bacillus subtilis* ATCC 6633, *S. aureus* 6538P = *Staphylococcus aureus* 6538P.

E. coli K-12 = *Escherichia coli* K-12, *Myc. smeg.* 607 = *Mycobacterium smegmatis* ATCC 607.

K. pneumo. 7 = *Klebsiella pneumoniae* 7.

But when compound I was treated heterogeneously with Amberlite IR-120 (H^+) ion exchange resin in an aqueous acetone solution, a mixture of three components was obtained. By a silica gel column chromatography, the components were successfully separated. These three components were a 6-O-acetyl derivative (J), 5-O-acetyl derivative (K) and the starting material, H obtained in 22, 25, and 21% yields, respectively (5) (Fig. 4).

The structures of J and K have been established by mesylation and subsequent degradation, followed by acetylation (Fig. 5) (6). NMR spectra were also consistent with the proposed structures of J and K.

After that, it was found that the hydroxyl group on C-6 is more reactive than the group on C-5 toward acylation. And when 3',4'-di-O-acetyl-1,3,2',6'-tetrakis-N-(benzyloxycarbonyl)neamine was treated with acetic anhydride in pyridine in a refrigerator, 6-O-acetyl derivative was obtained in a fairly good yield (7).

5-Deoxyneamine (R) can be prepared from the 6-O-acetyl derivative. Chlorination of compound J with sulfuryl chloride in pyridine gave the corresponding 5-chloro-5-deoxy derivative (P) as crystals in 64% yield. Chlorination took place at the C-5 position with an inversion of the configuration, similar to the reaction mechanism proposed by Jones et al. (29).

Dehalogenation of P with tributylstannane gave 5-deoxyneamine derivative (Q) in 83% yield, which was further converted to R by removing the protective groups in boiling aqueous barium hydroxide solution (6) (Fig. 6).

On the other hand, starting from the 5-O-acetyl derivative, K, an analogous reaction process afforded 6-deoxyneamine (U) (Fig. 7) (6).

In the case of S, the chlorine atom on the C-6 was readily removed by catalytic hydrogenation in the presence of Raney nickel (6).

To establish the structures of R and U, each compound was degraded in concentrated hydrobromic acid. An optically inactive 2,5-dideoxystreptamine derivative was obtained from R, and an optically active (+)-2,6-dideoxystreptamine derivative was obtained from U.

5,6-Dideoxyneamine (Y) was prepared (Fig. 8). When compound H was treated with an excess amount of methanesulfonyl chloride in pyridine, 5,6-di-O-mesyl derivative (V) was obtained in 64% yield as crystals.

Compound V was heated with zinc powder and sodium iodide in DMF to give a 5-ene derivative (W) in 43% yield. Catalytic hydrogenation of W, followed by hydrolysis in aqueous barium hydroxide solution afforded Y (6).

Directly from compound W, 5,6-dideoxyneamine-5-ene (Z) was prepared by hydrolysis (6).

Finally, all the four hydroxyl groups in neamine, G were removed (Fig. 9) to give 3',4',5,6-tetradeoxyneamine (DD) (8).

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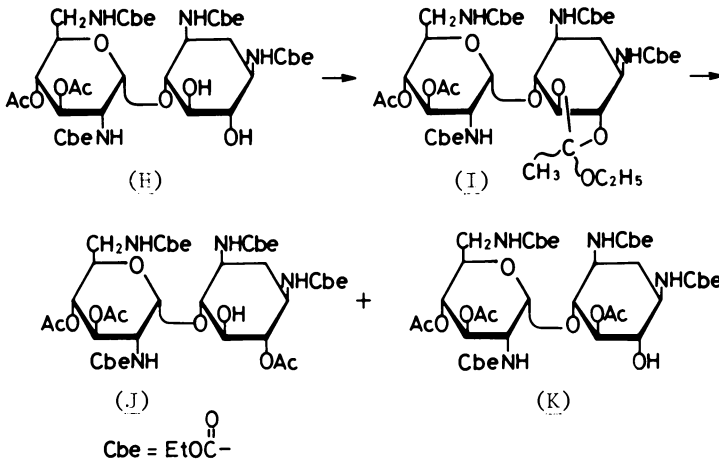


Figure 4. Selective protection of hydroxyl groups in neamine

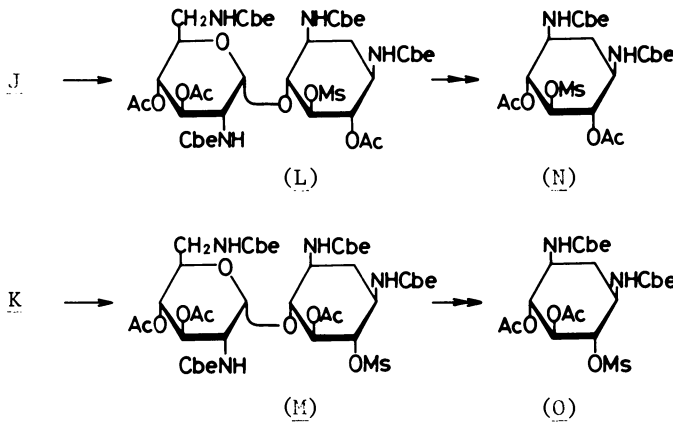


Figure 5. Degradation of J and K

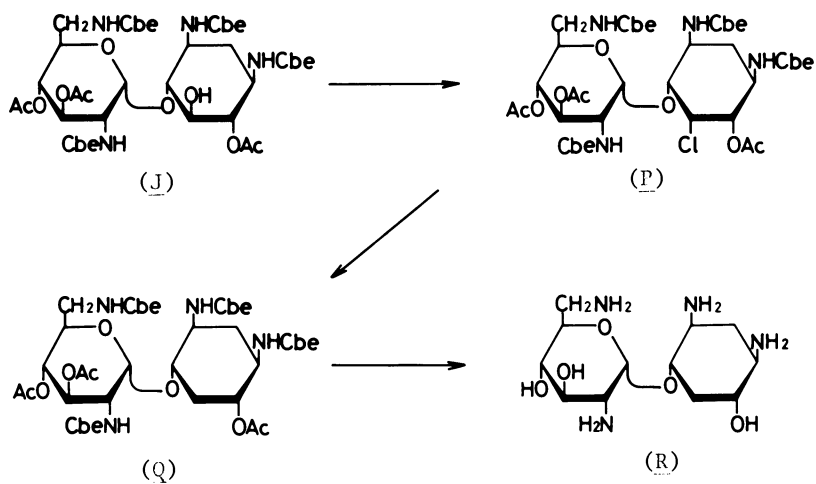


Figure 6. *Synthesis of 5-deoxyneamine (R)*

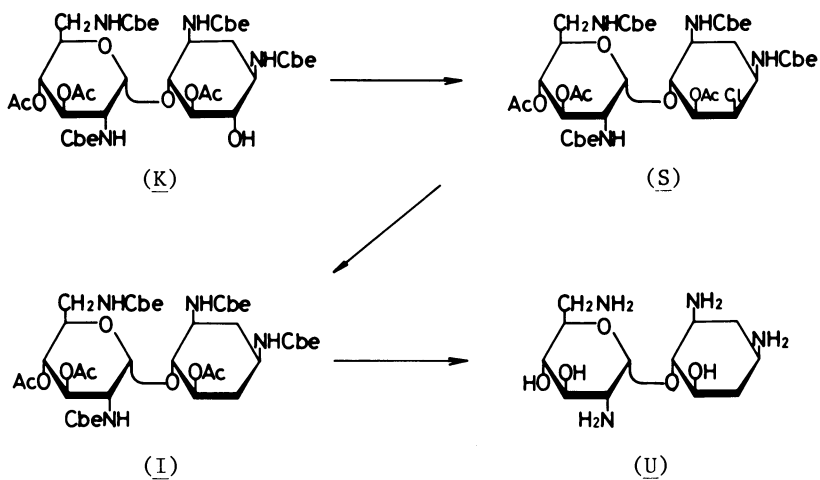


Figure 7. *Synthesis of 6-deoxyneamine (U)*

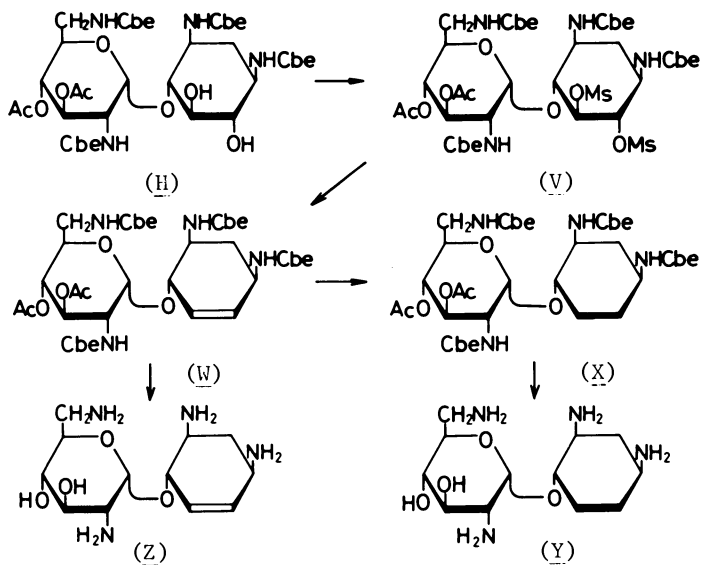


Figure 8. Synthesis of 5,6-dideoxyneamine (Y) and 5,6-dideoxyneamine-5-ene (Z)

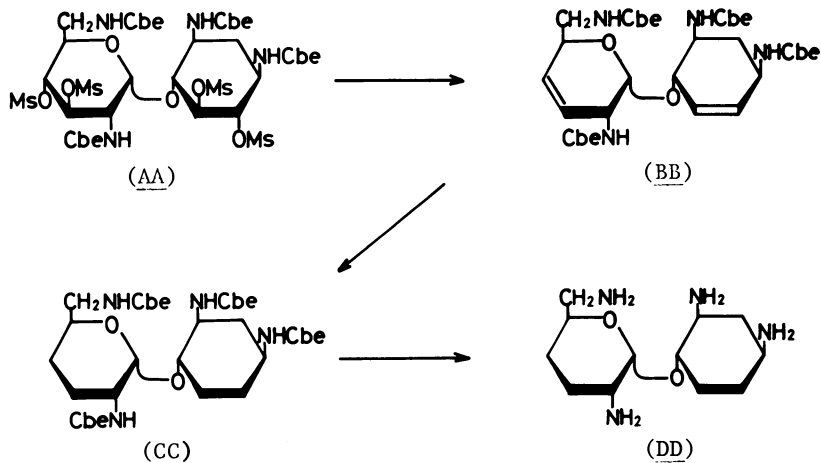


Figure 9. Synthesis of 3',4',5,6-tetraideoxyneamine (DD)

Compound DD was also described in a literature by other investigators (9,10)

Now we have five deoxyneamines and their antimicrobial activities were determined against several microorganisms (Table 2). The results showed that 5-deoxyneamine, R was most effective against microorganisms tested, especially against resistant strains of bacteria. But deoxygenation of the hydroxyl group on C-6 position did not improve the activity, compared to the parent neamine, G (6). The existence of a hydroxyl group in neamine, G is not essential for antimicrobial activity, but a hydroxyl group might play an important role for a reduction of toxicity, since tetra-deoxyneamine, DD is 3-fold more toxic than neamine, G (11).

Epimerization of the hydroxyl group has been attempted to demonstrate a relationship between the configuration of the hydroxyl group on C-5 or 6 and their respective antimicrobial activity, since an inversion of a configuration of a hydroxyl group in an aminocyclitol moiety of an antibiotic has not been well studied, except streptomycin and epistreptomycin in the case of hybrimycins (3) and 2-epiactinamine in the case of 7-epipectinomycin (12,13).

When compound J was oxidized with ruthenium tetroxide, 5-oxo derivative (EE) was obtained. Catalytic hydrogenation of EE in the presence of platinum oxide gave a 5-epineamine derivative (FF) in 52% yield, together with a neamine derivative in 42% yield. Compound FF was hydrolyzed as usual to give 5-epineamine (GG) (Fig. 10) (14).

Starting from compound K, a 6-oxo derivative (HH) was obtained as crystals in 76% yield by an analogous oxidation with ruthenium tetroxide. Hydrogenation of HH with tri-*sec*-butylborohydride afforded 6-epineamine derivative (II) in 31% yield, along with a neamine derivative in 13% yield. Removal of the protective groups of II gave 6-epineamine (JJ) (14) (Fig. 11).

While, compound M was treated with sodium alkoxide and subsequently acetylated in the usual way, a 5,6-anhydro derivative (KK) was obtained in 44% yield. Nucleophilic opening of the epoxide ring by an acetate ion, followed by aqueous barium hydroxide hydrolysis gave 5,6-diepineamine (LL) in 22% yield, and neamine, G was also recovered in 10% yield (14) (Fig. 12).

These epineamines were tested against several microorganisms. Only 5-epineamine, GG showed a hopeful result against a resistant strain of bacteria, but 6-epineamine, JJ and 5,6-diepineamine, LL did not show any improvement of the activity against a resistant strain of bacteria, moreover they showed a marked decrease of the activity against ordinary bacteria, compared to the parent neamine, G (Table 3) (14).

Considering from the results obtained, it was revealed that deoxygenation or epimerization of the hydroxyl group on C-5 in neamine, G achieved an improvement of the activity against a resistant strain of bacteria. This might be consistent with the fact that 5-deoxysisomicin: "Mutamicin 2" is not inactivated by an N-acetylating enzyme which acetylates the amino group on the

Table 2. Relative antimicrobial activity determined by paper disk method

Compound (Concentration: 1 mg/ml)	<i>B. subtilis</i> 6633	<i>S. aureus</i> 6538p	<i>E. coli</i> K-12	<i>Myc. smeg.</i> 607	<i>E. coli</i> * ML-1629	<i>K. pneumo.</i> * 7
Neamine	1	1	1	1	-	-
5-Deoxyneamine	1.7	1.4	1.5	1	+	+
6-Deoxyneamine	1.2	0.8	1	0.6	-	-
5,6-Dideoxyneamine	1.8	1	1	0.5	+	+
5,6-Dideoxyneamine-5-ene	0.7	0.6	0.5	0	-	+
3',4',5',6-Tetraoxyneamine	0.6	1	0.3	0.3	+	+

* Kanamycin resistant strains. + Active. - Inactive.
B. subtilis 6633 = *Bacillus subtilis* ATCC 6633, *S. aureus* 6538p = *Staphylococcus aureus* 6538p,
E. coli K-12 = *Escherichia coli* K-12, *Myc. smeg.* 607 = *Mycobacterium smegmatis* ATCC 607, *E. coli*
 ML-1629 = *Escherichia coli* K-12 ML-1629, *K. pneumo.* 7 = *Klebsiella pneumoniae* 7.

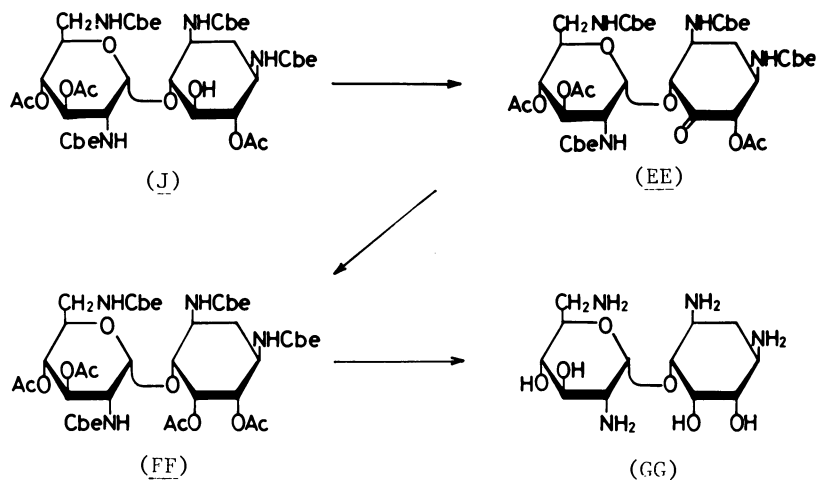


Figure 10. *Synthesis of 5-epineamine (GG)*

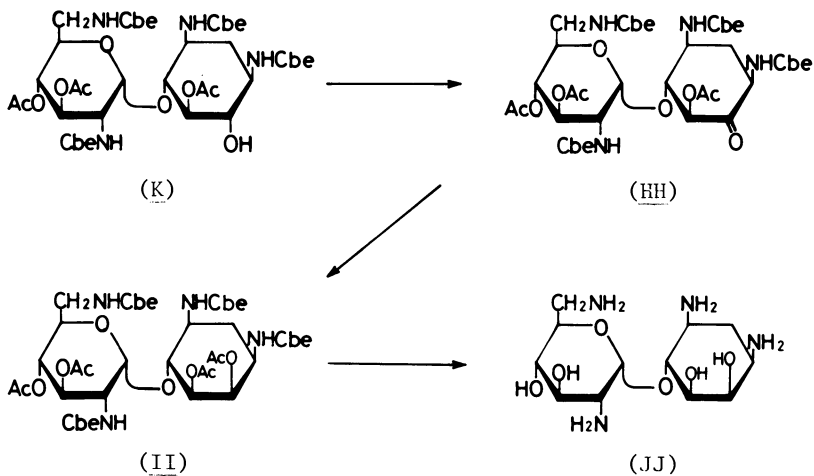


Figure 11. *Synthesis of 6-epineamine (JJ)*

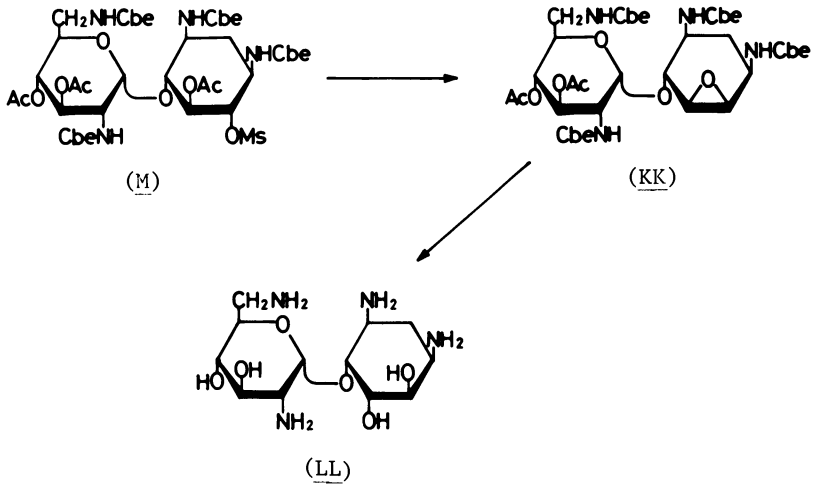


Figure 12. Synthesis of 5,6-diepineamine (LL)

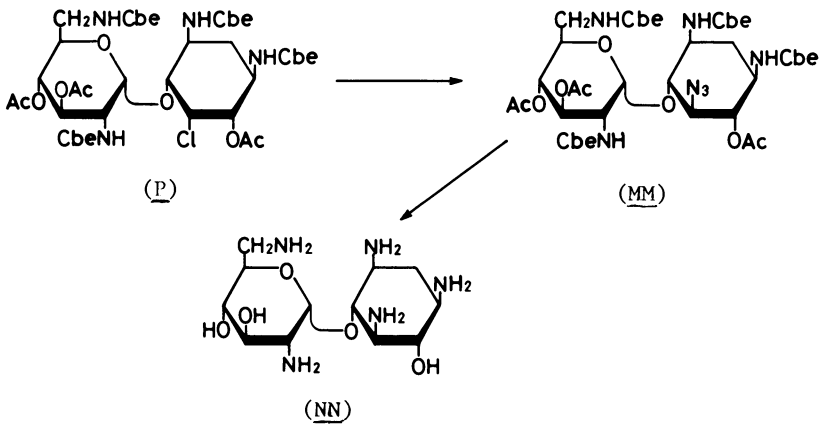


Figure 13. Synthesis of 5-amino-5-deoxyneamine (NN)

C-3 position (15).

Microorganisms used in the present experiments have not been specifically characterized in their deactivating enzyme systems, but probably, by the mechanism demonstrated with 5-deoxystreptomycin (15), an enzyme inactivation by a resistant strain of bacteria might be prevented.

Each one of these two hydroxyl groups of neamine, G has been replaced by an amino group. When 5-chloro-5-deoxyneamine derivative, P was deacetylated and subsequently treated with sodium azide in DMF, 5-azido-5-deoxyneamine derivative (MM) was obtained as crystals in 54% yield. Catalytic hydrogenation of compound MM in the presence of platinum oxide, followed by usual hydrolysis gave 5-amino-5-deoxyneamine (NN) in 58% yield (16) (Fig. 13).

By an analogous reaction process, 6-amino-6-deoxyneamine (PP) was prepared, starting from 6-chloro-6-deoxyneamine, S (16) (Fig. 14).

When the 5,6-anhydro derivative, KK was treated with freshly distilled boron trifluoride etherate in acetonitrile, a mixture of two products (QQ and RR) was obtained.

These two components, QQ and RR were isolated by column chromatography. It was suspected that these two components might be positional isomers, since compounds, QQ and RR gave a same correct elemental analysis and an introduction of an acetamido group might occur on C-5 or C-6 position.

However, degradations of these compounds gave a same triamino derivative: 1L-1,3,5-triacetamido-4,6-di-O-acetyl-1,2,3,5-tetra-deoxy-*allo*-inositol (16). This fact clearly indicated that an attack of an acetamido group occurred at the C-5 position in a manner of trans diaxial opening of the epoxide ring.

A plausible reaction mechanism for an introduction of an acetamido group into a sugar moiety of nucleosides by this reagent has been described in the literature (17), and the same mechanism is proposed in the present reaction (16). Thus, the structures of the two intermediary compounds, QQ and RR are comprehensible in terms of a migration of an ethoxycarbonyl group between an amino group on C-1 and a vicinal hydroxyl group on C-6. An intact mixture of QQ and RR was hydrolyzed to give 5-amino-5-deoxy-5,6-dipeamine (SS) as a sole product in 29% yield (Fig. 16).

Also, compound SS was prepared by an alternative route (Fig. 15). When the epoxide, KK was heated with sodium azide and ammonium chloride in 2-methoxyethanol, an azido derivative was obtained, which was further converted to SS in a yield of 7%, via compound TT (16).

Antimicrobial activities of the amino-deoxyneamine were determined (Table 4). All the compounds tested showed a marked decrease of the activity and therefore, an introduction of one more amino group into the 2-deoxystreptomine moiety of neamine, G is not promising.

When all the four amino groups of neamine were methylated, the product (VV) was devoid of activity (18) (Fig. 17).

Table 3. Relative antimicrobial activity determined by paper disk method

Compound (Concentration: 1 mg/ml)	<i>B. subtilis</i> 6633	<i>S. aureus</i> 6538P	<i>E. coli</i> K-12	<i>Hyco. meg.</i> 607	<i>E. coli</i> [*] ML-1629	<i>K. pneumoniae</i> [*] 7
Neamine	1	1	1	1	-	-
5-Epineamine	1	1	1.6	0.7	+	+
6-Epineamine	0.06	0	0.8	0.05	-	-
5,6-Diepineamine	0.05	0	0.05	0	-	-

* Kanamycin resistant strains. + Active. - Inactive.

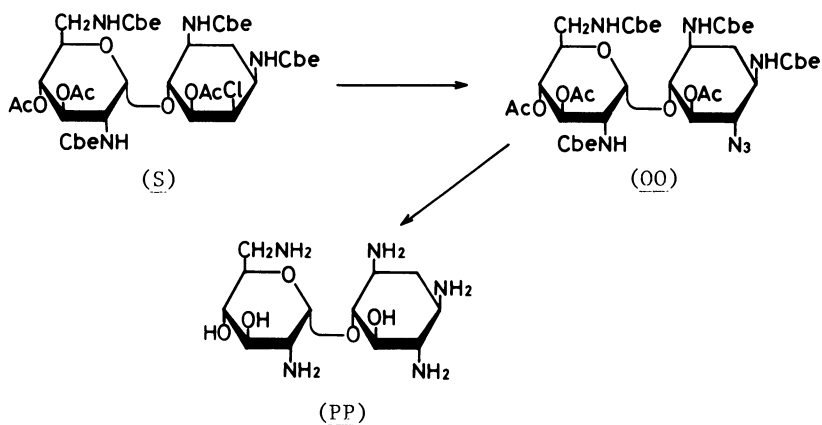


Figure 14. *Synthesis of 6-amino-6-deoxyneamine (PP)*

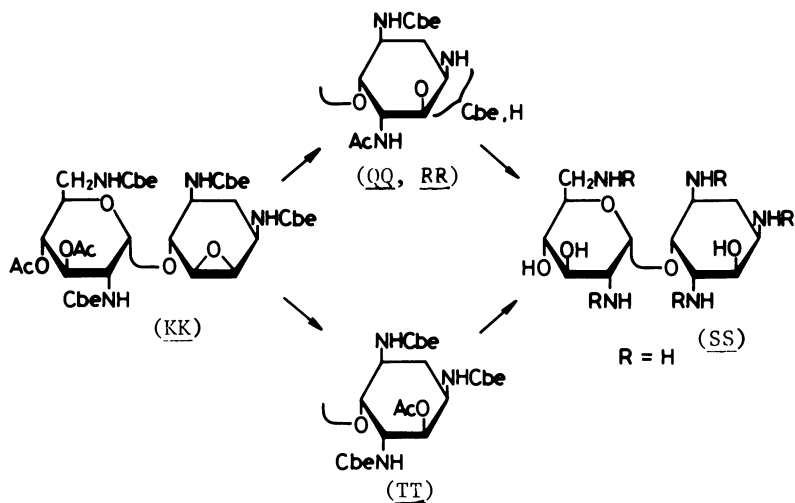


Figure 15. *Synthesis of 5-amino-5-deoxy-5,6-diepineamine (SS)*

Table 4. Relative antimicrobial activity determined by paper disk method

Compound (Concentration: 1 mg/ml)	<i>B. subtilis</i> 6633		<i>S. aureus</i> 6538P		<i>E. coli</i> K-12		<i>Myc. smeg.</i> 607		<i>E. coli</i> ^a ML-1629		<i>K. pneumoniae</i> 7
	1	0.4	1	0.3	1	0.3	1	0.5	1	0	
Neamine	1	0.4	1	0.3	1	0.3	1	0.5	1	0	-
5-Amino-5-deoxyneamine	0.4	0.1	0.3	0.2	0.3	0.08	0.5	0	0	+	-
6-Amino-6-deoxyneamine	0.1	0.02	0.2	0	0.08	0	0	0	0	-	-
5-Amino-5-deoxy-5,6-di-epineamine	0.02		0		0		0		0	-	-

* Kanamycin resistant strains. + Active. - Inactive.

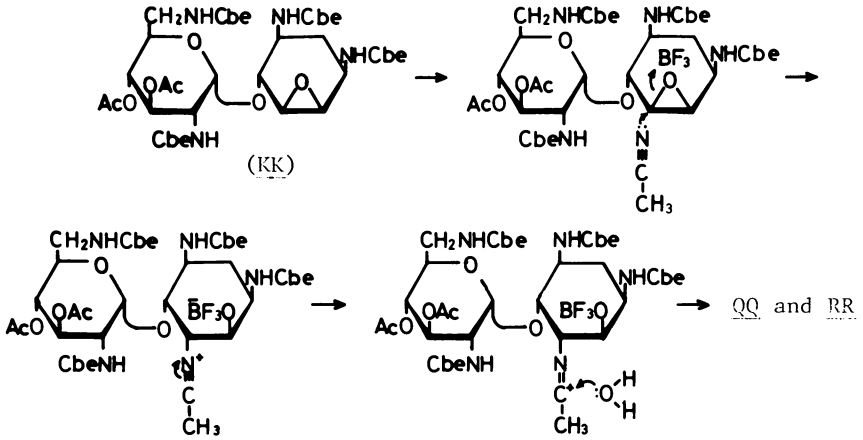


Figure 16. Reaction mechanism for the introduction of acetamido group

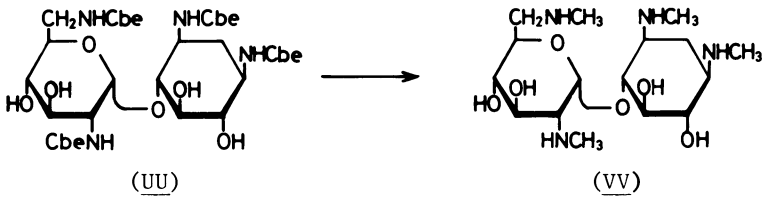


Figure 17. Synthesis of tetra-N-methylneamine (VV)

When the cyclohexane ring of neamine, G was cleaved by periodate oxidation and then reduced with NaBH_4 , a product (WW) is no longer an antibiotic (19) (Fig. 18).

Pseudotrisaccharides were prepared by introducing another sugar to the 5-O or 6-O position, using compound J or K as a starting material.

Ribostamycin (ZZ) is an antibiotic produced by *Streptomyces ribosidificus* and was discovered in 1970 (20). Its synthesis has been described in the literatures (21,22). Ribostamycin, ZZ which is 5-O- β -D-ribofuranosyl-neamine, was prepared selectively from compound J.

When compound J was condensed with 2,3,5-tri-O-benzoyl- α -D-ribofuranosyl chloride (XX) in benzene in the presence of mercuric cyanide and "Drierite", a condensation product (YY) was obtained in 75% yield. Hydrolysis of YY in aqueous barium hydroxide solution afforded ZZ in 63% yield (Fig. 19) (23).

A positional isomer (BBB) of ribostamycin in which β -D-ribofuranosyl group was attached to the 6-O position was prepared from compound K by an analogous reaction process in 29% yield (Fig. 20) (5).

The positional isomer, BBB showed a marked decrease of the activity and was devoid of the activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* (Table 5). This result was coincident with the fact that in the case of β -D-ribofuranosyl-paromamins, 6-O-substituted isomer was much less active than a corresponding 5-O-substituted isomer (24,25).

Since an antibiotic that has an α -D-glucopyranosyl moiety on the 5-O of neamine was prepared by a transglycosidation reaction with neamine and maltose under the presence of an enzyme in a buffer solution (26) and this antibiotic was 2 to 4 times more active than the parent neamine, it was interesting to prepare its anomeric isomer: 5-O-(β -D-glucopyranosyl)neamine (DDD).

Compound DDD was prepared from compound J by the following reaction process as shown in Fig. 21 (23).

The β -anomer, DDD showed less activity against microorganisms tested, except *Mycobacterium smegmatis*, than the parent neamine. The β -anomer, DDD was much less active than the α -anomer (Table 6) (23).

A hexopyranosyl group was then introduced into the 6-O of neamine. When compound K was condensed with acetobromoglucose (EEE) in the presence of mercuric cyanide, a mixture of two products was obtained. The one is an α -D-glucopyranosyl derivative and another one is the corresponding β -anomer. The ratio of α and β anomers was approximately 1:5. The former derivative was converted to 6-O-(α -D-glucopyranosyl)neamine (GGG), which exhibited considerably high activity against *E. coli*, compared to neamine. Compound GGG was also isolated from a culture broth of *Streptomyces kanamyceticus* (27). The latter β -anomer was converted to 6-O-(β -D-glucopyranosyl)neamine (FFF), which showed much less activity than neamine (Table 6) (5) (Fig. 22).

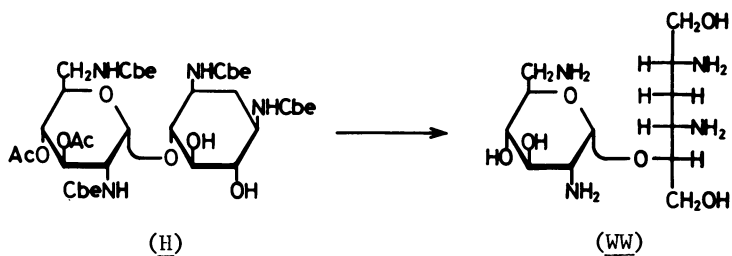


Figure 18. *Synthesis of Compound WW*

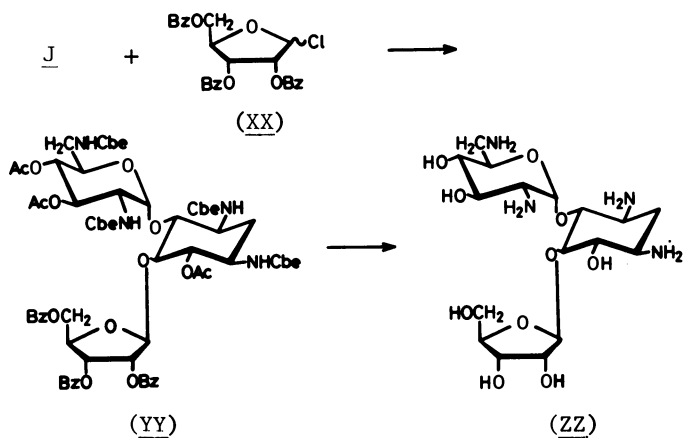


Figure 19. *Synthesis of ribostamycin (ZZ)*

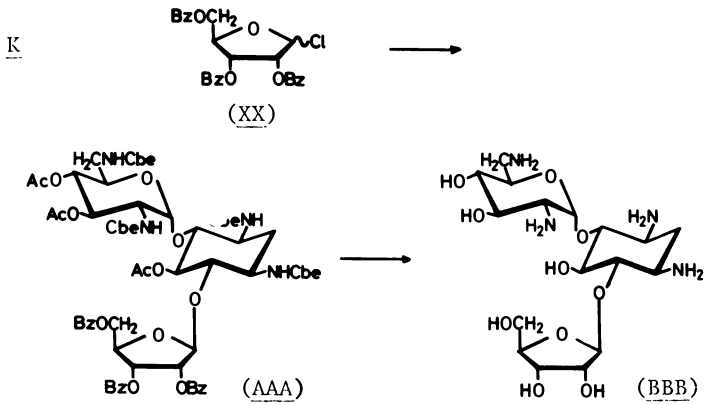


Figure 20. Synthesis of 6-O-β-D-ribofuranosylneamine (BBB)

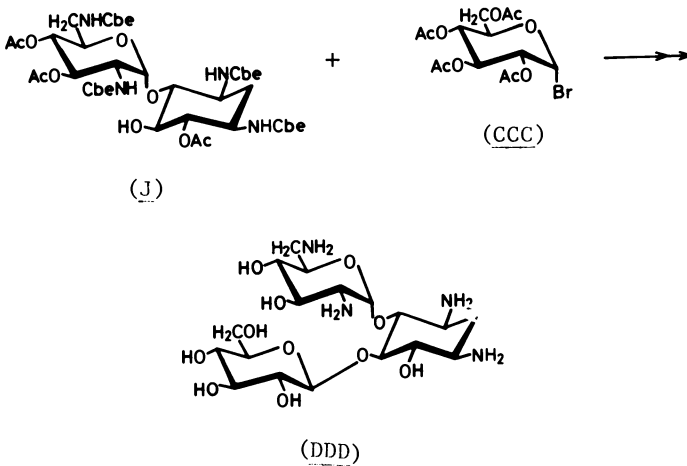


Figure 21. Synthesis of 5-O-(β-D-glucopyranosyl)neamine

Table 5. Relative antimicrobial activity determined by paper disk method

Compound (Concentration: 1 mg/ml)	<i>B. subtilis</i> 6633	<i>S. aureus</i> 6538P	<i>E. coli</i> K-12	<i>Myc. meg.</i> 607
Neamine	1	1	1	1
Ribosamycin	2.4	2.2	3.7	10
6-O-(β-D-Ribofuranosyl)-neamine	0.02	0	0.08	0

Table 6. Relative antimicrobial activity determined by paper disk method

Compound (Concentration: 1 mg/ml)	<i>B. subtilis</i> 6633	<i>S. aureus</i> 6538P	<i>E. coli</i> K-12	<i>Myc. shag.</i> 607
Neamine	1	1	1	1
5-O-(β -D-Glucopyranosyl)-neamine	0.3	0.3	0.7	1.2
6-O-(α -D-Glucopyranosyl)-neamine	0.7	0.7	2	0.4
6-O-(β -D-Glucopyranosyl)-neamine	0	0	0.03	0
6-O-(α -D-Galactopyranosyl)-neamine	0.6	1.4	2.4	0.9
6-O-(β -D-Galactopyranosyl)-neamine	0.04	0.06	0.06	0.4

When a D-galactopyranosyl group was introduced into the 6-0 position of neamine, a mixture of the corresponding two anomeric isomers was obtained. In this case, the ratio of α : β was ca. 1 : 3. Their antimicrobial activity was analogous to those of the corresponding D-glucopyranosyl analogs, respectively (Table 6) (5).

Finally, a modification of an aminocyclitol moiety of kanamycin B was attempted. Since 5-deoxyneamine, R and 5-epineamine, GG showed an improved activity against a resistant strain of bacteria in the present study (6,14), and the 5-deoxygentamicin complex (28) and 5-deoxyisomicin (15) also exhibited an improved activity against resistant strains of bacteria which contain a 3-N-acetylating enzyme, 5-deoxykanamycin B (KKK) and 5-epikanamycin B (NNN) might show an improved activity against a resistant strain of bacteria.

When pentakis-N-(ethoxycarbonyl)kanamycin B (HHH) (32) was treated with 22 molar equivalents of benzoyl chloride in pyridine, a penta-O-benzoyl derivative (III) was obtained in 63% yield. Since a hydroxyl group on C-5 in the 2-deoxystreptamine moiety is sterically highly hindered, the pentabenzoate, III was expected to have a free hydroxyl group on the C-5 position. The proposed structure of III was demonstrated by successive reactions leading to 5-deoxykanamycin B, KKK.

Chlorination of the hydroxyl group in compound III by sulfonyl chloride in pyridine afforded a chloro deoxy derivative (JJJ). Considering the reaction mechanism described by Jones et al (29), the chlorination would occur on the C-5 position with an inversion of the configuration.

Dehalogenation of compound JJJ was performed with tributylstannane in toluene solution and subsequently, all the protective groups were removed by the usual method to give 5-deoxykanamycin B, KKK (Fig. 23) (30). The structure of KKK was determined by mass and carbon-13 NMR spectra (Fig. 24 and Table 7).

5-Epikanamycin B (NNN) was prepared from the same pentabenzoate, III by the following reactions. When compound III was treated with an excess of methanesulfonyl chloride in pyridine, a corresponding sulfonate (LLL) was obtained in 71% yield. Compound LLL was heated with sodium acetate in DMF and the product was purified by column chromatography to give an epikanamycin B derivative (MMM) in 54% yield. The NMR spectrum of MMM revealed a sharp signal of acetoxyl-methyl protons at δ 2.20, indicating the existence of an axial acetoxyl group in compound MMM, since the corresponding 5-O-acetylkanamycin B derivative shows an equatorial acetoxyl-methyl signal at δ 2.07. Therefore, an inversion of the configuration on the C-5 was proved.

Compound MMM was converted to 5-epikanamycin B, NNN in 11% yield by removing the protective groups in the usual way (Fig. 25) (31). The structure of NNN was further confirmed by carbon-13 NMR spectroscopy (Table 7).

Antimicrobial activities of 5-deoxykanamycin B, KKK and 5-epikanamycin B, NNN were determined against several microorganisms

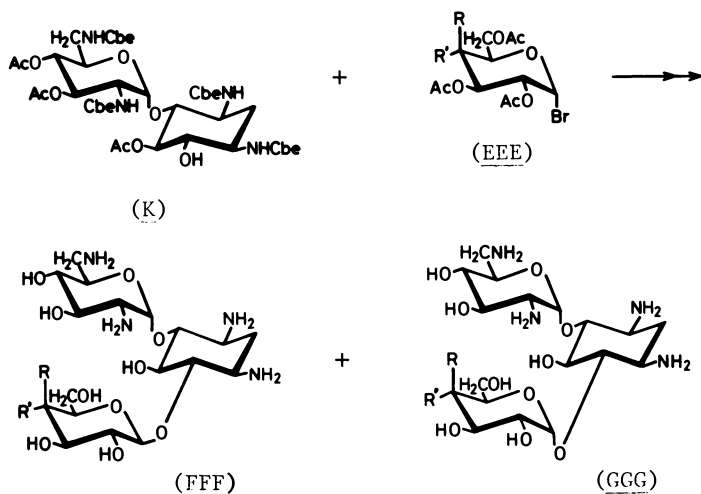


Figure 22. Synthesis of 6-O-(D-hexopyranosyl)neamines (FFF and GGG). D-Gluco: R = H, R' = OH; D-Galacto: R = OH, R' = H

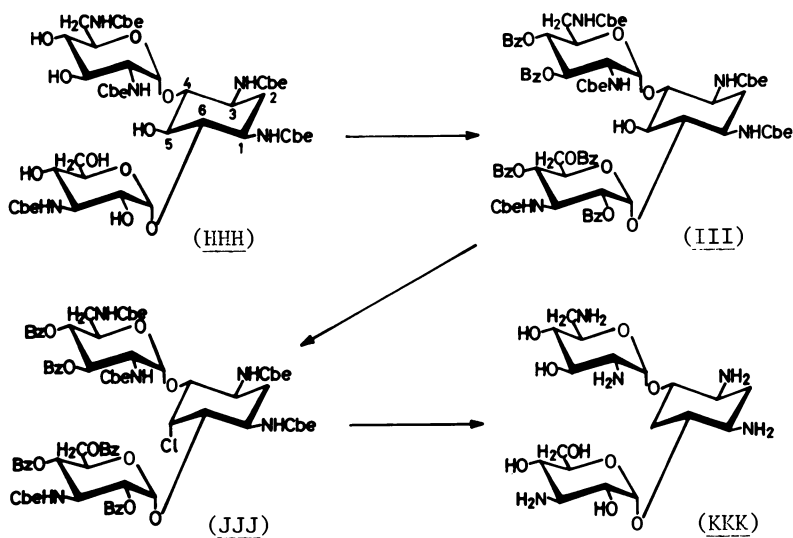


Figure 23. Preparation of 5-deoxykanamycin B (KKK)

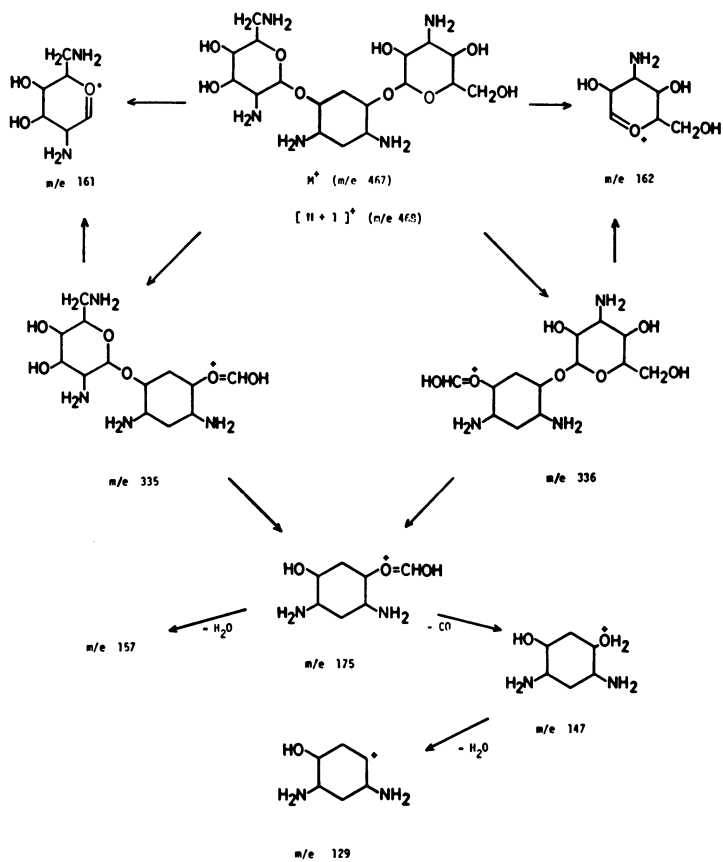


Figure 24. Fragmentation reactions in the mass spectrum of 5-deoxykanamycin B (KKK)

Table 7. Carbon-13 NMR spectra

	5-Deoxykanamycin B		5-Epikanamycin B		Kanamycin B		5-Deoxyneamine	
	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1
C-1	53.4	52.2	48.0	48.2	50.5	50.5	54.9	53.5
C-2	36.9	29.1	36.6	28.8	36.5	28.6	37.0	29.3
C-3	52.6	51.7	47.3	47.6	50.3	49.3	53.0	52.0
C-4	78.1	71.7 ^{c)}	79.9	73.8 ^{e)}	87.4	77.5	78.7	71.8 ⁱ⁾
C-5	34.9	32.9	68.4	65.7	75.2 ^{f)}	75.1	37.6	34.6
C-6	84.1	78.6	86.1	81.0	88.6	84.5	73.7	68.1
C-1'	96.2	91.4	96.0	91.3	100.5	96.2	96.4	91.6
C-2'	55.9	54.0	55.2	54.1	56.3	54.4	56.2	54.0
C-3'	75.4	69.6	74.8	69.5	74.9 ^{f)}	69.0	75.9 ^{h)}	69.5 ^{j)}
C-4'	73.0 ^{b)}	71.3 ^{c)}	72.9 ^{d)}	71.5	73.3 ^{g)}	71.8	73.3	71.6 ⁱ⁾
C-5'	74.4	69.8	73.9	69.9	74.7	70.1	75.1 ^{h)}	69.7 ^{j)}
C-6'	42.7	41.1	42.6	41.0	42.8	41.2	42.9	41.1
C-1''	101.8	100.2	101.9	100.8	101.4	101.4		
C-2''	72.8 ^{b)}	68.8	72.4 ^{d)}	68.8	73.0 ^{g)}	69.0		
C-3''	55.9	55.7	55.8	55.7	56.5	55.8		
C-4''	71.5	67.0	71.1	66.4	71.3	66.3		
C-5''	73.8	73.3	73.5	73.7 ^{e)}	74.0	73.7		
C-6''	62.3	61.4	62.1	61.3	62.0	60.8		

a) In parts per million downfield from tetramethylsilane.

b - i) The signals may be reversed.

of aminocyclitol antibiotics

6-Deoxyneamine		5,6-Dideoxyneamine		5,6,3',4'-Tetradeoxy- neamine		Neamine	
pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1
45.6	45.3	48.6	48.0	48.7	48.2	51.8	50.7
42.1	32.6	41.9	32.9	41.9	32.9	36.9	29.0
50.7	50.0	53.1	52.4	53.3	52.7	50.3	49.5
91.1	80.4	80.4	73.8	80.5	73.1	88.9	77.7
72.1	70.9	27.6	25.2	28.0	25.8	77.4	75.9
41.6	37.2	33.6	27.8	33.7	27.9	78.6	73.3
102.3	96.6	96.2	91.5	96.6	90.5	102.2	96.1
56.6	54.5	56.3	54.0	50.2	49.1	56.8	54.5
75.3 ^{k)}	69.2	76.0	69.5 ^{l)}	71.0	66.3	75.6	69.9
73.2	71.7	73.4	71.6	28.4	26.6	73.4	71.7
75.0 ^{k)}	69.9	75.2	69.6 ^{l)}	71.0	66.3	75.6	69.9
42.9	41.3	43.0	41.0	46.0	43.7	43.0	41.3

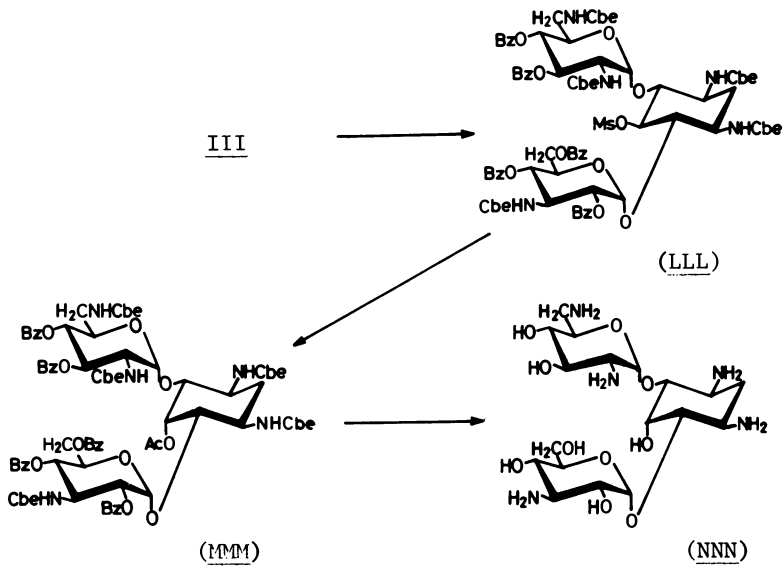


Figure 25. Preparation of 5-epikanamycin B (NNN)

Table 8. Antimicrobial activity of kanamycins B

Test Organisms	MIC (mcg/ml) ^{a)}		
	5-Deoxykanamycin B	5-Epikanamycin B	Kanamycin B
<i>Staphylococcus aureus</i> ATCC 6538P	0.78	1.56	0.39
<i>Staphylococcus epidermidis</i> ATCC 12228	0.39	0.78	0.2
<i>Diplococcus pneumoniae</i> Type 3	0.2	0.39	0.1
<i>Bacillus subtilis</i> ATCC 6633	0.2	0.39	0.1
<i>Escherichia coli</i> NIH JC-2	3.12	6.25	1.56
<i> Klebsiella pneumoniae</i> 602	3.12	3.12	0.78
<i>Pseudomonas aeruginosa</i> IAM 1007	100	100	12.5
<i> Proteus vulgaris</i> OX-19	0.39	0.78	0.2
<i> Salmonella paratyphi</i> A 1015	1.56	1.56	0.39
<i> Salmonella paratyphi</i> B	12.5	3.12	1.56
<i> Shigella flexneri</i> 2a.SH-74-1	6.25	12.5	3.12

a) MIC (minimum inhibition concentration) was determined by a dilution method.

and the results are listed in Table 8. Compounds KKK and NNN showed somewhat lower activities, compared to the parent kanamycin B, and they did not show any improvement of activity against a resistant strain of bacteria. The result is against the expectation as was expected from the data for 5-deoxygentamicin complex (28) and 5-deoxysisomicin (15). The inconsistency might be attributable to the existence of the hydroxyl group on C-6" in 5-deoxykanamycin B, KKK, and a preparation of 5,6"-dideoxykanamycin B is under way to demonstrate the point.

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Literature Cited

1. Shier, W. T.; Rinehart, Jr., K. L.; Gottlieb, D. Proc. Nat. Acad. Sci., U.S., 1969, 63, 198.
2. Shier, W. T.; Ogawa, S.; Hichens, M.; Rinehart, Jr., K. L. Antibiot., 1973, 26, 551.
3. Rinehart, Jr., K. L. Pure and Appl. Chem., 1977, 49, 1361.
4. Ogawa, S.; Funaki, Y.; Iwata, K.; Suami, T. Bull. Chem. Soc. Jpn., 1976, 49, 1975.
5. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. Carbohydr. Res., 1977, 53, 187.
6. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. Carbohydr. Res., 1977, 53, 239.
7. Suami, T.; Umemura, E. unpublished results.
8. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Umemura, E. Bull. Chem. Soc. Jpn., 1978, 51, 2354.
9. Price, K. E.; Godfrey, J. C.; Kawaguchi, H. Adv. Appl. Microbiol., 1974, 18, 225.
10. Canas-Rodriguez, A.; Ruiz-Poveda, S. G. Carbohydr. Res., 1977, 58, 379.
11. Price, K. E.; Godfrey, J. C.; Kawaguchi, H. Adv. Appl. Microbiol., 1974, 18, 293.
12. Rosenbrook, Jr., W.; Carney, R. E. J. Antibiot., 1975, 28, 953.
13. Rosenbrook, Jr., W.; Carney, R. E.; Egan, R. S.; Stanaszek, R. S.; Cirovic, M.; Nishinaga, T.; Mochida, K.; Mori, Y. J. Antibiot., 1975, 29, 960.
14. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. Carbohydr. Res., 1978, 65, 57.
15. Testa, R. T.; Wagan, G. H.; Daniels, P. J. L.; Weinstein, M. J. J. Antibiot., 1974, 27, 917.

16. Nishiyama, S.; Ishikawa, Y.; Yamazaki, M.; Suami, T. Bull. Chem. Soc. Jpn., 1978, 51, 555.
17. Reichman, U.; Hollenberg, D. H.; Chu, C. K.; Watanabe, K. A.; Fox, J. J. J. Org. Chem., 1976, 41, 2042.
18. Suami, T.; Kato, T. unpublished results.
19. Suami, T.; Iwata, K. unpublished results.
20. Shomura, T.; Ezaki, N.; Tsuruoka, T.; Niwa, T.; Akita, E.; Niida, T. J. Antibiot., 1970, 23, 155.
21. Ito, T.; Akita, E.; Tsuruoka, T.; Niida, T. Agr. Biol. Chem. 1970, 34, 980.
22. Fukami, H.; Kitahara, K.; Nakajima, M. Tetrahed. Lett., 1976, 48, 545.
23. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. Carbohydr. Res., 1977, 56, 415.
24. Ogawa, T.; Takamoto, T.; Hanessian, S. Tetrahed. Lett., 1974, 46, 4013.
25. Takamoto, T.; Hanessian, S. Tetrahed. Lett., 1974, 46, 4009.
26. Endo, T.; Perlman, D. J. Antibiot., 1972, 25, 681.
27. Murase, M.; Ito, T.; Fukatsu, S.; Umezawa, H. Progress in Antimicrobial and Anticancer Chemotherapy, Univ. of Tokyo Press, 1970, 2, 1098.
28. Rosi, D.; Gross, W. A.; Daum, S. J. J. Antibiot., 1977, 30, 88.
29. Jennings, H.; Jones, J. K. N. Can. J. Chem., 1965, 43, 2372.
30. Suami, T.; Nishiyama, S.; Ishikawa, Y.; Umemura, E. Bull. Chem. Soc. Jpn., 1978, 51, 2354.
31. Suami, T.; Nakamura, K. Bull. Chem. Soc. Jpn., 1979, 52, 955.
32. Umezawa, S.; Umezawa, H.; Okazaki, Y.; Tsuchiya, T. Bull. Chem. Soc. Jpn., 1972, 45, 3624.

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Syntheses of a Few Branched-Chain Aminocyclitol Antibiotics

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Recently, some biologically and optically active branched-chain aminocyclitol antibiotics such as validamycin A (1,2), orizoxymycin (3,4), G 7063-2 (5), enaminyomycin (6), and cyclitol antibiotics such as crotepoxide (7,8), LL-Z 1200 (9), simondsins (10), glyoxalase I inhibitor (11, 12), rancinamycins (13) have been found successively (Figure 1). These compounds except validamycin have relatively simple structures and seem to have a close relationship with biosynthesis of shikimic acid. However, the reliable synthetic methods for the stereoselective introduction of carbon-branching into inositols are yet few, compared with the recent advances in the usual sugar field. Among the several methods so far reported, the derivation from cyclohexenepolyols or nitromethane cyclization products (14) provide the only general methods for a total synthesis of branched-chain cyclitols.

In the case of cyclohexenepolyols, Diels-Alder adducts (15, 16) and hydrogenation products of benzene derivatives (17,18,19) can be freely selected depend on the structure of aimed compounds, although resolution is necessary in the course of the synthesis. Thus, the synthesis of D,L-validamine from the Diels-Alder adduct of furane and acrylic acid (20) and of D,L-gabaaculine (5-amino-cyclohexa-1,3-dienyl carboxylic acid) from propiolic acid and butadiene (21) were reported recently.

On the other hand, nitromethane cyclization has been extensively used for the synthesis of aminocyclitols (14,22,23,24,25, 26). Although this method is the sole one to obtain optically active compounds by which the part of asymmetric configurations can be previously designed, it is known that the cyclization of 6-deoxy-6-nitro-hexoses under basic conditions affords thermodynamically equilibrated mixture (27,28). Kovar and Baer disclosed that the barium hydroxide-catalyzed cyclization of the 6-deoxy-3-O-methyl-6-nitro-D-allose under conditions favoring kinetic control (pH 8, 0°C) gave mainly 1D-5-deoxy-2-O-methyl-5-nitro-*allo*-inositol together with the 1L-*epi*-1 and *epi*-6 stereoisomers as minor products, whereas that of L-talose derivative afforded *myo*-5 isomer in a high yield, which can be isomerized to *neo*-2

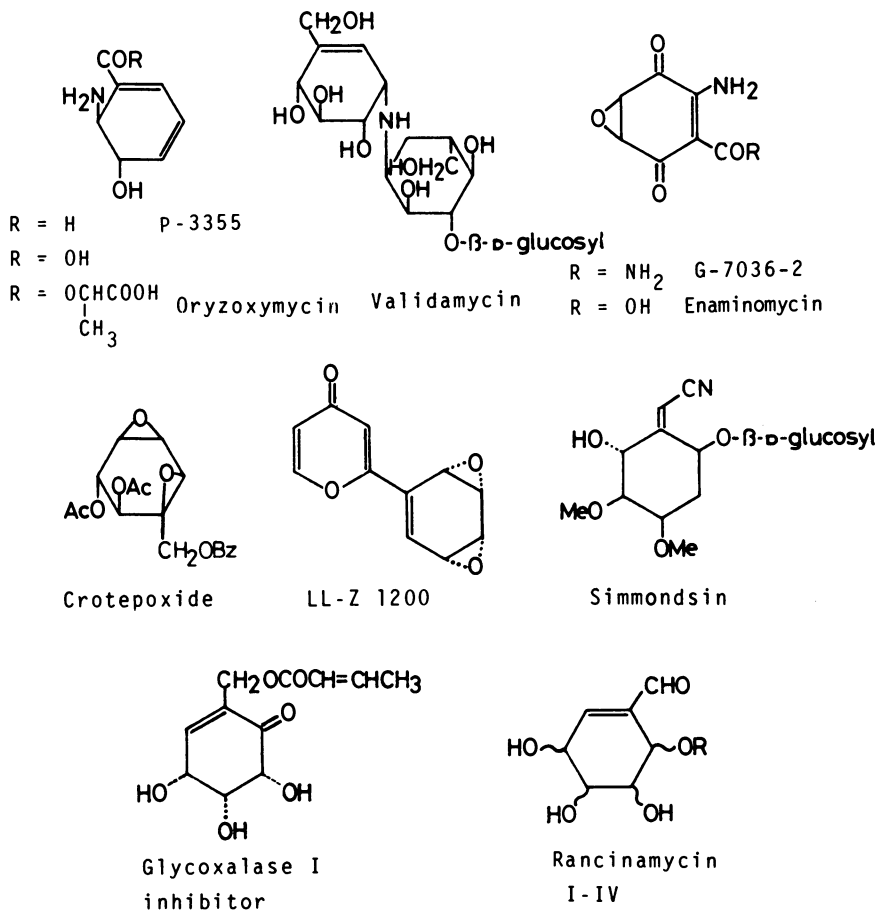


Figure 1. *Optically and biologically active branched-chain aminocyclitols and cyclitols*

epimer (29). For the purpose of the stereoselective synthesis of branched-chain nitrocyclitols which can be easily converted into aminocyclitols, it was supposed that the introduction of a bulky carbon-branching instead of a hydroxy group into C-5 of 6-deoxy-6-nitro-hexoses limits the number of possible isomers by fixing both nitro group and 5-C-substituent in a trans-diequatorial orientation.

In this report we will describe some new synthetic approaches to branched-chain aminocyclitols from Diels-Alder adducts of ethyl 3-nitro-2-alkenoate and 1,3-butadiene which include both nitro and alkoxy-carbonyl groups as the precursors of an amino and carbon-branching groups, and also from cyclization products of 5,6-dideoxy-5-C-(1,3-dithian-2-yl)-nitro-hexoses. No special route to a proper antibiotic could be shown, but the steric course in the above cyclization was extensively examined.

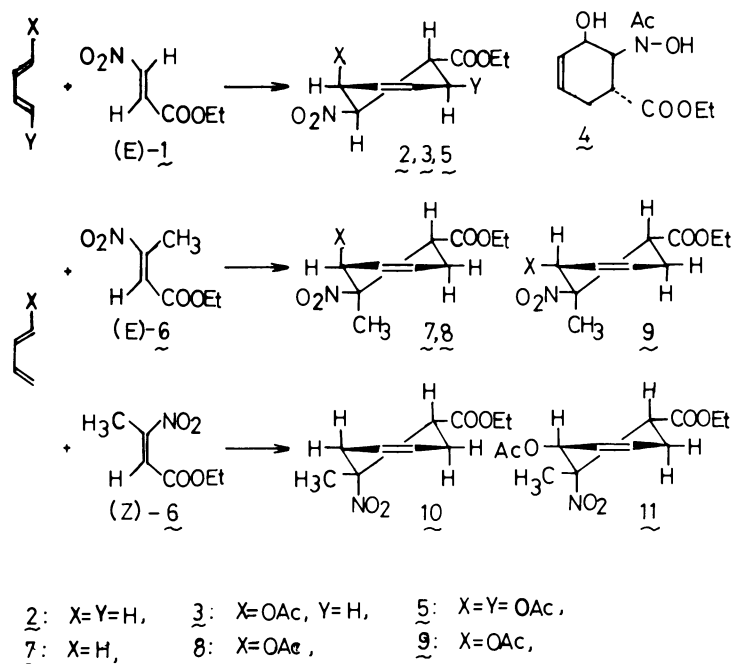


Figure 2. Diels-Alder adducts from ethyl 3-nitro-2-alkenoate and 1,3-butadiene

Conversion of Diels-Alder Adducts from Ethyl 3-Nitro-2-alkenoate and 1,3-Butadiene

In previous papers, we reported on the synthesis and configuration of Diels-Alder adducts from ethyl 3-nitro-2-alkenoate and cyclopentadiene (30) and 1,3-butadiene (31). Among the adducts from 1,3-butadiene (Figure 2), that from 1-acetoxy and 1,4-

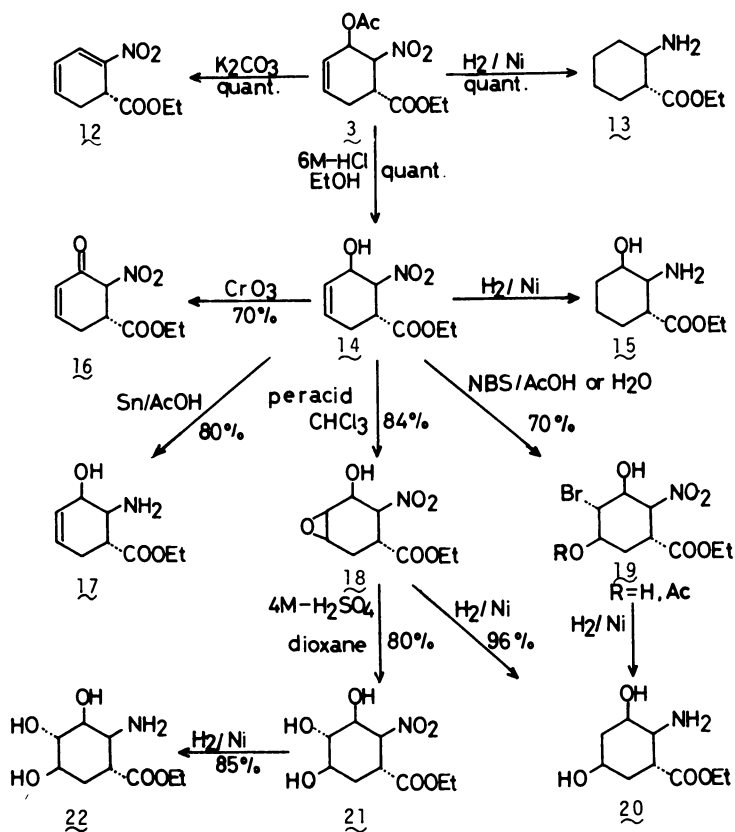


Figure 3. Conversion of ethyl t-5-acetoxy-t-6-nitro-3-cyclohexene-r-1-carboxylate

diacetoxy derivatives, 3 and 5 (57 and 91% Yields), are useful intermediates to branched-chain deoxyaminocyclitols. The positional relation between nitro and acetoxy groups in 3 was confirmed by the *O-N* acetyl migration during the reduction with aluminium amalgam to give *N*-acetylhydroxamic acid, 4. Other adducts having two carbon branchings, 7-11, may be used for a special type of aminocyclitols, but the yields of 8-11 were not so good.

As an example, chemical conversions of 3 were shown in Figure 3. Due to the strong electron withdrawing effect of the nitro group, easy elimination of the acetoxy group occurred under alkaline conditions to give the dihydrobenzene derivative, 12. Hydrogenation of 3 in the presence of Raney nickel also proceeded via the initial elimination to give 13, and the normal hydrogenation product, 15, could be obtained through de-acetylated compounds, 14. Oxidation of the hydroxyl group and reduction of the nitro group with tin and acetic acid in 14 gave the corresponding

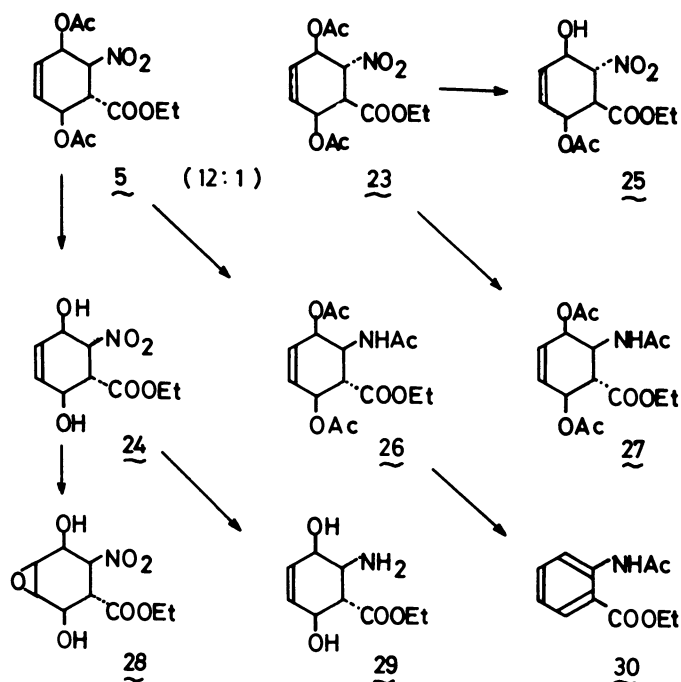


Figure 4. Conversion of 5 and 23

enone, 16, and aminocyclohexene, 17, respectively. Peroxy acid oxidation or addition of bromine atom of NBS in acetic acid or water to the olefinic function of 14 gave the corresponding epoxide 18 or bromo derivative 19, respectively. The stereospecificity in these conversions is controlled by the axial hydroxyl group in 14 (32). Hydrogenolysis of both 18 and 19 gave the common dideoxyaminocyclitol, 20. Hydrolytic opening of the epoxy function in 18 gave the corresponding cyclohexanetriol, 21, which was converted into aminocyclitol, 22. Similar conversions of the olefinic function and nitro group in 2 were also successful.

Although it is not yet extensively explored, the Diels-Alder adduct, 5, seems to be a suitable starting material for the synthesis of enaminomycin and oryzoximycin analogue shown in Figure 1, if the above conversions are applicable. Recently, a diastereomer, 23, of 5 having trans-orientation between the nitro and vicinal acetoxy groups was isolated as a minor product (Figure 4). Deacetylation of 5 and 23 with 6M-HCl in methanol gave the corresponding diols, 24 and 25, respectively. Direct hydrogenation of 5 and 23 with tin and acetic acid gave unexpectedly the corresponding *N,O*-triacetate, 26 and 27, in 67% yield, respectively. Compound 24 was also converted into the corresponding epoxide, 28, and amino derivative, 29, by similar ways shown in Figure 3. However, treatment of 26 with DBU at room temperature gave anthranilic acid derivative, 30, together with a small amount of acethoxybenzoates. Therefore, conversion of 29 into an oryzoximycin analogue is now under investigation.

Cyclization of 5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitrohexoses

As a rule, the starting materials were synthesized by successive reactions, the Michael addition (34) of 2-lithio-1,3-dithiane to 5,6-dideoxy-6-nitro-hex-5-enofuranoses and then the removal of 1-*O*-protecting group. The Michael addition of 2-lithio-1,3-dithiane to 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro- α -D-xylo-hex-5-enofuranose, 31, gave the corresponding *L-ido*, 32, and *D-gluco*, 33, products in the ratio of 4:3 (Figure 5). The crystalline 32 was de-*O*-isopropylidened, and then cyclized. When sodium carbonate was used as a catalyst, two main products were detected on t.l.c. and one of which showed typical absorption bands of nitroolefin at 1650 and 1520 cm^{-1} . The *scyllo*-nitroinositol, 34, and sirupy nitroolefin, 35, were isolated as *O*-acetyl derivatives in the ratio of 1:1. While, the same cyclization with sodium hydrogencarbonate gave the corresponding *myo*-nitroinositol, 36, as the main product. Because the acetylation of 36 in the presence of boron trifluoride etherate or *p*-tolylsulfonic acid gave only 35, 36 was characterized as *O*-acetyl-*O*-isopropylidene derivative 37. Cyclization of de-*O*-isopropylidened 33 in the presence of sodium carbonate or sodium hydrogencarbonate gave only *muco*-product which was characterized as tri-*O*-acetyl, 38, and *O*-acetyl-*O*-isopropylidene, 39, derivatives.

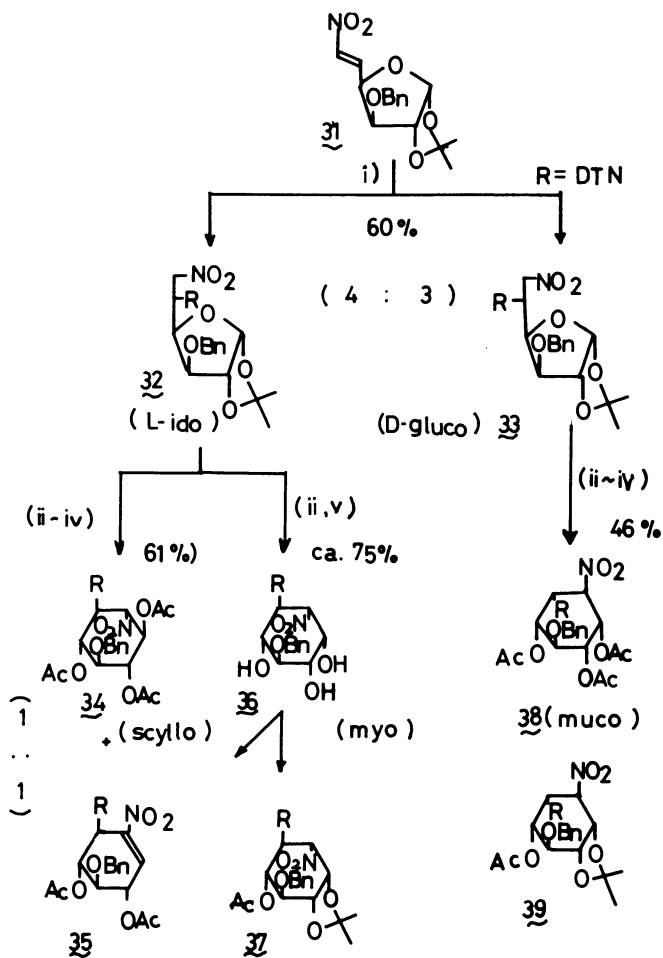


Figure 5. Cyclization of 3-O-benzyl-5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitro-L-idose and -D-glucose. (i) Li-DTN/THF/ -45°C ; (ii) 75% AcOH reflux 2.5 hr; (iii) 2% Na_2CO_3 ; (iv) $\text{Ac}_2\text{O}/\text{TsOH}$; (v) 0.5% NaHCO_3 .

Another possible *chiro*-isomer could not be detected. Conversion of 1,3-dithian-2-yl group in 39 into dimethoxymethyl group was successfully carried out by the treatment with mercuric oxide, mercuric chloride, and boron trifluoride etherate in methanol (35).

The reason why the different basic conditions gave strong influence on the stereodirection of cyclization of 32, but not of 33, is ambiguous. However, these phenomena could be understood on the following assumptions: (i) in the case of 32, *myo*-36 is the kinetically controlled product and the epimerization to *scyllo* form is very slow in the presence of sodium hydrogencarbonate, whereas the epimerization is faster at a higher pH where more labile 36 than *scyllo* form partly changed to the nitroolefin, (ii) in the case of 33, the *muco*-inositol is the kinetically and also thermodynamically controlled product.

For *L-talo* and *D-allo* compounds (Figure 6), the nitroolefin, 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro- α -*D-ribo*-hex-5-enofuranose, 40, was newly prepared from the corresponding *D*-allofuranoside, by periodate oxidation, condensation of nitromethane, and then dehydration in 75% total yield. Addition of 2-lithio-1,3-dithiane to the olefinic function of 40 gave the corresponding products of *L-talo*, 41, and *D-allo*, 42, configurations in the ratio of 2:3. Cyclization of de-*O*-isopropylidenated product of 41 with sodium hydrogencarbonate gave one nitroinositol of *myo*-configuration, 43, whereas that of 42 gave two nitroinositols of *epi*, 44, and *allo*, 45, configurations in the ratio of 1:1. When the cyclization was conducted with sodium carbonate, nitroolefin formation took place rather preferentially and the yields of nitroinositols were much decreased. Compound 44 was also converted into the corresponding *O*-isopropylidene-*O*-acetyl derivative, 46, indicating the presence of *cis*- α -diol function (36).

NMR parameters of tri-*O*-acetyl derivatives of 44 and 45 are shown in Table 1, together with those of 34, 38, 43, and 37. H-1 and H-5 proton signals (a common numbering was tentatively used for easy understanding) were distinguishable from others by their chemical shifts, and other signals were assigned with the double resonance technique. As expected, $J_{1,2}$ values indicate a trans-diequatorial orientation of nitro and 1,3-dithian-2-yl groups. From the number of larger trans-diaxial couplings, *scyllo*-34, *myo*-43, and *allo*-45 configurations were predicative. *Muco*-38 and *epi*-44 configurations were deduced from that of the parent 6-nitrohexoses. It will be worthy to note that 37 exists in a twist-boat conformation.

The stereodirection of the above cyclization will be considered from the conformation in the transition state (Figure 7). When the benzyloxy group must occupy an axial position such as in the case of *D-gluco* and *L-talo* derivatives, a newly formed hydroxyl group should take an equatorial position to avoid 1,3-nonbonded interaction. Thus, the thermodynamically stable isomer, *muco* and *myo*, respectively, is formed as a single product.

In the next experiment (Figure 8), 3-*O*-benzyl-5,6-dideoxy-6-

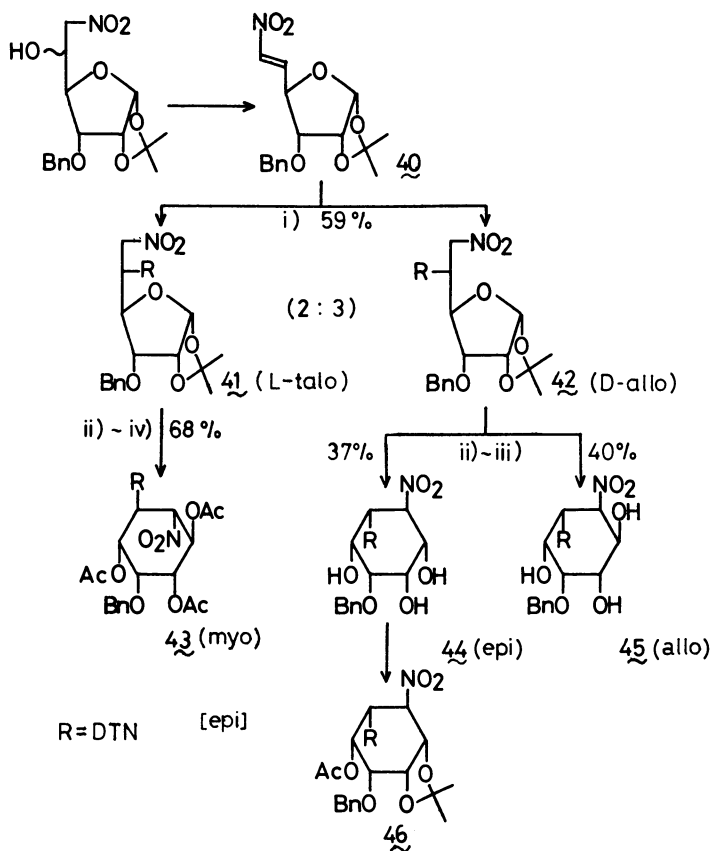
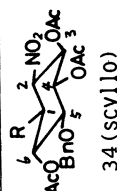
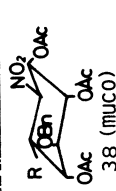
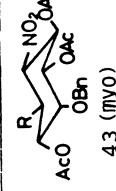
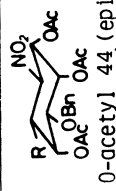
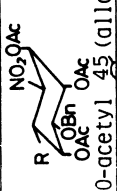
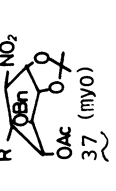


Figure 6. Cyclization of 3-O-benzyl-5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitro-L-talose and -D-allose. (i) Li-DTN/THF/ -50°C ; (ii) 90% $\text{CF}_3\text{CO}_2\text{H}$; (iii) NaHCO_2 ; (iv) $\text{Ac}_2\text{O}/\text{TsOH}$

Table I NMR parameters of branched-chain nitrocyclitols

nitroinositols	H ₁ (J _{1,2})	H ₂ (J _{2,3})	H ₃ (J _{3,4})	H ₄ (J _{4,5})	H ₅ (J _{5,6})	H ₆ (J _{6,1})	H ₁ '	Ac or (Ip)
 34 (scv110)	2.95 dt (10)	5.06 t (10)	5.61 q (7.5)	5.15 t (7.5)	3.70 q (7.5)	5.43 (10)	3.98 d	1.88 1.95 2.00
 38 (mucO)	3.20 oct (10.5)	5.35 t (10.5)	5.70 q (3.0)	~5.5 (2.5)	3.78 t (2.5)	~5.5 (2.5)	4.13 d	1.97 2.05 2.09
 43 (myO)	3.42 sex (12)	4.92 q (10)	6.00 t (10)	5.0 q (2.8)	4.06 t (2.8)	4.9 (12)	4.06 d	1.96 2.00 2.06
 0-acetyl 44 (ep1)	2.9 oct (10)	5.38 t (10)	5.28 q (3.0)	5.75 q (3.2)	3.56 t (3.2)	5.92 q (3.0)	4.10 d	1.99 2.13 2.15
 0-acetyl 45 (allo)	3.1 m (11)	5.37 q (3.0)	5.37 t (3.0)	5.66 q (3.6)	3.67 q (3.0)	5.92 q (3.0)	4.46 d	2.03 2.12 2.12
 37 (myO)	3.21 oct (12)	5.25 q (3.2)	4.83 q (7.0)	4.42 oct (3.0)	3.79 t (2.4)	5.44 sex (5.6)	4.44 J _{4,6} =1.6	1.30 1.53

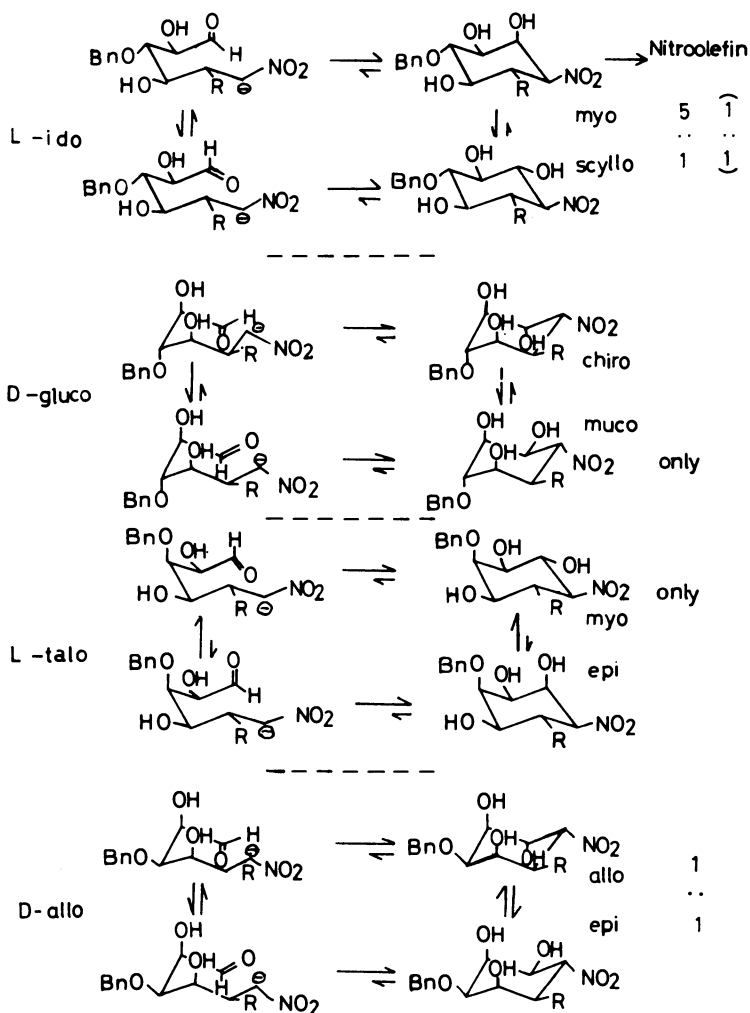


Figure 7. Steric effect of axial benzyloxy group in the cyclization of 3-O-benzyl-5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitrohexoses

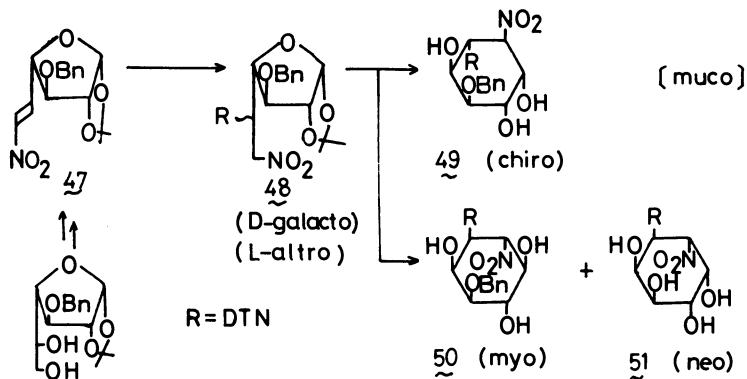


Figure 8. Cyclization of 3-O-benzyl-5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitro-D-galactose and -L-altrose

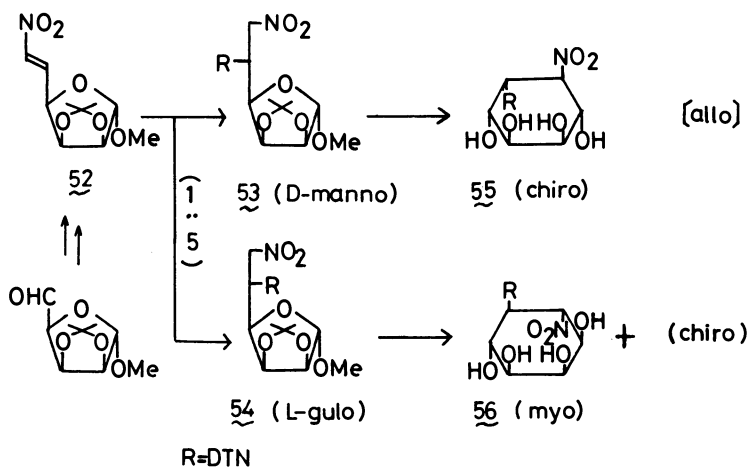


Figure 9. Cyclization of 5,6-dideoxy-5-C-(1,3-dithian-2-yl)-6-nitro-D-mannose and -L-gulose

nitro- β -L-arabino-5-enofuranose, 47, was synthesized from the corresponding galactose derivative by the usual methods. Addition of 2-lithio-1,3-dithiane gave a mixture of the corresponding addition products, 48, of D-galacto and L-alto configurations in 30% yield. Because these isomers could not be separated, the mixture was de-O-isopropylidened and cyclized with sodium hydrogen carbonate. From the reaction mixture, two main products, 49 and 50, of *chiro* and *myo* configurations and one minor products, 51, of *neo* configuration were obtained (37). No *muco*-isomer will be produced by the 1,3-nonbonded interaction in the transition state.

In the cases of the D-manno and L-gulo derivatives (Figure 9) methyl 5,6-dideoxy-2,3-O-isopropylidene- α -D-lyxo-hex-5-enofuranose, 52, was synthesized in the usual manner in a fairly good yield. Addition of 2-lithio-1,3-dithiane to 52 gave the corresponding addition products of D-manno, 53, and L-gulo, 54, configurations in the ratio of 1:5. This stereoselectivity will be explained by the steric hindrance of the isopropylidene group. Cyclization of deprotected sirupy 53 gave only *chiro*-nitroinositol, 55, as expected, whereas in the case of crystalline 54, *myo*-inositol, 56, was obtained in a pure state and *chiro*-isomer was only detectable. In these experiments, the yields of nitroinositols were lower than others mentioned before, we will re-examine this by use of 3-O-benzyl derivatives.

On the other hand, addition of nitromethane to 5,6-dideoxy-6-nitro-hex-5-enoses, 31, 40, 47, and 52 gave the corresponding 5-C-nitromethyl derivatives, 57, 58, 59, and 60, in good yields (Figure 10). Cyclization of these derivatives was expected to proceed in the same manner as 5-C-(1,3-dithian-2-yl) derivatives mentioned before. However, cyclization of de-O-isopropylidened 57 gave many products, in which the presence of nitroolefin and another unidentified main product and a small amount of *muco*-nitroinositol, 61, could be detected, whereas in the case 58, *epi*-62 and *myo*-63, were obtained in fairly good yields as expected. Cyclization of de-O-isopropylidened 59 gave also the expecting nitrocyclitols, 64 and 65, of *chiro* and *myo* configurations. The possible *myo* and *chiro* products, 66 and 67, from 60 are now under separation. In general, isopropylideneation of rough products gave a better result in these experiments.

A successful example of the nitromethane cyclization for the preparation of a designed compound having several asymmetric centers is now described. As the key compound for total synthesis of tetrodotoxin, 1L-(1,2,3',4,5/3,6)-3-hydroxymethyl-4,5-O-isopropylidene-3,3'-O-methylene-6-nitro-2,3,4,5-tetrahydroxycyclohexanecarboxaldehyde dimethylacetal, 75, was synthesized as shown in Figure 11.

The stereoselective epoxidation (38) of 3-deoxy-1,2:5,6-di-O-isopropylidene-3-C-methylene- α -D-ribo-hexofuranose, followed by ring-opening with M sodium hydroxide in tetrahydrofuran gave 68 in a good yield. α -Diol function of 68 was protected with methylenidene group to give 69 which was then converted into 5,6-dideoxy-

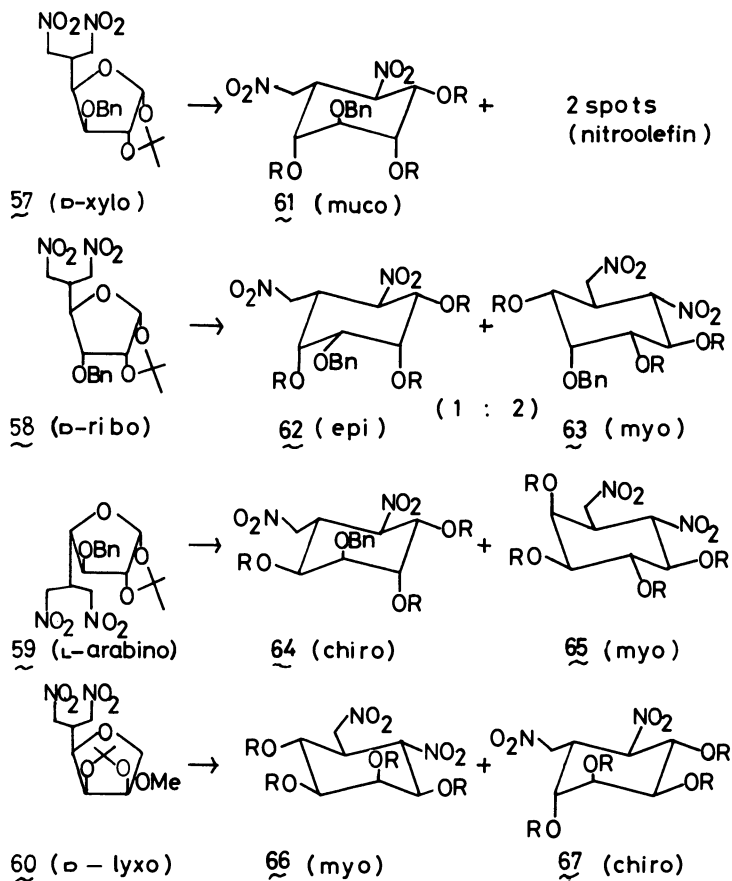


Figure 10. Cyclization of 5,6-dideoxy-6-nitro-5-C-nitromethylhexoses

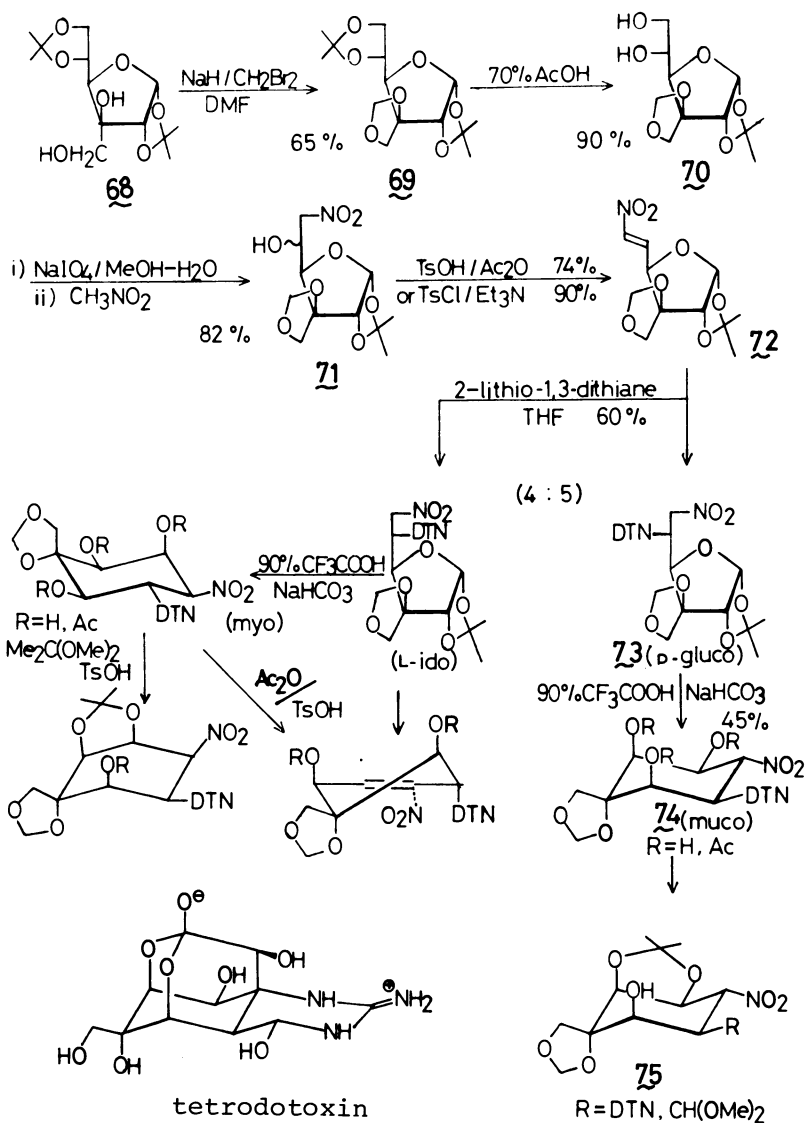


Figure 11. Synthesis of 1-L-(1,2,3',4,5/3,6)-3-hydroxymethyl-4,5-O-isopropylidene-3,3' O-methylene-6-nitro-2,3,4,5-tetrahydrocyclohexancarboxaldehyde dimethyl-acetal

6-nitro-hex-5-enofuranose derivative 72 via 70 and 71, by the usual process mentioned before. Addition of 2-lithio-1,3-dithiane and intramolecular cyclization of the product proceeded in a similar manner mentioned in Figure 5. Thus the aimed compound 75 containing six of seven asymmetric centers of tetradoxin was obtained from 68 through nine steps in 5% overall yield (39).

For the development of utilization of the nitromethane cycli-

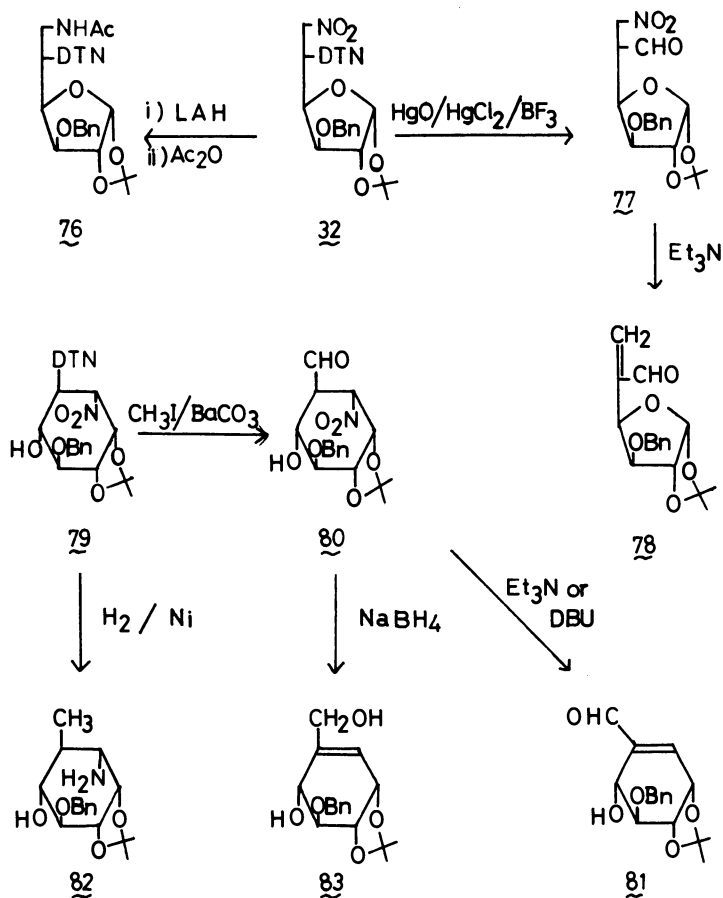


Figure 12. Conversion of DTN and nitro groups in optically active nitrocyclitols

zation, a few transformations of 1,3-dithian-2-yl and nitro groups were examined in the last section. Hydrogenation of 32 with lithium aluminium hydride and then acetylation of the product gave the corresponding acetamido derivative, 76, in good yield. Treatment of 32 with mercuric oxide, mercuric chloride, and boron trifluoride etherate in aqueous acetone gave the 5-C-formyl derivative, 77, of which the nitro group was quantitatively eliminated to give crystalline 78 by treatment with triethylamine. These transformations were also applicable to nitrocyclitols. Treatment of the de-O-acetyl derivative, 79, of 37 with methyl iodide and barium carbonate in aqueous acetone at refluxing temperature gave the corresponding aldehyde, 80, in a good yield, which was characterized as the crystalline 2,4-dinitrophenyl hydrazone. Compound 80 was easily converted into the elimination compound, 81, which is an analogue of rancinamycin. Catalytic hydrogenation of 79 with Raney nickel gave the corresponding aminocyclitol, 82, whereas reduction of 80 with sodium borohydride gave the cyclohexene alcohol, 83 (Figure 12).

Summary

As the useful starting materials for a total synthesis of branched-chain aminocyclitols, Diels-Alder adducts from 3-nitro-2-alkenoates and 1,3-butadienes, and 5,6-dideoxy-5-C-substituted-6-nitro-hexoses were chosen and their transformations were explored. In the former part, various aminocyclitols and their derivatives were derived by the usual transformation of olefinic function and nitro group, and a possible route to oryzoxylicin were shown. However, there will be some stereochemical limitation in this process. In the latter part, stereochemical course in the intramolecular cyclization of this class compounds was extensively examined, and the usefulness of this method for synthesis of a compound designed as a synthetic intermediate of tetrodoxin was shown. Elimination of the nitro group in the compounds of both classes shown in this paper will be applicable for synthesis of unsaturated cyclitol derivatives.

Literature Cited

1. Hori, S., Iwasa, T., Mizuta, E., and Kameda, Y., *J. Antibiotics* (1971), 24, 59.
2. Kameda, Y. and Hori, S., *J.C.S. Chem. Commun.* (1972), 746, 747.
3. Hashimoto, T., Takahashi, S., Naganawa, H., Takita, T., Maeda, K., and Umezawa, H., *J. Antibiotics* (1972), 25, 350.
4. Hashimoto, T., Kondo, S., Naganawa, H., Takita, T., Maeda, K., and Umezawa, H., *J. Antibiotics* (1974), 27, 86.
5. Noble, M., Noble, D., and Sykes, R. B., *J. Antibiotics* (1977), 30, 455.
6. Ito, Y., Haneishi, T., Arai, M., Hata, T., Aiba, K., and Tamura, C., *J. Antibiotics* (1978), 31, 838 and earlier works cited

therein.

7. Kupchan, S. M., Hemingway, R. J., Coggon, P., McPhail, A. T., and Sim, G. A., J. Am. Chem. Soc., (1968), 90, 2982.
8. Kupchan, S. M., Hemingway, R. J., and Smith, R. M., J. Org. Chem. (1969), 34, 3898.
9. Borders, D. B. and Lancaster, J. E., J. Org. Chem. (1974), 39, 435.
10. Elliger, C. A., Waiss, A. C., Jr., and Lundin, R. E., J. Org. Chem. (1974), 39, 2930.
11. Takeuchi, T., Chimura, H., Hamada, M., Umezawa, H., Yoshioka, O., Oguchi, N., Takahashi, Y., and Matsuda, A., J. Antibiotics (1975), 28, 737.
12. Chimura, H., Nakamura, H., Takita, T., Takeuchi, T., Umezawa, H., Kato, K., Saito, S., Tomisawa, T., and Iitake, Y., J. Antibiotics (1975), 28, 743.
13. Argoudelis, A. D., Spvague, R. W., and Mizesak, S. A., J. Antibiotics (1976), 29, 387.
14. Lichtenthaler, F. W., Newer Methods preparative Org. Chem. (1968), 4, 155, and literatures cited therein.
15. Sarel, S. and Kowarsky, H., Bull. Res. Council Israel (1960), A9, 72.
16. Criegee, R. and Becher, P., Chem. Ber. (1957), 90, 2516.
17. Kurihara, N., Hayashi, K., and Nakajima, M., Agr. Biol. Chem. (Tokyo), (1969), 33, 256 and earlier papers cited therein.
18. Suami, T., Lichtenthaler, F. W., Ogawa, S., Nakashima, Y., and Sano, H., Bull. Chem. Soc. Jpn. (1968), 41, 1014.
19. Dijkstra, D., Rec. Trav. Chim. (1968), 87, 161.
20. Suami, T., Ogawa, S., Nakamoto, K., and Kasahara, I., Carbohydr. Res. (1977), 58, 240.
21. Kobayashi, K., Miyazawa, S., Terahara, A., Mishima, H., and Kurihara, H., Tetrahedron Lett. (1976), 537.
22. Hasegawa, A. and Sable, H. Z., J. Org. Chem. (1968), 33, 1604.
23. Baer, H. H. and Wang, M. C. T., Can. J. Chem. (1968), 46, 2793.
24. Hasegawa, A. and Sable, H. Z., Tetrahedron (1969), 25, 3567.
25. Nakagawa, T., Sakakibara, T., and Lichtenthaler, F. W., Bull. Chem. Soc. Jpn. (1970), 43, 3861.
26. Kitagawa, I., Kadota, A., and Yoshikawa, M., Chem. Pharm. Bull. (1978), 25, 3825.
27. Baer, H. H. and Rank, W., Can. J. Chem. (1965), 43, 3462.
28. Lichtenthaler, F. W., Angew. Chem. (1963), 75, 93.
29. Kovar, J. and Baer, H. H., Carbohydr. Res. (1975), 45, 161 and earlier papers cited therein.
30. Shin, C., Kosuge, y., Yamaura, M., and Yoshimura, J., Bull. Chem. Soc. Jpn. (1978), 51, 1137.
31. Shin, C., Yamaura, M., Inui, E., Ishida, Y., and Yoshimura, J., Bull. Chem. Soc. Jpn. (1978), 51, 2618.
32. Henbest, H. B. and Wilson, R. A. L., J. Chem. Soc. (1957), 1958.
33. McCormick, J. R. C., Reichenthal, J., Hirsch, U., and Sjolander, N. O., J. Am. Chem. Soc. (1962), 84, 3711.
34. Iida, T., Funabashi, M., Yoshimura, J., Bull. Chem. Soc. Jpn.

- (1973), 46, 3203.
35. Funabashi, M. and Yoshimura, J., *J. Chem. Soc. Perkin Trans. 1* (1979), 1425.
36. Funabashi, M., Kobayashi, K., and Yoshimura, J., *J. Org. Chem.* (1979), 44, 1618.
37. Iwakawa, M., Funabashi, M., and Yoshimura, J., *Bull. Chem. Soc. Jpn.* (submitted).
38. Funabashi, M., Sato, H., and Yoshimura, J., *Bull. Chem. Soc. Jpn.* (1976), 49, 788.
39. Funabashi, M., Wakai, H., Sato, K., and Yoshimura, J., *J. Chem. Soc. Perkin Trans. 1* (accepted).

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A New Synthesis of Branched-Chain Epi-Configuration Deoxyhalogeno- and Deoxyaminocyclitols

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The ring forming step in the biogenesis of the important cyclitols myo-inositol (1-6) and 2-deoxystreptamine (7,8), and the cyclitol carboxylic acids shikimic acid and quinic acid (9, 10, 11), in each instance appears to involve an enzyme promoted intramolecular aldol condensation of an appropriate delta-dicarbonyl sugar precursor. The resultant substituted cyclohexanones (cycloses) are then biosynthetically converted along discrete pathways to the appropriate cyclitols. This type of cyclization should, in general, also be promoted by base, although one would not expect the stereochemical control that is observed in the enzyme driven cases. A significant example of a cyclization of this variety is the hydroxide induced conversion of 3,7-dideoxy-D-threo-hepto-2,6-diulosonic acid to crystalline dehydroquinic acid as reported by Aldersberg and Sprinson (11). This cyclization also chemically mimicked the ring forming step in the biogenesis of shikimic and quinic acids.

Our interest in this method of cyclose formation was initiated with the hydroxide catalyzed cyclization of "5-ketoglucose" (Kiely and Fletcher, 12) and its 6-phosphate (Kiely and Sherman, 13) to mixtures of cycloses and cyclose phosphates, respectively. These reactions were carried out as chemical modeling experiments for the ring forming step in the biosynthesis of L-myo-inositol 1-phosphate. More recently we have directed our efforts to employing this cyclization in organic solvents on protected 2,6-heptodiuloses for the synthesis of some branched-chain cyclitols, the subject of this report.

Branched-chain Dihalocyclitols

The diketone tri-O-acetyl-1,7-dideoxy-1,7-bis(diazo)-xylo-2,6-heptodiulose (2) prepared from D-xylose (1) in a reaction scheme (Figure 1) that utilized the diazomethane chain extension

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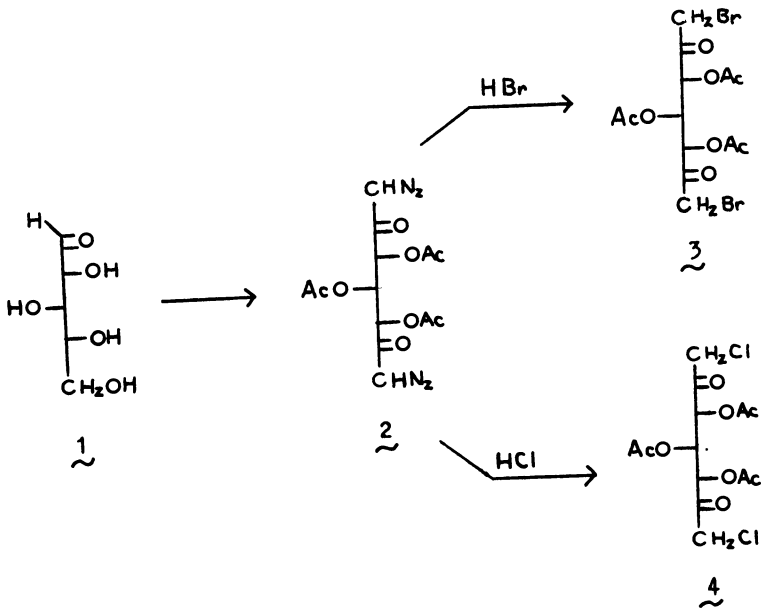


Figure 1.

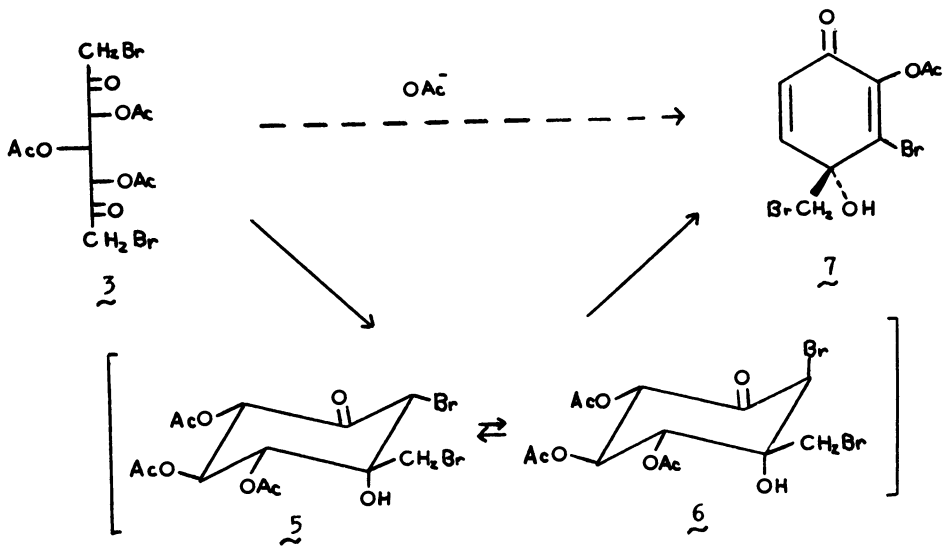


Figure 2.

of tri-*O*-acetyl xylaryl dichloride, served as the key precursor in the overall sequence. This crystalline diazoketone **2** (**14**), when treated with an ether solution of hydrogen bromide or hydrogen chloride, was readily converted to crystalline 1,7-dibromo-1,7-dideoxy-tri-*O*-acetyl-xylo-2,6-heptodiulose (**3**, 74%, **15**) or the 1,7-dichloro derivative **4** (94%, **16**). In an attempt to displace the bromines of **3** with acetate, we observed that stirring an acetone solution of **3** at room temperature in the presence of suspended sodium acetate yielded the crystalline dibromocyclohexadienone derivative **7** (Figure 2) as virtually the only product. The formation of **7** from **3** suggested that an aldol condensation was the first reaction in a series of reactions that produced the product, and that the likely equilibrating initial cyclic products from the condensation were the racemic equatorial and axial α -bromocyclohexanone derivatives **5** and **6**. The conversion of **3** to **7** was repeated, but in order to avoid complications that might result from the presence of water in the reaction mixture, the reagent grade acetone used as the solvent was dried over molecular sieves, and freshly fused sodium acetate used as the basic catalyst. The course of the reaction was monitored by ^1H NMR spectrometry and the results from a typical experiment are shown in Figure 3. After stirring the reaction mixture for 3 h, the acyclic diketone **3** (Figure 3, spectrum A) was consumed and converted to essentially one product, dibromocyclose **5** (Figure 3, spectrum B). Extending the reaction period to 18 h led to the formation of **7** (Figure 3, spectra C and D). The expanded spectrum of **5** was matched with the theoretically generated version (**17**) and clearly showed the large coupling (10 Hz) between the vicinally coupled axial ring protons as well as a single peak from H-2. The spectrum of **5** did not denote the stereochemistry at the new chiral carbons, C-2 and C-3, but the bulkiness of the bromine atom and bromomethyl group suggested that both of these ring substituents are sterically less constrained in equatorial positions.

Treatment of the acyclic dichlorodiketone **4** with acetate (Figure 4) resulted in an even cleaner cyclization in forming the dichlorocyclose **8** (> 90% by ^1H NMR). As with **5** the suggested stereochemistry of **8** at C-2 and C-3 was grounded on steric considerations.

The first branched-chain cyclitol we prepared from the cyclization routine was DL-1,5,6-tri-*O*-acetyl-3-chloro-2-C-chloromethyl-3-deoxy-epi-inositol (**9**). Catalytic reduction of **8** in acetic acid at 80° C over platinum at 3 atm for 20 h gave **9** in 72% yield after a single crystallization, although **9** appeared to be the only product formed from the reduction. Sodium borohydride reduction of **8** in methanol-ether produced the C-4 axial alcohol isolated as its crystalline tetraacetate **10** (63%). This acetate was also prepared by acetylation of **9**. The unprotected dichlorocyclitol DL-3-chloro-2-C-chloromethyl-3-deoxy-epi-inositol (**11**, mp 222-223°C) was then prepared by acidic

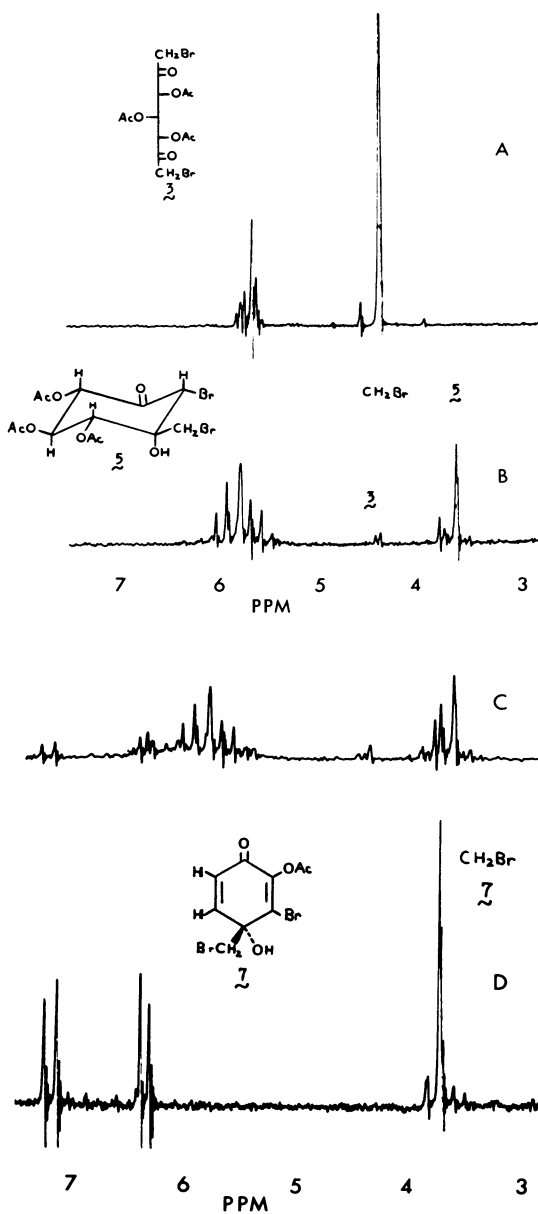


Figure 3. The 90 MHz $^1\text{H-NMR}$ spectra (nonacetyl region) from monitoring the reaction of **3** with sodium acetate: Spectrum A—compound **3**; Spectrum B—reaction mixture after 3 hr; Spectrum C—reaction mixture after 10 hr; Spectrum D—reaction mixture after 24 hr

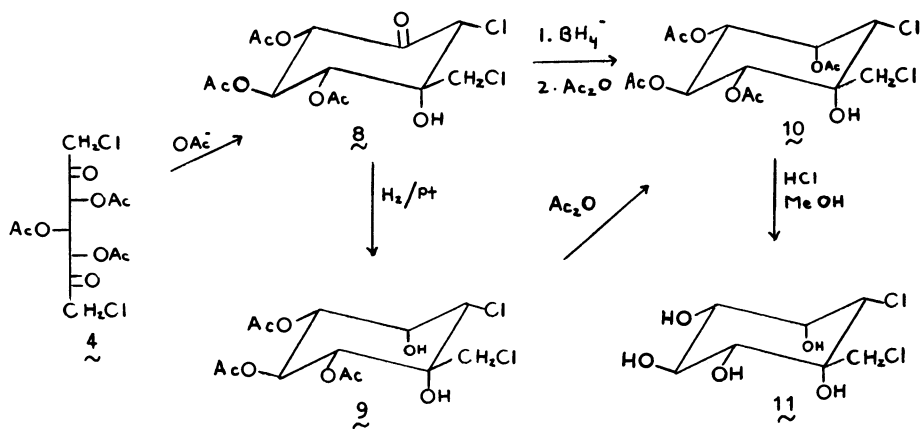


Figure 4.

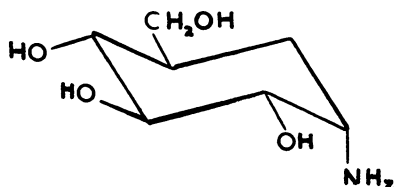
methanolysis of the tetraacetate 10. Conversion of 4 to 11 by the borohydride reduction route was attained in an overall yield of 63% if both 8 and 10 were isolated along the way.

Figure 5 contains a portion of the 90 MHz ^1H NMR spectrum of 9 recorded in $\text{Me}_2\text{SO}-d_6$ (spectrum A) and in the same solvent with D_2O added (spectrum B). These spectra clearly show large coupling between the 1,2-anti-diaxial ring protons H-1, H-6 and H-5, H-6, as compared to the coupling between the axial-equatorial combinations H-5, H-4 and H-3, H-4. Even the hydroxyl protons at C-4 and C-2 of 9 were resolved in the spectrum of the molecule prior to the addition of D_2O . In converting 9 to 10 the C-4 hydroxyl group was acetylated, causing the predicted downfield shift of the H-4 proton (Figure 6).

Returning to the dibromocyclose 5, we found that sodium borohydride reduction of the ketone produced an axial alcohol, isolated as the tetraacetate 12 (Figure 7). Deprotection of this crystalline compound with methanolic HCl provided the final product in the sequence, DL-3-bromo-2-C-bromomethyl-3-deoxy-epi-inositol (13, mp 231-232°C). Based on the starting diketone 3, the overall yield of crystalline 13 was only 27% when 5 and 12 were isolated along the way, but the yield was raised to 69% when crude 5 was not isolated and the reduction mixture was not acetylated. Clearly, all the acetyl groups are stripped from the ring during the course of the reduction.

Branched-chain Aminocyclitols

Microbiologically produced aminocyclitol antibiotics usually contain an unbranched aminocyclitol as a component part. However, the recently discovered validamycins (18-21) produced by Streptomyces hygrosopicus var. limoneus, are structured around the branched-chain aminocyclitol validamine, L-(1,3,4/2,6)-4-amino-6-(hydroxymethyl)-1,2,3-cyclohexanetriol (22). The validamycins are effective in controlling some plant diseases, but show no antimicrobial activity in vitro against



Validamine

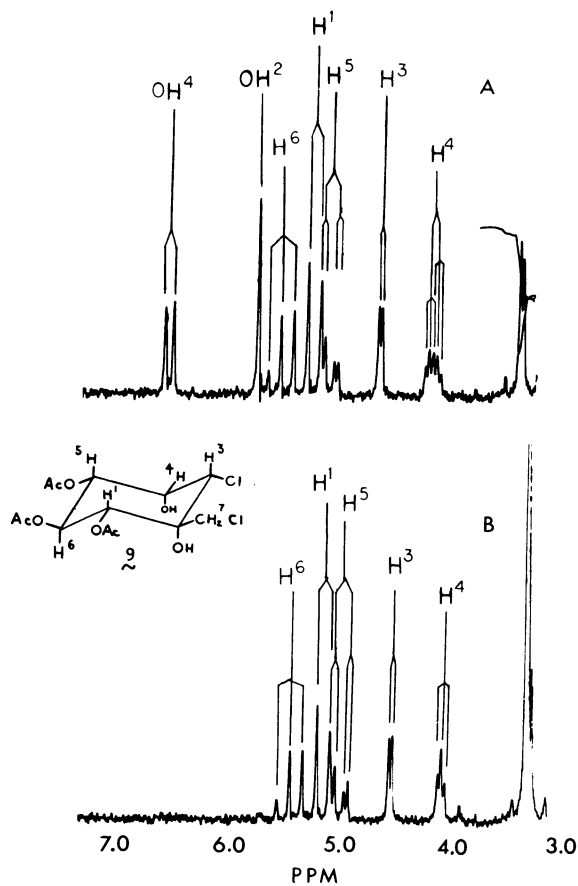


Figure 5. The 90 MHz $^1\text{H-NMR}$ spectra of **9** (nonacetyl region) in $\text{Me}_2\text{SO}-d_6$: Spectrum A—no D_2O added; Spectrum B— D_2O added

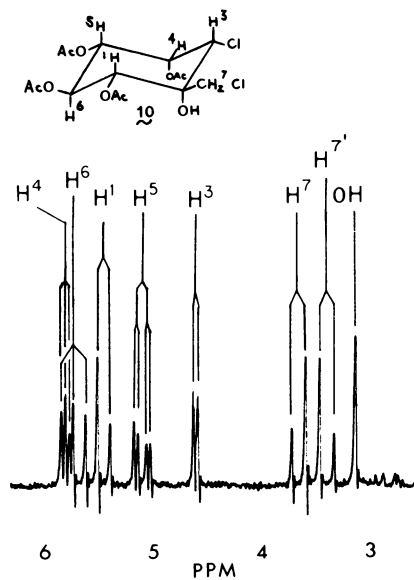


Figure 6. The 90 MHz ¹H-NMR spectrum of 10 (nonacetyl region) in CDCl₃.

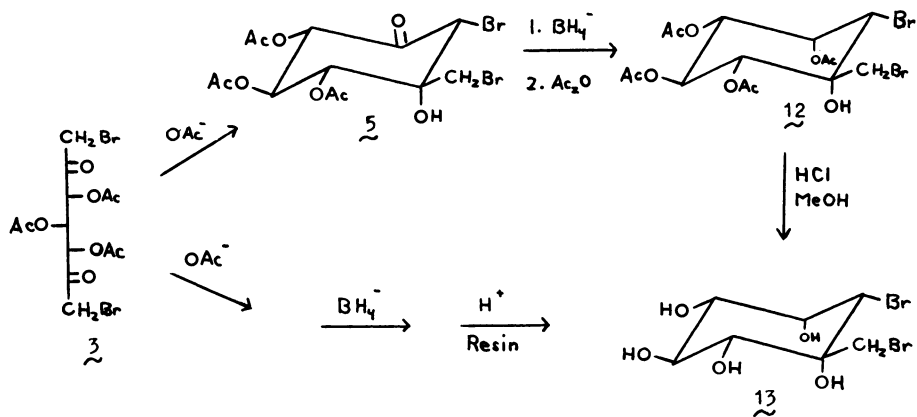


Figure 7.

bacteria and fungi (21). Syntheses of racemic validamine (23,24) and the naturally occurring enantiomer have been reported (24). The preparation of the branched-chain dihalocyclitols 11 and 13 via our cyclization-reduction route suggested that this synthetic approach might be extended to the synthesis of branched-chain aminocyclitols as well.

Our objective was to replace one or both of the halogens from the dihalocyclitols with azide, and then convert the resultant azidocyclitols to aminocyclitols. Using an experimental procedure patterned after the one described by Suami *et al.* for azide displacement of halide from halocyclitol acetates (25), we refluxed an aqueous 2-methoxyethanol solution of 10 with suspended sodium azide in anticipation of displacing one or both of the chlorines on the molecule with azide (Figure 8). Acetylation of the reaction mixture produced the azidochlorocyclitol tetraacetate 14 (73%), which was quantitatively converted to unblocked cyclitol 16 (mp 208-210°C) in methanolic sodium methoxide solution. An alternate route to 14 utilized the epoxide 15, in *tert*-butyl alcohol containing potassium *tert*-butoxide. Treating the *tert*-butyl alcohol solution of crude 15 with suspended sodium azide and ammonium chloride opened the oxirane ring and formed the azidomethyl branch on the cyclitol. Acetylation of the crude product gave 14 (60% yield from 10). The branched-chain aminocyclitol 17 was then quantitatively generated from 16 in aqueous solution by catalytic hydrogenolysis of the azido function over platinum black.

The fact that the ring chlorine of neither 10 nor 15 was displaced by azide under vigorous reaction conditions, supported the notion that this halogen was equatorial and consequently difficult to displace directly or by anchimeric assistance from the C-4 acetoxy group. Verification of the structure of 10 as shown, which also established the structure of 17, was finally achieved by an X-ray crystallographic study (16).

Azide displacement of the side-chain bromine of 12 in aqueous 2-methoxyethanol, followed by acetylation rendered the azidobromocyclitol tetraacetate 18 (84%, Figure 9). Base catalyzed transesterification then removed the acetates from 18 to produce the unprotected cyclitol 19. By carrying out the catalytic hydrogenolysis of 19 in aqueous solution using a platinum catalyst, the azido group was converted to an amine but the resultant basic solution fostered hydrogenolysis of the C-3 bromine. Consequently, the sole product from the hydrogenolysis under these conditions was the deoxyaminocyclitol 20. The ¹H NMR spectrum of 20 as its hydrochloride (20a) is shown in Figure 10. The axial H^{3a} and equatorial H^{3e} protons on C-3 are chemical shift nonequivalent and exhibit slightly different coupling with equatorial H-4. The intended conversion of the azidobromocyclitol 19 to the aminobromocyclitol 21 was accomplished using acetic acid as the solvent during the hydrogenolysis.

Since azide displacement of halogens at the acetylated

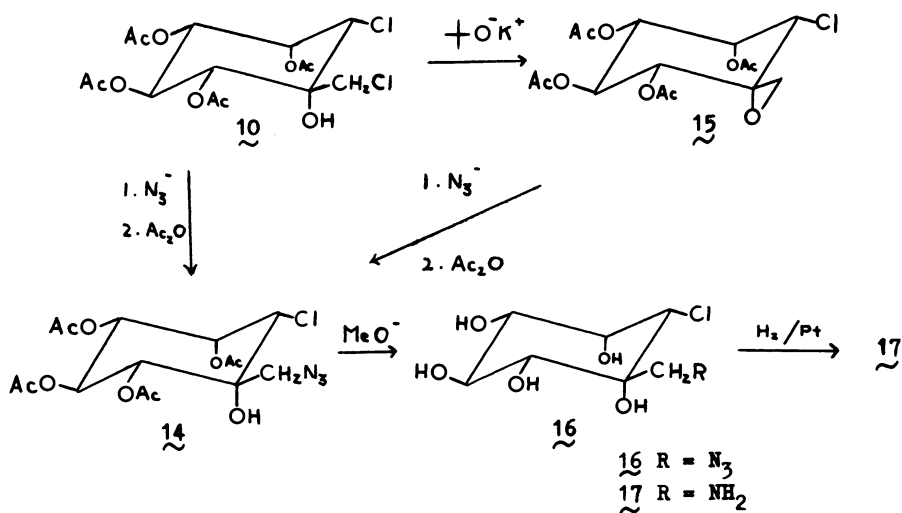


Figure 8.

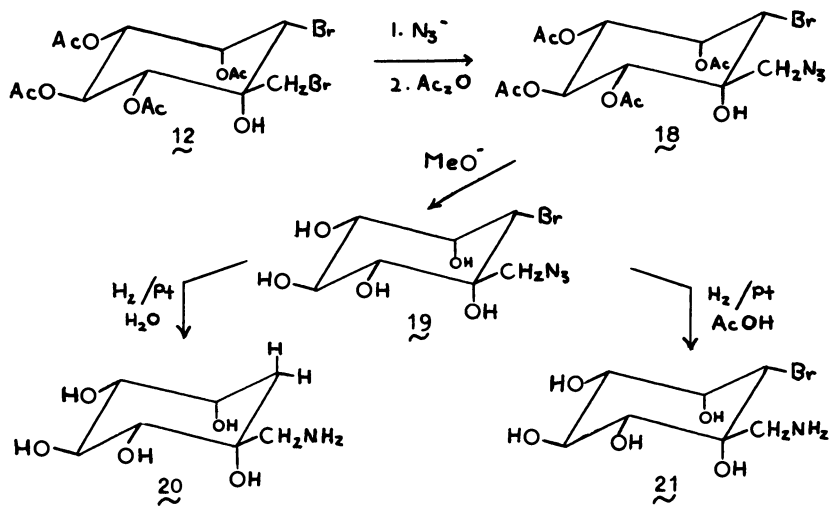


Figure 9.

cyclitol stage produced only azidohalocyclitols, we undertook the displacement of the halogens prior to cyclization, i.e., at the acyclic diketone stage, and followed the course of the reaction by ^1H NMR. The results from spectroscopic monitoring of the reaction of the dibromodiketone 3 with sodium azide are shown in Figure 12, and the accompanying reaction scheme is in Figure 11. An acetone solution of 3 was initially stirred with suspended sodium azide at room temperature. After the reaction mixture was stirred for an hour, the single peak from the bromomethyl protons of 3 at 4.4 ppm (Figure 12, spectra A-C) was gone, and a new singlet was observed at 4.5 ppm. Accompanying this spectral change was a change in the signals from the backbone protons centered at about 5.75 ppm. It seemed likely that the 4.5 ppm signal was due to the azidomethyl protons of the acyclic diazidodiketone 22. By allowing the reaction mixture to remain unstirred at 5°C for an additional 3 h, the spectrum of the mixture underwent a second major alteration; the singlet at 4.5 ppm disappeared, a new singlet at 4.65 ppm erupted, and the downfield backbone proton region (5.75 - 5.85 ppm) changed again (Figure 12, spectra C-E). We have assigned the single peak at 4.65 ppm (spectrum E) to the isolated C-2 proton of the cyclose 23, and the 5.85 ppm region to the ring protons of the molecule. We made no attempt to purify either 22 or 23, but chose to stabilize the ring system by sodium borohydride reduction of 23. The acetylated diazidocyclitol 24 was obtained as a crystalline product (64% yield from 3) after a single crystallization from ethyl acetate-hexane. Methoxide catalyzed deacetylation of 24 produced the nicely crystalline branched diazidocyclitol 25, which was readily converted by catalytic hydrogenolysis to the final branched diaminocyclitol, DL-3-amino-2-C-aminomethyl-3-deoxy-epi-inositol (26). We also found that we could obtain 25 in 64% yield by simple crystallization of the reduction-deacetylation product from 23. Thus, in three simple steps it was possible to convert the acyclic dibromodiketone 3 to the diaminocyclitol 26 in better than 60% overall yield. The stereochemistry of the cyclitols 24-26 was assigned on the basis of the ^1H NMR spectra, illustrated in the spectrum of 25 (Figure 13), and by way of analogy with the previously determined stereochemistry of the dichlorocyclitol 10.

Table I contains a list of the unprotected branched-chain cyclitols that have been prepared from D-xylose by the cyclization-reduction process described in this report. The dichloro, dibromo, and azidocyclitols were crystalline compounds, as were the picrates of the branched-chain aminocyclitols.

Acknowledgment

This work was supported by Grant No. GM-19252 from the National Institute of General Medical Sciences, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, MD., 20014.

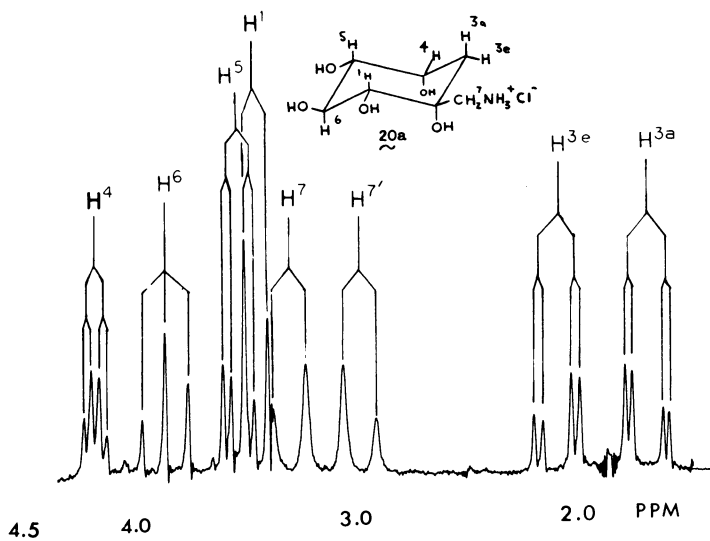


Figure 10. The 90 MHz ¹H-NMR spectrum of 20a in D₂O

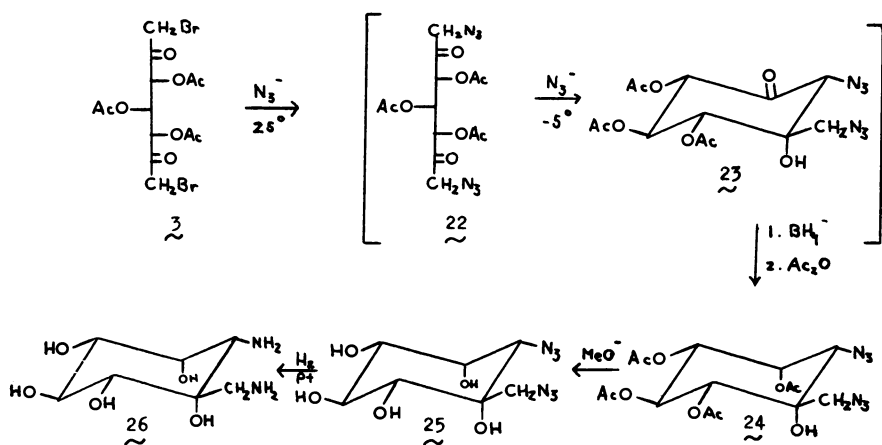


Figure 11.

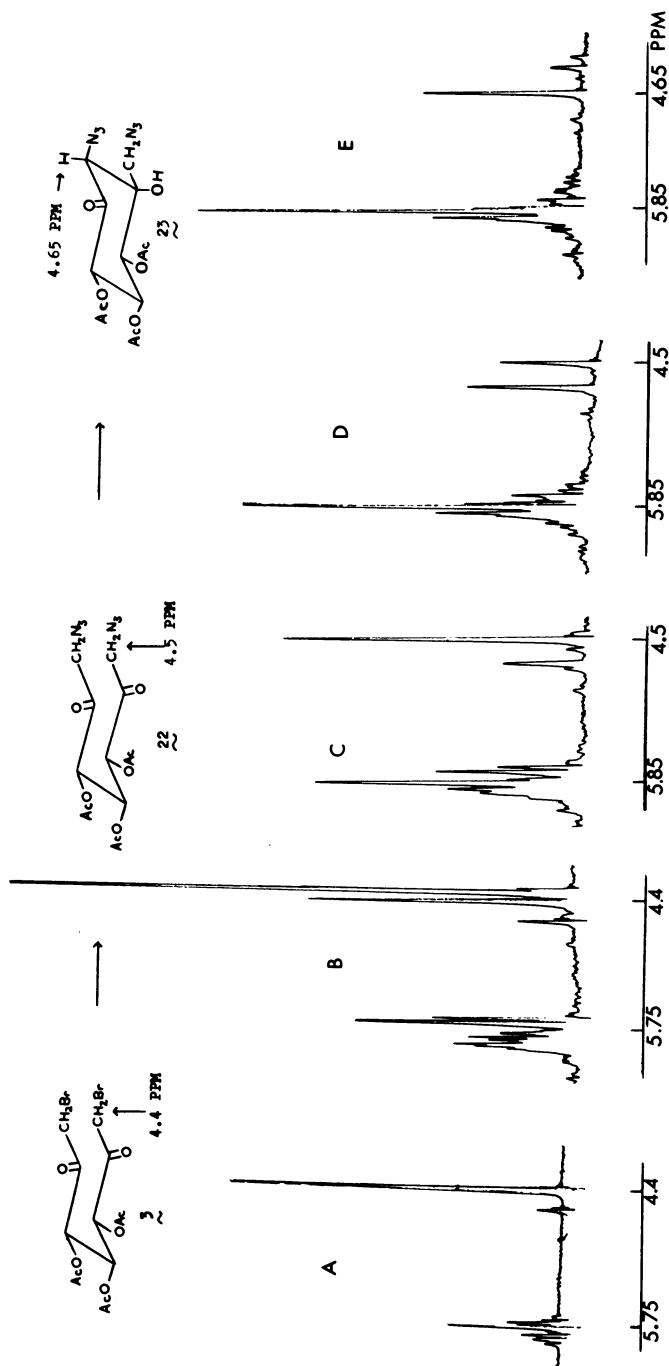


Figure 12. The 90 MHz $^1\text{H-NMR}$ spectra (nonacetyl region) from monitoring the reaction of **3** with sodium azide: Spectrum A—compound **3**; Spectra B and C—reaction mixture after approximately .5 hr and 1 hr; Spectra D and E—reaction mixture after approximately 2 hr and 3 hr

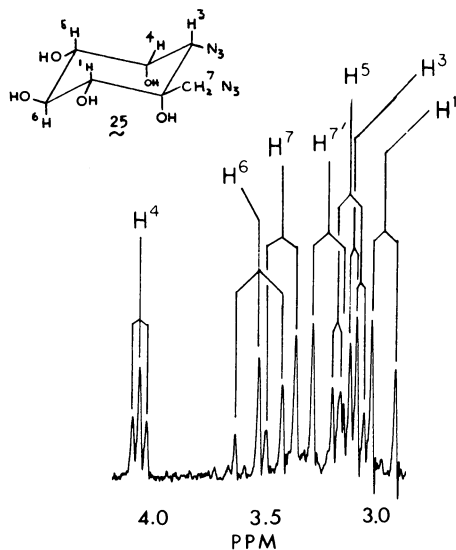


Figure 13. The 90 MHz ^1H -NMR spectrum of $\underline{25}$ in D_2O

Table I. Branched-Chain Cyclitol Melting Points

<u>Cyclitol</u>	<u>Melting Point</u> $^{\circ}\text{C}$
$\underline{11}$ R=R'=Cl	222-223
$\underline{13}$ R=R'=Br	231-232
$\underline{16}$ R=Cl, R'=N ₃	208-210
$\underline{19}$ R=Br, R'=N ₃	211-214
$\underline{25}$ R=R'=N ₃	160-161
$\underline{17a}$ R=Cl, R'=NH ₂ · Picric Acid	225-226
$\underline{21a}$ R=Br, R'=NH ₂ · Picric Acid	225-228
$\underline{20a}$ R=H, R'=NH ₂ · Picric Acid	204-206
$\underline{26a}$ R=R'=NH ₂ · Picric Acid	243-245

Literature Cited

1. Loewus, F. A., and Kelly, S., Biochem. Biophys. Res. Commun., 1962, 7, 204.
2. Chen, I. W., and Charalampous, F. C., J. Biol. Chem., 1964, 239, 1905.
3. Eisenberg, F. Jr., and Bolden, A. H., Biochem. Biophys. Res. Commun., 1965, 21, 100.
4. Barnett, J. E. G., Rasheed, A., and Corina, D. L., Biochem. J., 1973, 131, 21.
5. Sherman, W. R., Rasheed, A., Mauck, L. A., and Wiecko, J., J. Biol. Chem., 1977, 252, 5672.
6. Eisenberg, F., Jr. in "Cyclitols and Phosphoinositides", Wells, W. W., and Eisenberg, F., Jr., Eds., Academic Press: New York, 1978, 269.
7. Rinehart, K. L., Jr., Malik, J. M., Nystrom, R. S., Stroshane, R. M., Truitt, S. T., Taniguchi, M., Rolls, J. P., Haak, W. J., and Ruff, B. A., J. Am. Chem. Soc., 1974, 96, 2263.
8. Rinehart, K. L., Jr., and Stroshane, R. M., J. Antibiot., 1976, 29, 319.
9. Sprinson, D. B., Advan. Carbohyd. Chem., 1960, 15, 235.
10. Weiss, U., Davis, B. D., and Mingioli, E. S., J. Am. Chem. Soc., 1953, 75, 5572.
11. Aldersberg, M., and Sprinson, D.B., Biochemistry, 1964, 3, 1855.
12. Kiely, D. E., and Fletcher, H. G., Jr., J. Org. Chem., 1968, 34, 1386.
13. Kiely, D. E., and Sherman, W. R., J. Am. Chem. Soc., 1975, 97, 6810.
14. Cantrell, C. E., Kiely, D. E., Abruscato, G. J., and Riordan, J. M., J. Org. Chem., 1977, 42, 3562.
15. Cantrell, C. E., Ph.D. dissertation, University of Alabama in Birmingham, 1975.
16. The experimental details for the work described in this report will appear elsewhere. Riordan, J. M., Kiely, D. E., DeLucas, L., Einspahr, H. M., and Bugg, C. E., submitted for publication in Carbohydr. Res.
17. Riordan, J. M., and Kiely, D. E., "Abstracts of Papers", 172nd National Meeting of the American Chemical Society, San Francisco, CA., Aug. 1976; CARB 11.
18. Iwasa, T., Yamamoto, H., and Shibata, M., J. Antibiot., 1970, 23, 595.
19. Iwasa, T., Kameda, Y., Asai M., Horii, S., and Mizuno, K., J. Antibiot., 1971, 24, 119.
20. Horii, S., Kameda, Y., and Kawahara, K., J. Antibiot., 1972, 25, 48.
21. Horii, S., Kameda, Y., Iwasa, T., and Yamamoto, H., U. S. Patent 4 011 356, 1977.

22. Kamiya, K., Wada, Y., Horii, S., and Nishikawa, M., J. Antibiot., 1971, 24, 317.
23. Suami, T., Ogawa, S., Nakamoto, K., and Kashara, I., Carbohydr. Res., 1977, 58, 240.
24. Ogawa, S., Shibata, Y., Chida, N., and Suami, T., "Abstracts of Papers", American Chemical Society/Chemical Society of Japan Chemical Congress, Honolulu, HA., April 1979; CARB 89.
25. Suami, T., Ogawa, S., and Uchida, M., Bull. Chem. Soc. Jpn., 1970, 43, 3577.

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The Stereospecific Synthesis of Spectinomycin

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Spectinomycin (1) is an aminocyclitol antibiotic marketed by The Upjohn Company as the dihydrochloride salt under the name of Trobicin[®]. It is a broad spectrum antibiotic of moderate potency which has become especially important for the treatment of penicillin-resistant strains of gonorrhoeae. Spectinomycin does not have the oto- and nephrotoxicity which is usually associated with the 2-deoxystreptamine containing aminocyclitols. The structure of spectinomycin was determined at Upjohn by Wiley, Hoeksema, and Argoudelis (1, 2). This structure (1), shown on Figure 1, is unique among the aminocyclitols in that it contains a glycosylated actinamine ring which is cyclized to form a third ring by hemiketal formation. Spectinomycin (1) has nine asymmetric centers. It also has a carbonyl group at C-3' and two masked carbonyl groups at C-1' and C-2'. This electrophilic portion of the molecule is sensitive to mild base which causes benzylic acid type rearrangement to give actinospectinoic acid (2).

The aminoglycoside numbering system will be used in the interest of consistency when intermediates are discussed.

As shown on the Figure 2, stepwise reduction of spectinomycin using H₂/Pt (1) gives dihydrospectinomycins (4), compounds of diminished biological activity (3). Further reduction with NaBH₄ gives tetrahydrospectinomycins (5) which are inactive (3). Hydrolysis of spectinomycin (1) with mineral acid gives actinamine (3) (1) which has been synthesized by Suami, *et al.* (4) from myoinositol (6). In 1977 Suami reported (5) a synthesis of a tetrahydrospectinomycin (5). However, the conversion of tetrahydrospectinomycin (5) to spectinomycin (1) has not been reported. In their work on spectinomycin modification, Rosenbrook and coworkers have demonstrated (6) the oxidation of N-blocked dihydrospectinomycin analogs to N-blocked spectinomycin analogs. However the DMSO oxidation is not selective so that isolated yields are reported to be 14-18%. Synthesis of spectinomycin (1)

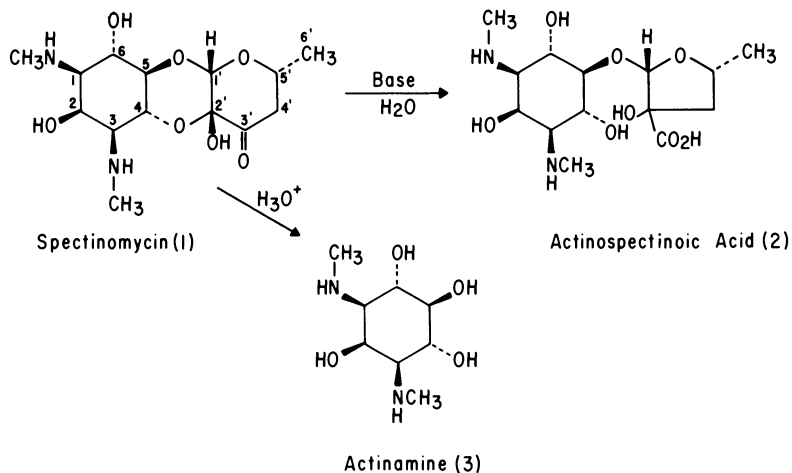


Figure 1. Structure and chemistry (1); absolute configuration (x-ray) (2)

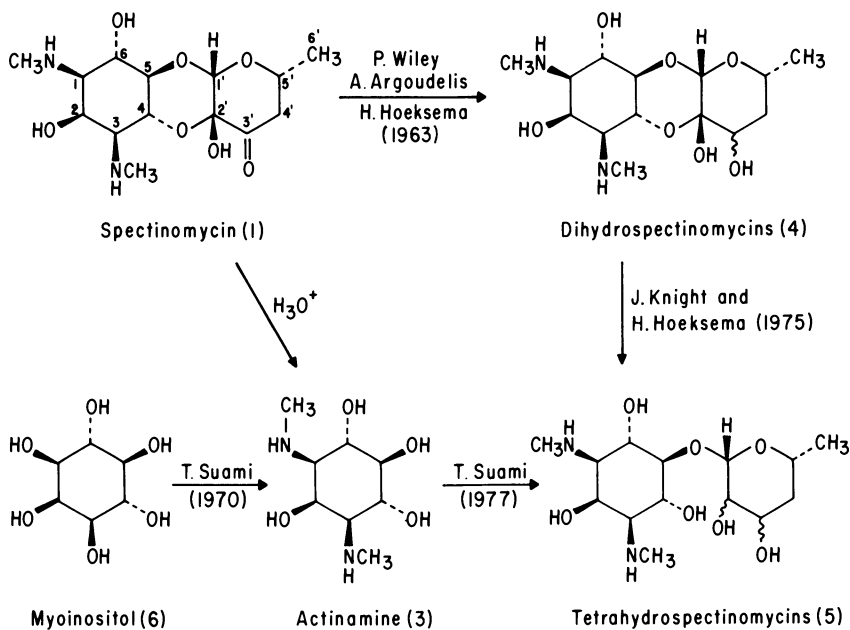


Figure 2.

from tetrahydro compounds (5) is not attractive, either, since it would require selective oxidation of the C-2' and C-3' hydroxyl groups or complex protecting group manipulation. Starting from fully functionalized sugars such a route would require first removal of hydroxyls at C-6' and C-4' and this, taken together with C-2' and C-3' oxidation, constitutes a highly involved adjustment in a single sugar ring.

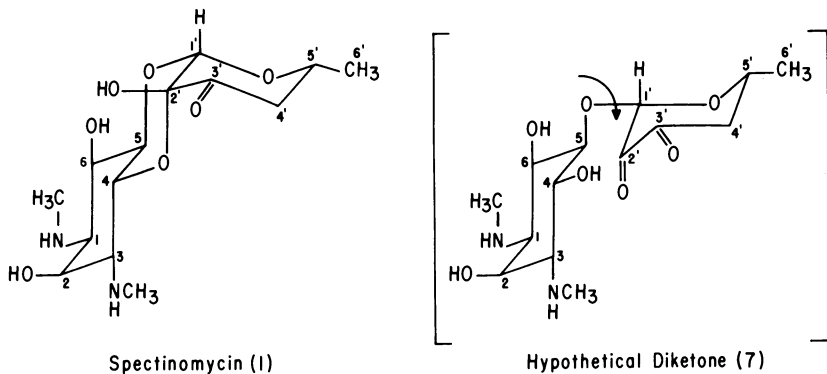
Synthetic Goals

For these reasons, one can see that a simple method for generation of carbonyl groups at C-2' and C-3' is an interesting and formidable synthetic challenge. This is listed on Figure 3 along with four other synthetic goals. Selective glycosylation at the C-5 hydroxyl is essential as is control of anomeric stereochemistry. In all planning, the base sensitivity of spectinomycin must be respected. For this reason, generation of the C-3' carbonyl toward the end of a synthesis is desirable. A fifth goal is to generate natural hemiketal folding of the hypothetical diketone (7) as shown on Figure 3. This is a very interesting problem from the theoretical point of view. I will discuss it in some detail.

The hypothetical diketone (7) would have free rotation around the glycoside bond and *a priori* might cyclize to a hemiketal involving either C-4 or C-6 hydroxyl giving either *cis* or *trans* fusion. The occurrence of one natural structure, to the apparent exclusion of three others, may be rationalized by evaluation of anomeric effects at the C-1' and C-2' centers as well as the preference for chair rings and an equatorial C-5' methyl group. These four effects are summarized on Figure 4.

Here we see the four possible hemiketals generated from the hypothetical diketone (7) by involving either the R or S hydroxyl group. Consider possible destabilization forces (a) through (d). In (a) the C-2' center is considered and destabilization by the anomeric effect is evident when the C-2' hydroxyl is forced into an equatorial position rather than the preferred axial orientation. This is the usual anomeric effect. In (b) the anomeric effect at the C-1' center is considered, and either one or two oxygen lone pairs may be eclipsed. No hemiketals are possible having no eclipsing of lone pairs. In (c) the axial or equatorial disposition of the C-5' methyl group is considered, and in (d) the necessity of boat rings is considered as destabilizing the system. From this discussion one can say that, while these influences (a) through (d) are probably not equivalent, they show why the natural configuration is most stable; furthermore the influence of epimerization at C-5' can be estimated as being great enough to destabilize natural hemiketal folding toward other modes of cyclization. This perturbation has been tested experimentally and is part of Dr. Thomas' manuscript.

Certain naturally derived protected dihydrospectinomycin diastereomers which have been trapped as unnaturally folded



SYNTHETIC GOALS

- I Generation of carbonyl groups at C-2' and C-3'.
- II Selective glycosidation at the C-5 hydroxyl.
- III Selective generation of natural anomeric stereochemistry.
- IV Sensitivity of the C-3' carbonyl must be respected.
- V Hemiketal "folding" must be natural.

Figure 3.

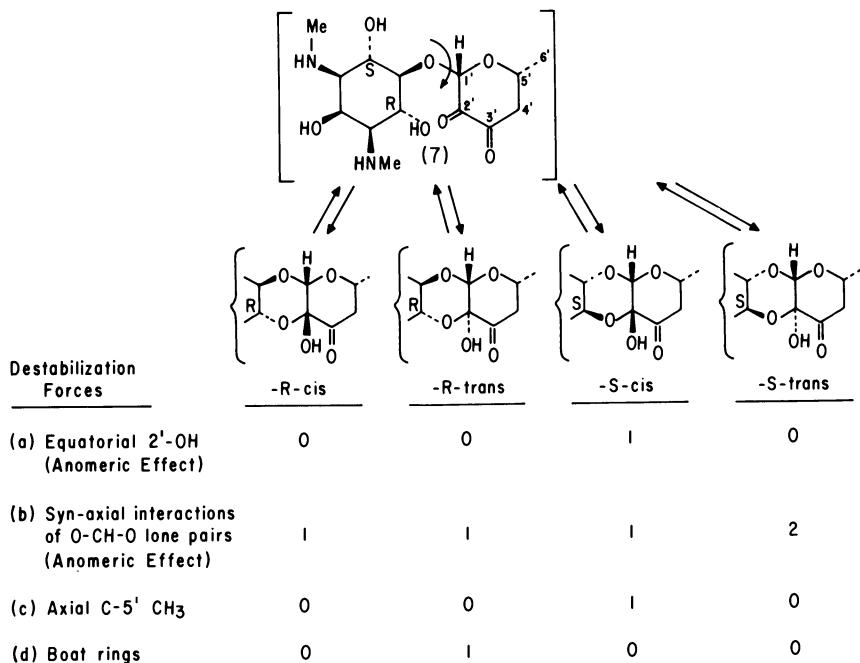


Figure 4.

acetonides have been found to reclose to the natural skeleton upon acetonide removal (7). However, natural folding is not intrinsic to the spectinomycin skeleton as evidenced by structure (12) (*vide infra*) and by the subject of Dr. Thomas's manuscript. Full consideration of anomeric effects and conformational effects for each case is essential to predict the mode of folding. Natural hemiketal folding is the last of five major goals of spectinomycin synthesis and anticipation of a favorable outcome allows the use of actinamine (3) as an achiral, stereochemically rich building block.

Synthesis

As shown on Figure 5, L-glucal triacetate (8) is used as our starting material; it can be made from L-glucose without purification of intermediates by the method of Roth and Pigman (8). Addition of NOCl gives 90% yield of the known (9), crystalline nitrosodimer (9). This nitrosodimer (9) is allowed to react with N,N'-dicarbobenzyloxyactinamine (10) in DMF at room temperature. Several 1:1 adducts are formed which are separated by chromatography. The major product, the α -glycoside (11), is formed in 48% yield. The preference for α -glycosylation is anticipated in the use of this method which was established by Lemieux (10). In this reaction natural chirality has been established at C-1'. Among the minor fractions, a few percent of the β -glycoside is usually formed in the reaction and removed in the chromatography. Also, up to 10% of glycosylation at C-4 and C-6 hydroxyls occurs. These compounds are reactive with periodate as expected. Finally, it should be noted that the oxime at C-2' is a carbonyl equivalent.

Figure 6 shows that deoxygenation occurs to give one product, a hemiketal (12) which is isolated in 87% yield after chromatography. NMR data show that the mode of hemiketal folding, which will be destroyed in the next step, is unnatural. Consideration of anomeric effects and conformational analysis suggests the structure (12) shown.

The next step, effected by reaction with anhydrous KHC_3 , accomplishes (a) removal of unwanted functionality at C-4' and C-6', (b) removal of unnatural stereochemistry at C-5', (c) generation of the sensitive carbonyl at C-3', and (d) introduction of natural folding of the hemiketal. Reaction conditions are chosen to avoid hydrolysis of the acetate at C-2' since the free hemiketal is rapidly converted to the related α -hydroxy- γ -pyrone (14) shown at the bottom of Figure 6. Evaluation of the crude reaction mixture by TLC and CMR shows that only one enone-acetate is formed. The enoneacetate (13) is crystallized directly from a chloroform solution. A second crop of product, giving a total of 55% yield, is obtained after chromatography of the mother liquor on silica gel.

A likely mechanism for the elimination reaction, which guided this synthesis, is shown on Figure 7. Base encourages hemiketal

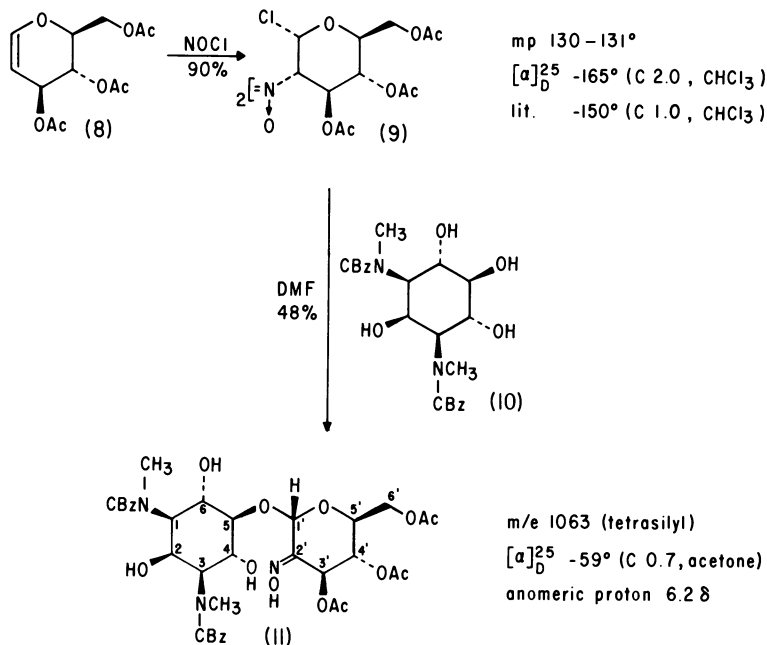


Figure 5.

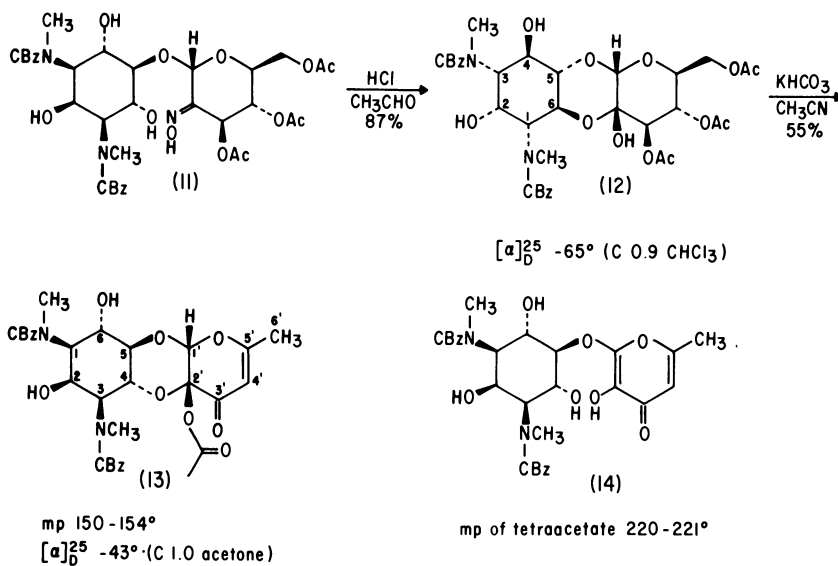


Figure 6.

opening to give a 2-ketosugar (15) which suffers two consecutive eliminations of acetate giving an α -acetoxydienone (17). This intermediate which contains only one asymmetric center, at the anomeric position, closes to a hemiketal (18). Migration of the acetyl group then generates the enoneacetate (13) with natural hemiketal folding as determined by nmr. Eight asymmetric centers are now in place, with one remaining to be established at C-5'.

As mentioned above the enoneacetate (13) is sensitive to hydrolytic conditions because of pyrone (14) formation. However, as shown on Figure 8, we have found that careful hydrolysis using K_2HPO_4 in methanol at room temperature for 1-2 hours gives the enone (19) having a free hemiketal. Even under these conditions the pyrone (14) is formed so that generally the reaction is not run to completion. In this way 55% of the enone (19) and 28% of recovered starting material (13) are obtained after column chromatography. One can see that the free hemiketal (19) and the pyrone (14) are related by hemiketal opening and subsequent enolization.

Figure 9 shows that the last step of the synthesis is hydrogenation of the olefin from the convex side of the molecule and concomitant hydrogenolysis of the carbobenzyloxy groups. At the conclusion of the reaction GC/MS shows the major peak corresponding to the natural antibiotic; no peaks correspond to possible stereoisomers. The product (1) is isolated in 40% yield by crystallization as the dihydrochloride salt. It has identical physical and biological properties as the natural antibiotic.

Figure 10 summarizes the synthesis of spectinomycin (1) from the known starting materials. I would like to point out that separate hydrolysis of the enoneacetate (13) is not necessary. Thus, direct hydrogenation of the enoneacetate (13) gives spectinomycin (1) in 35% yield since the acetylated hemiketal is reactive enough to be hydrolyzed by the isopropyl alcohol.

Summary

Although the antibiotic is highly functionalized, this approach requires minimum protection-deprotection chemistry since the scheme does not require subjecting the pseudodisaccharide to oxidants which would attack a hydroxyl group. Only four chemical steps from known starting material are required. This is possible because in all except the second step more than one change toward the goal is occurring; all of the changes are stereospecific. Extremely mild reagents are used, all at room temperature. Finally the scheme has flexibility to modify either half of the molecule by choice of different starting materials or by doing chemistry on some of the intermediates described.

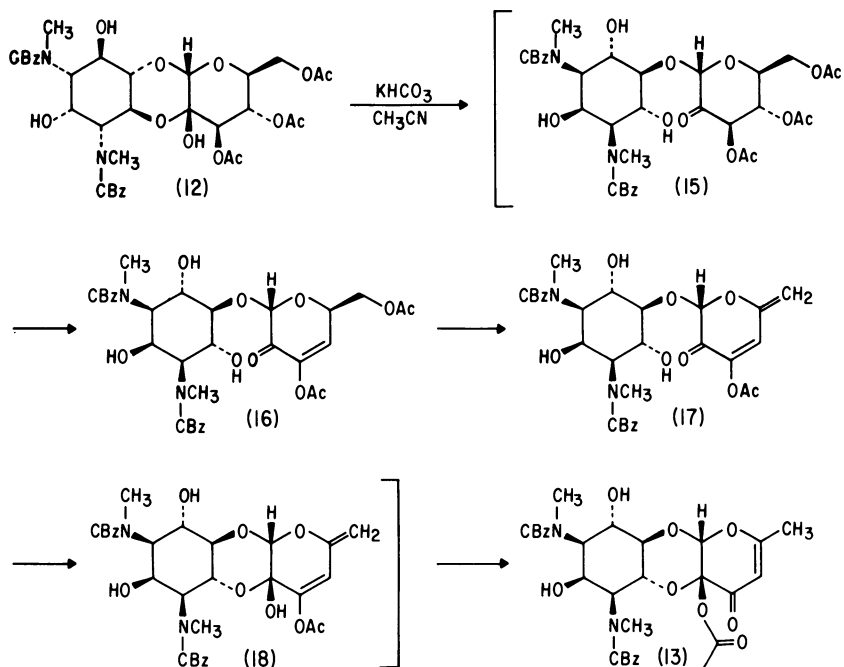
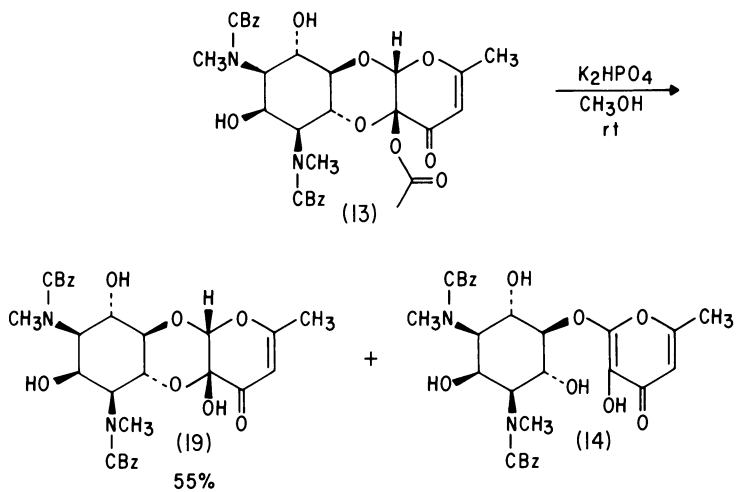


Figure 7.



(76% based on recovered starting material)

Figure 8.

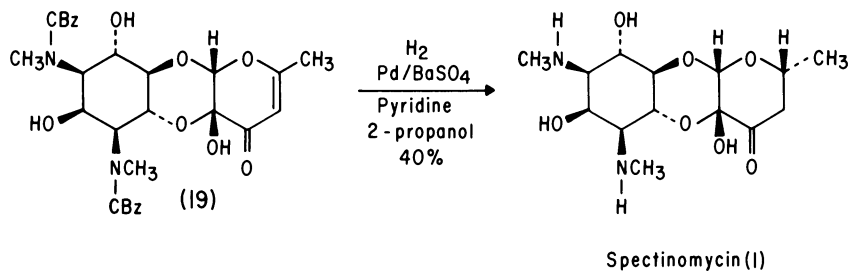


Figure 9.

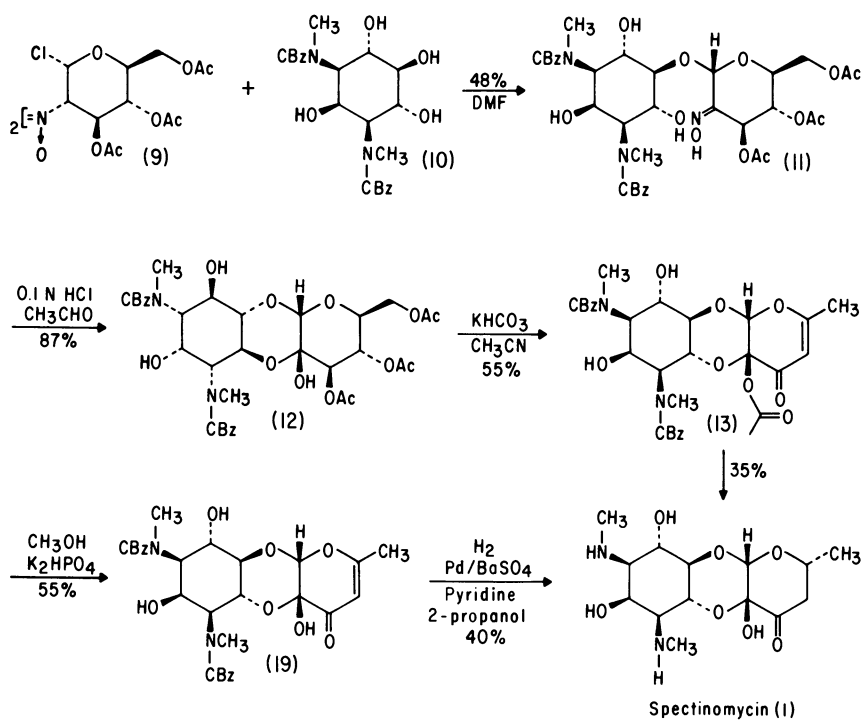


Figure 10.

Literature Cited

1. Wiley, P. F., Argoudelis, A. D., and Hoeksema, H., J. Amer. Chem. Soc. (1963) 85, 2652.
2. Cochran, T. G., Abraham, D. J., and Marton, L. L., J. Amer. Soc., Chem. Comm. (1972) 494.
3. Knight, J. C. and Hoeksema, H., J. Antibiot. (1975) 136.
4. Suami, T., Ogawa, S., and Sano, H., Bull. Chem. Soc. Japan (1970) 43, 1843.
5. Suami, T., Nishiyama, S., Ishikawa, H., Okada, H., and Kinoshita, T., Bull. Chem. Soc. Japan (1977) 50, 2754.
6. Rosenbrook, W., Carney, R. E., Egan, R. S. Stanaszek, R. S., Cirovic, M., Nishinaga, T., Mochida, K., and Mori, Y., J. Antibiot. (1978) 451.
7. Foley, L. and Weigele, M., J. Org. Chem. (1978) 43, 4355.
8. Roth, W., Pigman, W., "Methods in Carbohydrate Chemistry," Vol. II, p.405, Academic Press, New York, 1963.
9. Paulsen, H., Stadler, P., and Tödter, F., Chem. Ber. (1977) 110, 1925.
10. Lemieux, R. U., Nagabhushan, T. L., and Gunner, S. W., Can. J. Chem. (1968) 43, 405.

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Synthesis of Spectinomycin Analogs

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Spectinomycin (1) (Figure 1) is an aminocyclitol antibiotic of moderate potency which is useful in the treatment of gonorrhoeae, especially for penicillin resistant strains. It has antibacterial activity against gram-negative and gram-positive bacteria, and lacks the oto- and nephrotoxicity problems usually associated with the aminoglycoside family of antibiotics. This broad spectrum activity and lack of toxicity make spectinomycin an attractive candidate for structural modifications aimed at enhancing its potency. The sensitivity of spectinomycin to both acids and bases has severely hampered past synthetic efforts in this area.

Recently, a number of reports have appeared in the literature describing chemical modifications of the aminocyclitol portion of the antibiotic (2-7). These structural modifications have been inspired by aminoglycoside structure-activity relationships and by a knowledge of spectinomycin inactivation by R-factor mediated adenylation of the C-6 hydroxyl group. All changes in the actinamine portion of the molecule have resulted in the loss of antibiotic activity.

The only modification of the sugar portion of spectinomycin, to be reported to date, is the sequential reduction of the α -diketone system (Figure 2). The dihydrospectinomycins (1), prepared during the course of the structure determination studies, have diminished biological activity, but remain, after 16 years, the only active analogs of spectinomycin appearing in the literature. Further reduction affords the tetrahydrospectinomycins (8), which are devoid of antibiotic activity.

In the accompanying manuscript of Dr. David R. White, the features of the synthetic approach to spectinomycin analogs which has been developed at The Upjohn Company were outlined. The first synthesis of spectinomycin was reported. In this companion paper the flexibility of this synthetic scheme will be shown by detailing the synthesis of some analogs and the interesting structural

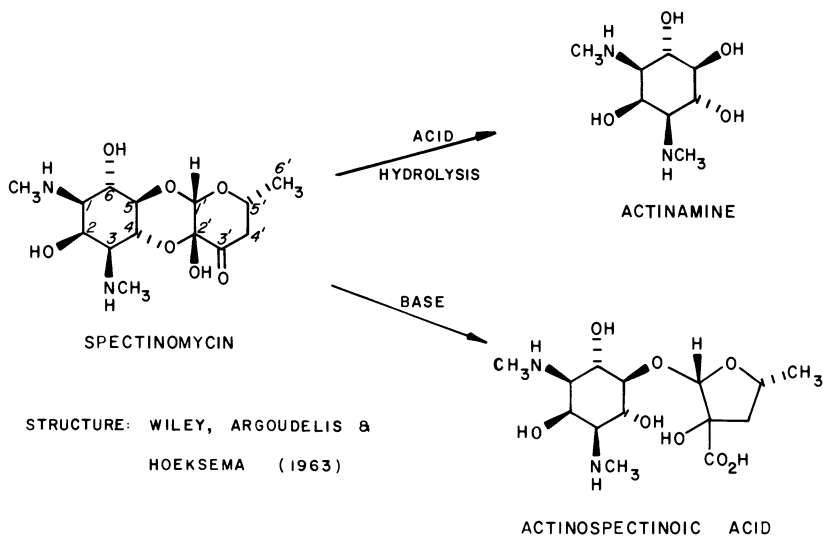


Figure 1.

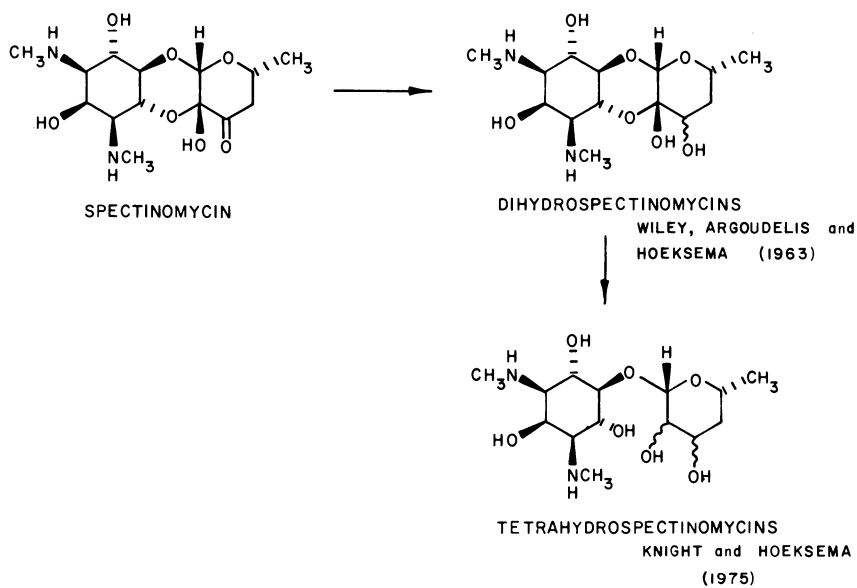


Figure 2.

consequences arising from modifications in the sugar fragment will be described.

Discussion

The first key steps of the synthetic sequence are shown in schematic form in Figure 3. The flexible nature of this approach is immediately apparent in that the aminocyclitol and sugar components may be varied at will and structural modifications in either can be made before or after the coupling of these pieces. N,N'-dicarbobenzyloxyactinamine is shown as a representative protected aminocyclitol in this instance. This paper will be limited to this example, while focusing on modification of the sugar component. A central feature of this scheme is the use of the Lemieux coupling process (9) for formation of the glycoside linkage. This process provides adequate selectivity for the actinamine C-5 hydroxyl group and introduces the requisite C-2' carbonyl group as its oxime. Subsequent deoxygenation affords the desired tricyclic hemiketal system which can be subjected to further modifications and deprotection.

In the previously described spectinomycin synthesis, L-glucose was employed as the sugar component due to the preference for α -glycosylation in the Lemieux coupling reaction. The α -L anomer corresponds to natural spectinomycin anomeric stereochemistry. Subsequent transformations inverted the stereochemistry at carbon -5' to that of the natural product. The consequences of employing the antipode D-glucose in this synthetic scheme are shown in Figure 4.

Reaction of the D-glucose derived glycosylating reagent with the actinamine derivative provides a mixture of symmetrical adducts, with the α -anomer predominating as anticipated. The ratio of α to β anomers being approximately 20 to 1 in this instance. Notice that the β -anomer has natural spectinomycin stereochemistry at both the anomeric carbon C-1' and at C-5'. The α -anomer, which is the mirror image of the intermediate employed in the spectinomycin synthesis, has unnatural anomeric stereochemistry. In the case of the α -anomer, subsequent deoxygenation, introduction of the C-3' carbonyl by β -elimination, and enone reduction with concomitant deprotection of the nitrogen atoms provides the enantiomer of spectinomycin (Figure 5).

This compound has physical characteristics identical to those of the natural isomer except for the optical rotation. It was found to be devoid of antibacterial activity even when tested at unusually high concentrations, thus establishing two very important points. First, the biological activity resides only in the natural antipode in this series of compounds and second, that the synthesis is in fact stereospecific. If any racemization of the anomeric center had occurred at any point in the synthesis, the resulting natural anomer would have yielded bioactive material. It should be recalled that all of the

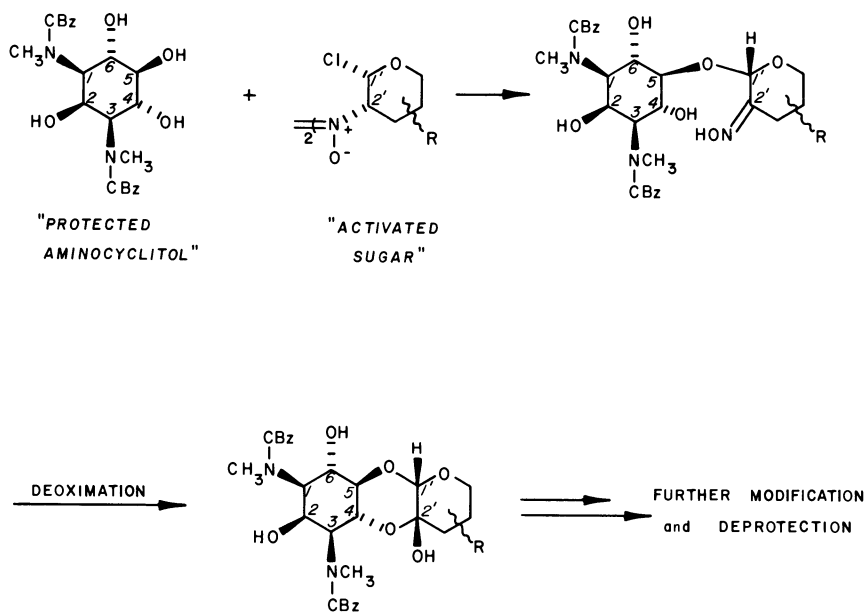


Figure 3.

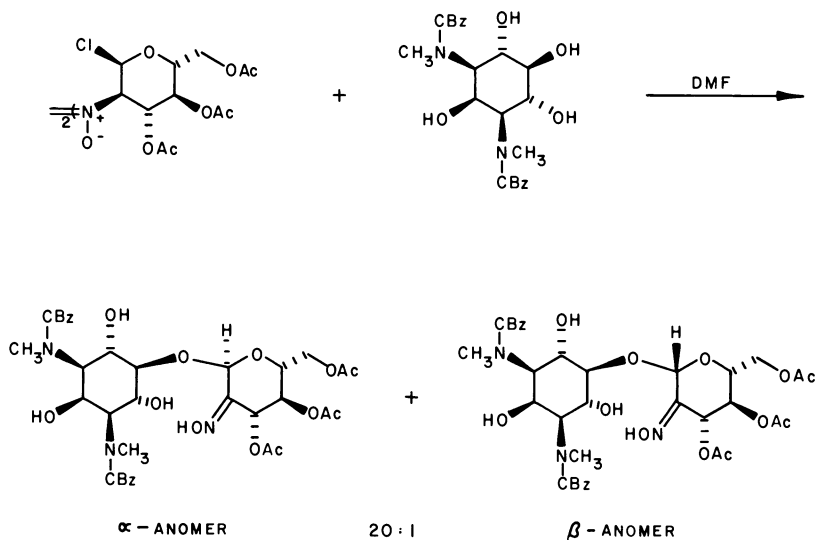


Figure 4.

asymmetric centers of the sugar except at the anomeric position are removed during the course of the synthesis and that the anomeric stereochemistry determines the configuration of all nine asymmetric centers in the final product.

As is shown in Figure 6, the minor oxime, the β -anomer, was sequentially deoximated, the acetates were methanolized and the protecting groups removed by catalytic hydrogenation to afford 4',6'-dihydroxydihydrospectinomycin. This totally synthetic spectinomycin analog was active against a variety of test organisms, with potency somewhat less than the corresponding nonhydroxylated dihydrospectinomycin which was derived from reduction of spectinomycin itself. Thus for the first time, a biologically active spectinomycin analog was synthesized, by fusing a sugar component onto an aminocyclitol unit.

The success achieved with both antipodes of glucose prompted the examination of the utility of other sugars in this synthetic scheme. The remaining two sugars to be discussed were examined as probes to answer chemical questions relating to the problem of hemiketal folding that was discussed earlier in Dr. White's manuscript. These sugars have provided interesting biological information as well.

The results obtained with D-arabinose are shown in Figure 7. One of the more attractive features of this pentose is the high proportion of β -D-anomer produced in the coupling process. Thus reaction of the nitrosyl chloride adduct of di-O-acetyl-D-arabinal with N,N'-dicarbobenzyloxyactinamine gives the desired β -anomer in 35% yield, along with lesser amounts of the α -anomer and products of reaction at the unsymmetrical hydroxyl groups. Deoximation gives one hemiketal structure established by spectral means as having natural spectinomycin folding. That is, the C-4 hydroxyl group forms the *cis*-hemiketal linkage as opposed to the C-6 hydroxyl. Application of either the β -elimination method of C-3' ketone introduction or acetate hydrolysis with subsequent N-deprotection in both cases affords the 5'-demethyl analogs shown. Both of these compounds possess moderate bioactivity with the keto compound being more active as expected.

Analysis of spectra establishes only the natural spectinomycin mode of hemiketal folding for 5'-demethylspectinomycin (Figure 8). This is an interesting result in light of the factors affecting the mode of hemiketal folding described in Dr. White's manuscript. In spectinomycin, the equatorial methyl group at C-5' is one of the factors that helps stabilize the natural folding involving the C-4 hydroxyl group, relative to other modes of folding which would require the methyl group to adopt an axial orientation. Even in the 5'-demethyl analog, which lacks this stereochemical bias, anomeric effects and other forces are effective in maintaining natural folding.

The effects of altered stereochemistry in the sugar ring have been further studied by exploring the application of L-rhamnose in this scheme. Figure 9 depicts the question at hand. That is,

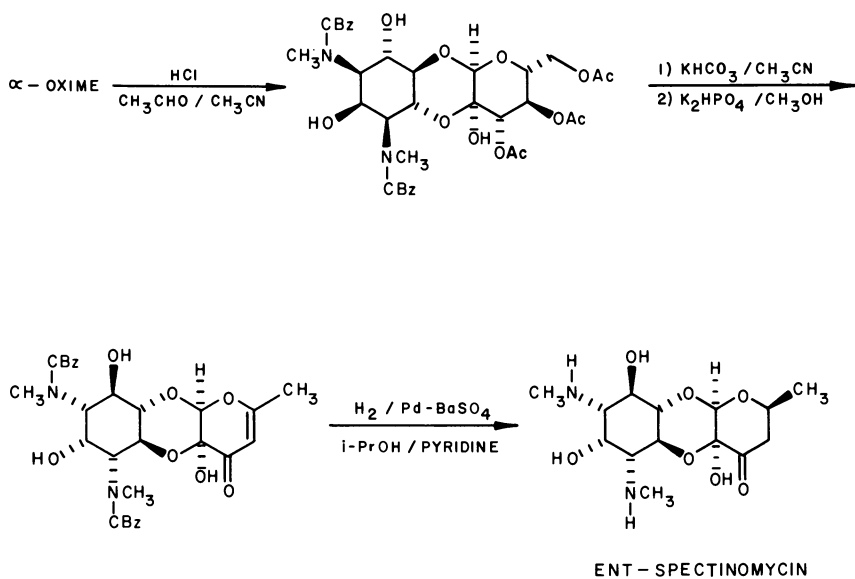


Figure 5.

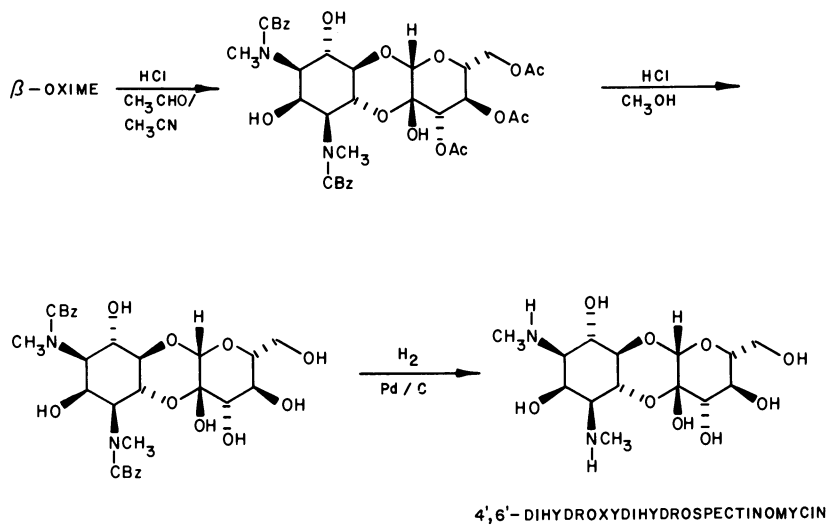


Figure 6.

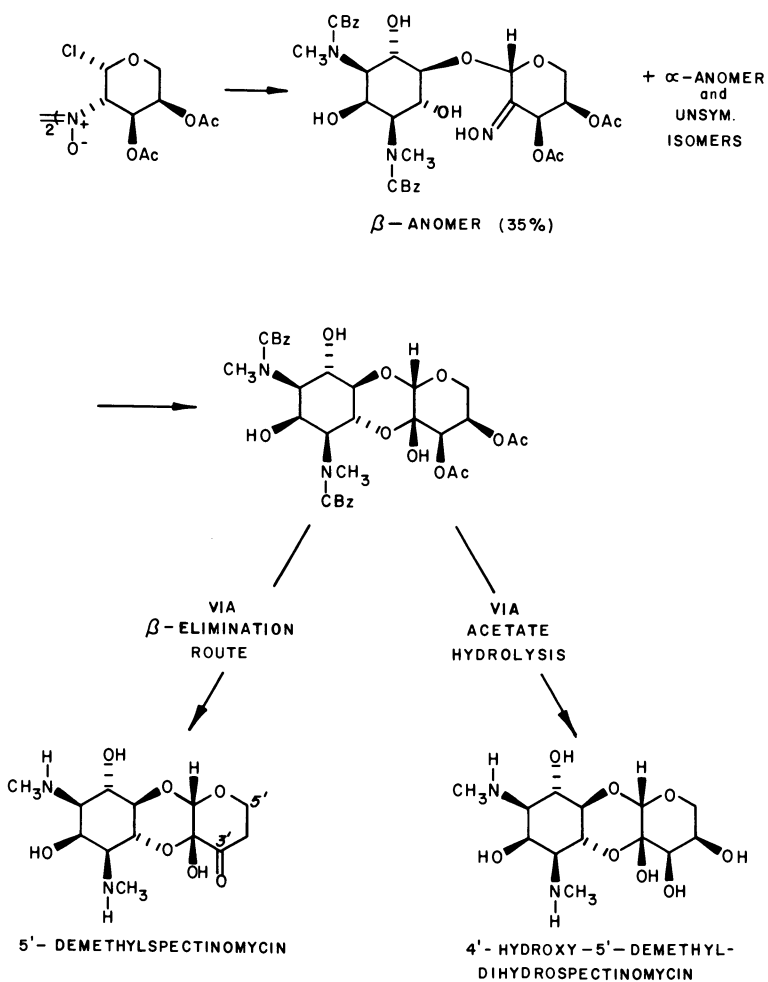


Figure 7.

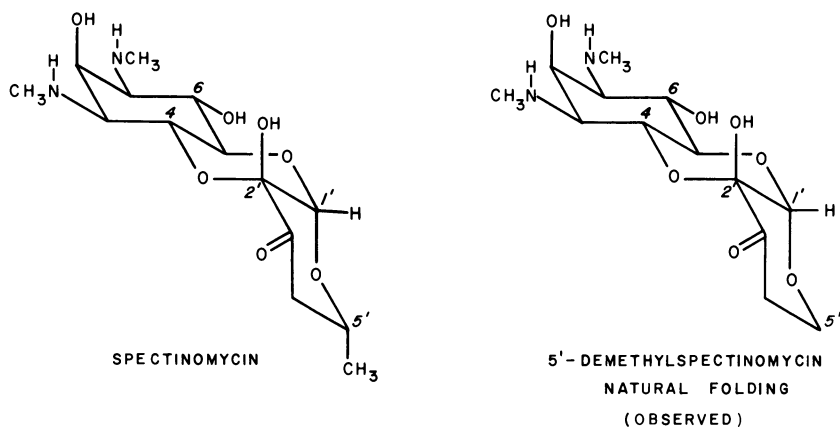


Figure 8.

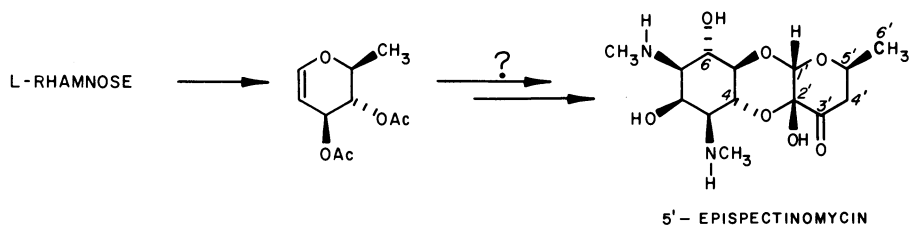
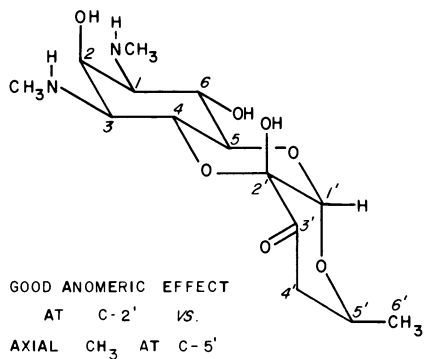
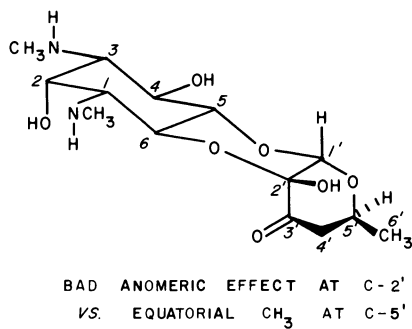
NATURAL SPECTINOMYCIN FOLDINGABNORMAL FOLDING

Figure 9.

what is the effect of an unnatural configuration at carbon 5? We see that natural folding in the 5'-epimeric series would require an axial orientation of the methyl group, a destabilizing factor. One of the alternative folding modes, which is shown in the Figure, would allow an equatorial C-5' methyl group, but the hydroxyl group at C-2' must now be in the less favored equatorial orientation. The anomeric effect at carbon 1' is equivalent in these two structures, and neither requires the presence of boat rings. The chemical steps used to evaluate this question are shown in Figure 10.

Treatment of commercially available 3,4-di-O-acetyl-L-rhamnal with nitrosyl chloride gives the usual *cis* adduct, isolated as the nitrosodimer. Reaction with N,N'-dicarbobenzyloxyactinamine affords the expected α -L-anomer in good yield. Deoxygenation affords a single hemiketal which does not have natural folding as established by NMR. Examination of spectral data and molecular models suggests the structure shown. Abnormal folding is expected at this stage because natural folding would require the substituents on carbons 3', 4', and 5' to all adopt axial orientations.

The stage is now set for the β -elimination process for generation of the C-3' ketone. In the present instance, the lack of an acetoxy group on C-6' precludes any second elimination and therefore precludes alteration of the unnatural C-5' stereochemistry. Spectral studies show that the resulting acetoxy-ketone and the derived hydroxy-ketone are both abnormally folded. Consideration of molecular models suggests the structures shown, which correspond to the mode of abnormal folding that was depicted in the previous figure. Removal of the protecting groups yields a product having only very weak antibiotic activity. Spectral analysis of this product shows mainly a single unnaturally folded compound, assigned the structure shown, along with minor impurities which could be alternative folded forms or acyclic compounds.

Taken together with the results obtained in the 5'-demethyl-spectinomycin case, the following trend emerges. The equatorial 5'-methyl group in spectinomycin, while certainly stabilizing the natural mode of hemiketal folding over other possibilities is not a requirement. The anomeric and other effects in the 5'-demethyl analog are sufficient to enforce natural folding. These effects, however, are not enough to overcome the bias of an axial substituent at carbon 5'. Thus, the 5'epi analog of spectinomycin adopts alternative hemiketal folding.

Summary

These results establish the generality of the total synthetic method for producing biologically active analogs of spectinomycin. It provides ready access to dihydro analogs as well as providing a versatile method for introducing the desirable C-3' carbonyl group without relying on selective protection or oxidation. It is a short efficient sequence, by which active analogs can be pre-

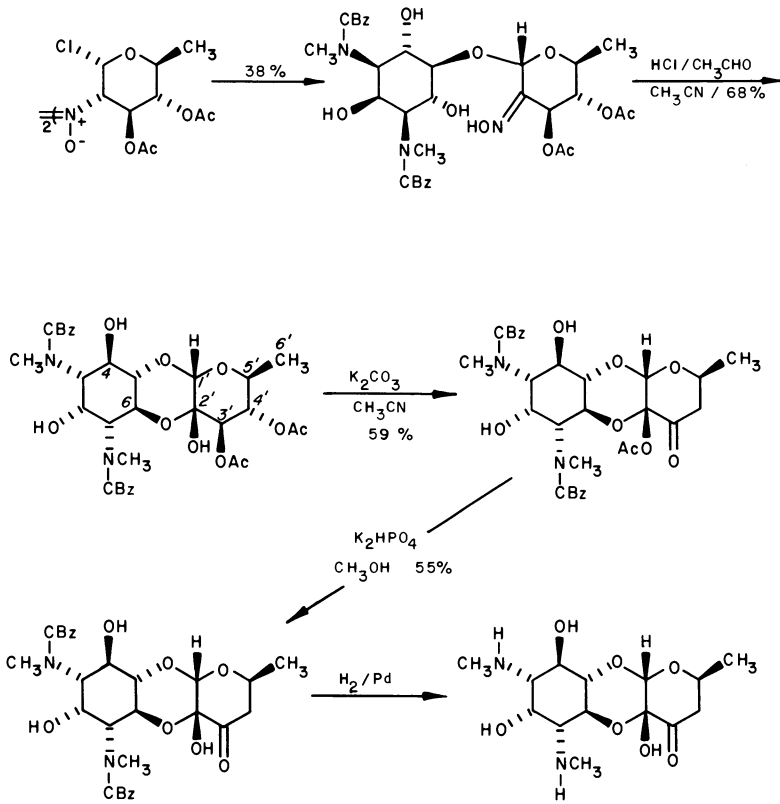


Figure 10.

pared from known starting materials in as few as four synthetic steps. Thus, it compares very favorably with approaches relying on protection and subsequent modification of intact spectinomycin precursors.

The synthesis of enantiomeric spectinomycin by this technique confirms the stereospecificity of the synthetic scheme and establishes the dependence of activity on natural absolute configuration. The results obtained in the D-arabinose and L-rhamnose derived analogs have shed some light on the factors affecting hemiketal folding and have shown the effects of abnormal folding on antibiotic activity.

Literature Cited

1. Wiley, P. F., Argoudelis, A. D., and Hoeksema, H., J. Amer. Chem. Soc., (1963), 85, 2652.
2. Carney, R. E. and Rosenbrook, W., J. Antibiotics, (1975), 28, 953.
3. Rosenbrook, W., Carney, R. E., Egan, R. S., Stanaszek, R. S., Cirovic, M., Nishinaga, T., Mochida, K., and Mori, Y., ibid., (1975), 28, 960.
4. Rosenbrook, W., Carney, R. E., Egan, R. S., Stanaszek, R. S., Cirovic, M., Nishinaga, T., Mochida, K., and Mori, Y., ibid., (1978), 31, 451.
5. Carney, R. E. and Rosenbrook W., ibid., (1977), 30, 960.
6. Foley, L., Lin, J. T. S., and Weigele, M., ibid., (1978), 31, 979.
7. Foley, L., Lin, J. T. S., and Weigele, M., ibid., (1978), 31, 985.
8. Knight, J. and Hoeksema, H., ibid., (1975), 28, 136.
9. Lemieux, R. U., Nagabhushan, T. L., and Gunner, S. W., Can. J. Chem., (1968), 46, 405.

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Spectinomycin Modification

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Spectinomycin is an aminocyclitol antibiotic unique both in structure (1,2) and in biological activity. The antibiotic (Figure 1) is a pseudodisaccharide in which a non-aminosugar moiety, actinospectose, is fused to an aminocyclitol, actinamine or N,N'-dimethyl-2-epi-streptamine by both a β -glycosidic bond and a hemiketal linkage. The bioactivity of spectinomycin resembles that of other aminocyclitol antibiotics only in that its antibacterial spectrum is broad and its mode of action is the inhibition of protein synthesis by an interaction with the 30s ribosomal subunit (3). Spectinomycin's action is generally bacteriostatic rather than bacteriocidal and, although its antibacterial spectrum is described as broad, in vitro potency is generally low. In vivo potency is, however, considerably better than one would predict on the basis of in vitro activity. Most importantly, spectinomycin is devoid of the ototoxic and nephrotoxic properties normally associated with the aminoglycosidic aminocyclitol class of antibiotics (4). In our hands, we have been unable to demonstrate ototoxicity in the rat at a dose of 820 mg/kg/day for 14 days or nephrotoxicity in the rat at a dose of 810 mg/kg/day for 14 days. This lack of toxicity makes spectinomycin an appealing substrate for chemical modification. Our major concern has been the enhancement of antibiotic potency and bacteriocidal action.

The rationale of our approach to the modification of spectinomycin is based on the observation that a 2-deoxystreptamine or streptamine moiety, while not sufficient, is generally necessary for antibiotic activity among the aminocyclitol antibiotics (Figure 2) (5). The stereochemistry at the 2-position of the aminocyclitol moiety is also important. The semisynthetic neomycin analogs, hybrimycins B₁ and B₂ of Rinehart and co-workers (6), in which 2-epi-streptamine has been incorporated, exhibit greatly reduced antibiotic activity. Spectinomycin has, therefore, been modified at the 7-position, which corresponds to the 2-position of streptamine, in an effort to enhance potency. In addition, the R-factor mediated inactivation of spectinomycin by

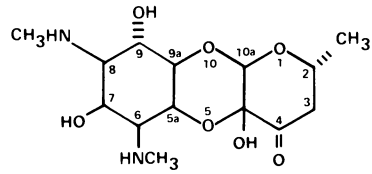


Figure 1. Spectinomycin

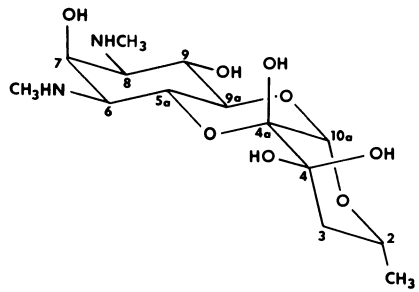


Figure 2.

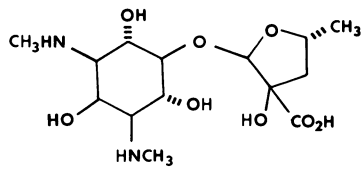


Figure 3. Actinospectinoic acid

adenylation of the 9-hydroxy group (7,8) prompted modifications at the 9-position.

The lability of the spectinomycin molecule severely limits direct chemical modification and a suitably blocked derivative was required to withstand the rigors of deoxygenation and epimerization. The most unstable feature of the molecule is the alpha keto hemi ketal functionality at 4a. In basic solution the incipient 1,2-diketone undergoes a benzilic acid rearrangement to the ring opened actinospectinoic acid (Figure 3), while in acid, both the hemi ketal and glycosidic bridges are cleaved to give the component actinospectose and actinamine. Although the C-4 carbonyl of spectinomycin normally exists as a gem diol, the usual ketone derivative can be formed and stabilize the molecule. Our attempts to regenerate the ketone function were, however, unsuccessful.

As reported by Wiley, et. al. (1,9), spectinomycin can be reduced to an epimeric mixture of 4-dihydrospectinomycins. In our hands, reduction of spectinomycin dihydrochloride with borohydride in methanol proceeded stereospecifically to the 4(S)- or equatorial epimer of dihydrospectinomycin (Figure 4). Hydrogenation, using a rhodium on carbon catalyst on the other hand, gave predominately (up to 95%) the 4(R)- or axial dihydrospectinomycin (10). The stereochemistry of the two epimers was established by analysis of their PMR spectra and a knowledge of spectinomycin stereochemistry as established by the x-ray study of Chochran and co-workers (2). 4(R)-Dihydrospectinomycin is a convenient starting material, considerably more stable than spectinomycin, and is amenable to protection of its alpha hydroxy hemi ketal functionality via the 4,4a acetonide. Also, the axial hydroxyl group of a suitably blocked derivative undergoes selective oxidation back to the 4-oxo or spectinomycin analog. The amino blocking group should be one which can be removed under conditions which maintain the integrity of the spectinomycin molecule. Reaction of an epimeric mixture of dihydrospectinomycins with 2,2-dimethoxy propane gives only the expected cis product, 4(R)-dihydrospectinomycin 4,4a-acetonide.

Both the 4(R)- and 4(S)-dihydrospectinomycins, although substantially less active than spectinomycin, exhibit both in vitro and in vivo antibiotic activity, with the R-epimer being somewhat more active than the S. While the dihydrospectinomycin antibacterial spectra are different than that of spectinomycin, organisms resistant to spectinomycin are also resistant to the dihydrospectinomycins. Extrapolation of structure-activity relationships from the dihydro-series to the 4-oxo or spectinomycin series is at best uncertain.

Epimerization at C-7 was achieved via reduction of a β -diketone (Figure 5). Oxidation of the N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide with dimethylsulfoxide-acetic anhydride for 16 hours at room temperature gave 7,9-dioxo-di-Z-acetonide. The crude diketone was immediately treated with sodium

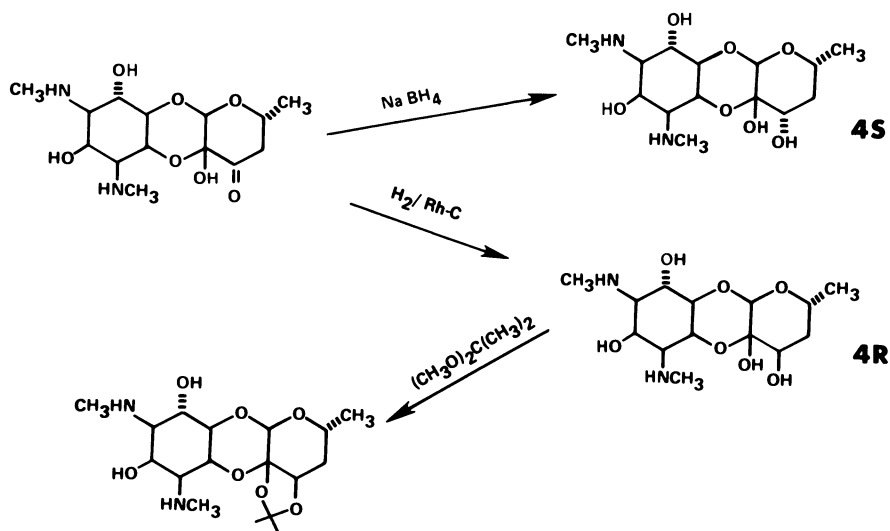


Figure 4.

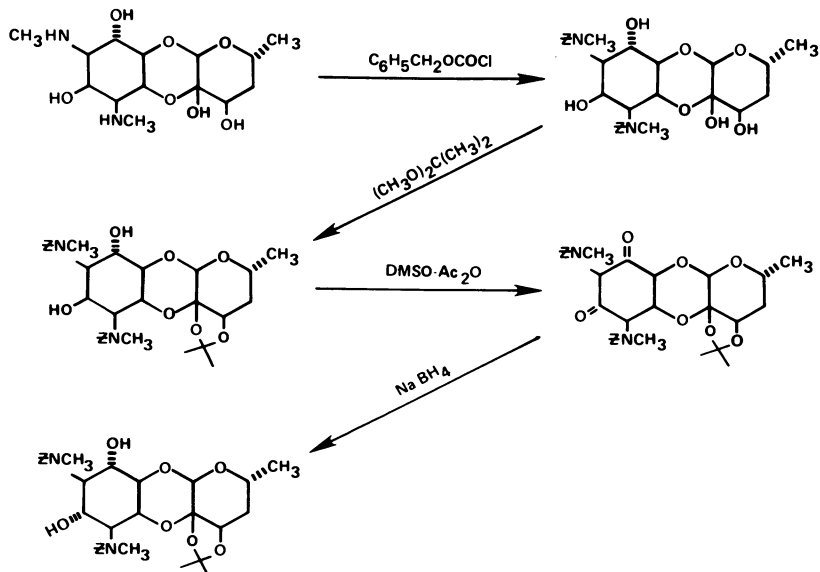


Figure 5.

borohydride to provide a stereospecific reduction at both sites to give the desired 7-epi-di-Z-4(R)-dihydro-spectinomycin-4,4a-acetonide (11).

Removal of the acetonide block followed by oxidation with a mixture of DMSO and acetic anhydride for a short period of time gave the 4-oxo-analog, 7-epi-di-Z-spectinomycin (Figure 6). Removal of the carbobenzoxy groups by catalytic hydrogenation to provide 7-epi-spectinomycin was achieved only after exhaustive purification. Catalyst poisons, apparently derived from the DMSO oxidation step, were finally separated by partition chromatography. Evidence for the C-7 stereochemistry was obtained from an analysis of the 270 MHz PMR spectrum of 7-epi-4(R)-dihydro-spectinomycin. Only the C-3 equatorial and C-4 protons exhibit small coupling constants, which requires that all the protons in the actinamine derived ring are now axial. Analysis of the PMR spectra of carbobenzoxy-blocked intermediates could not be achieved due to the presence of rotamers causing broad and ill-defined resonances. Extensive chemical shift overlap of ring protons precluded analysis of the spectrum of 7-epi-spectinomycin.

Di-Z-4(R)-Dihydro-spectinomycin-4,4a-acetonide was selectively oxidized in high yield by the Pfitzner-Moffatt technique (12) to give the 9-oxo-analog (Figure 7) (13). Complete characterization of this ketone was precluded by the ready elimination of water during silica gel column chromatography to give an α,β -unsaturated ketone. This intermediate was identified as the $\Delta^7,8$ -9-oxo-di-Z-4(R)-dihydro-spectinomycin-4,4a-acetonide. Treatment of this unsaturated ketone with sodium borohydride provided a stereospecific reduction with regeneration of the natural stereochemistry at both C-8 and C-9 to give 7-deoxy-di-Z-4(R)-dihydro-spectinomycin-4,4a-acetonide.

Removal of the carbobenzoxy groups (Figure 8) yielded 7-deoxy-4(R)-dihydro-spectinomycin-4,4a-acetonide, an intermediate whose structure and stereochemistry could be assigned from an analysis of its 270 MHz PMR spectrum.

The acetonide block was then removed from 7-deoxy-di-Z-4(R)-dihydro-spectinomycin-4,4a-acetonide to provide 7-deoxy-di-Z-4(R)-dihydro-spectinomycin, which was further deblocked to 7-deoxy-4(R)-dihydro-spectinomycin.

Selective oxidation of the C-4 axial hydroxyl group of 7-deoxy-di-Z-4(R)-dihydro-spectinomycin by DMSO-acetic anhydride again gave the desired 4-oxo-analog, 7-deoxy-N,N'-dicarbenzoxy-spectinomycin. All attempts to remove the carbobenzoxy groups by catalytic hydrogenation, however, failed to give the desired 7-deoxyspectinomycin.

N,N'-9-O-Triacetyl-4(R)-dihydro-spectinomycin-4,4a-acetonide was derived by selective acetylation of the 4(R)-dihydro-spectinomycin-4,4a-acetonide (Figure 9) (10). The amino groups in this compound are protected from oxidation and the 9-hydroxyl function is converted to a leaving group. Treatment of this triacetate

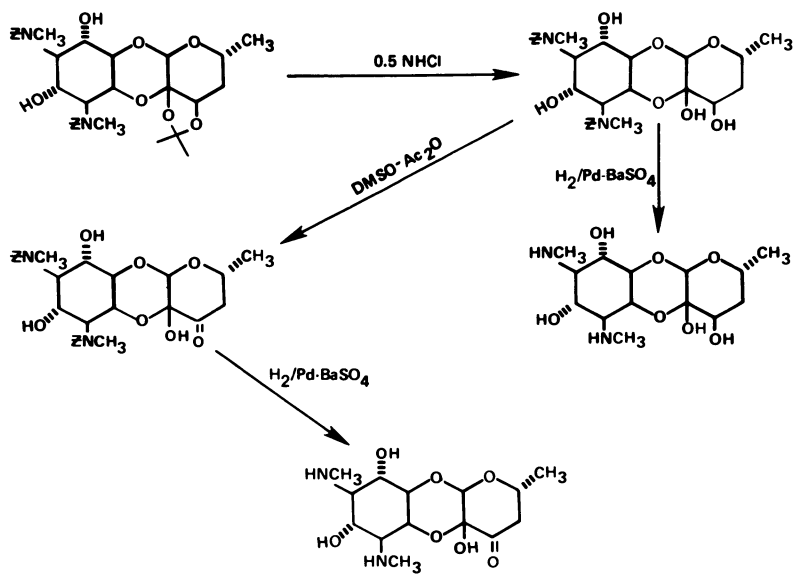


Figure 6.

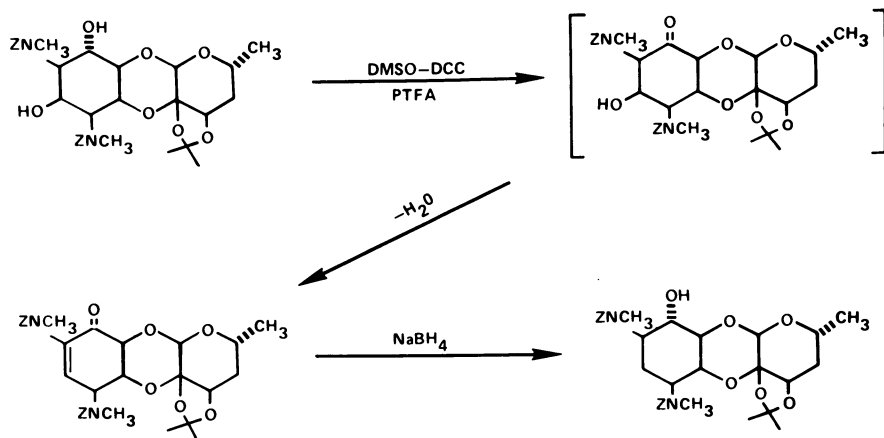


Figure 7.

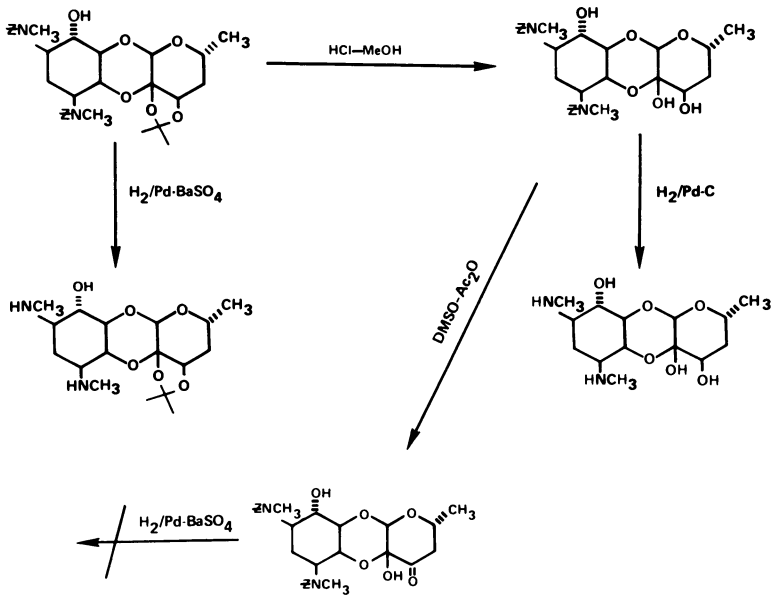


Figure 8.

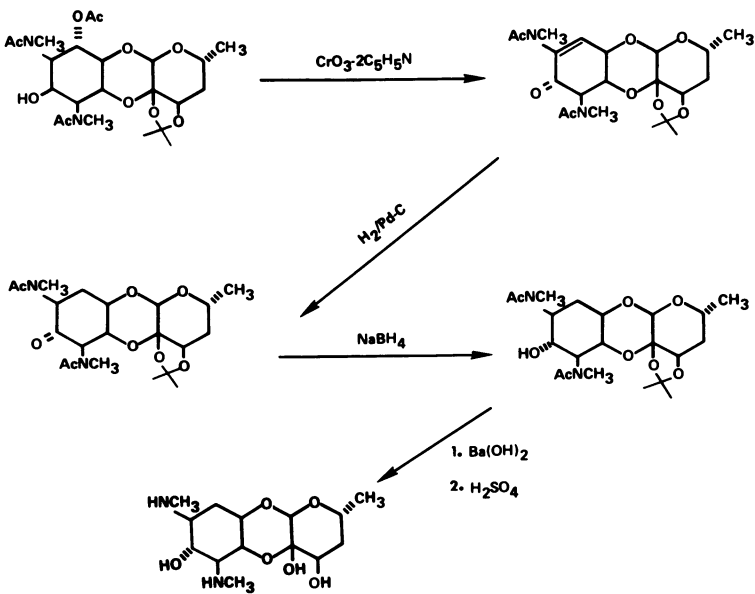


Figure 9.

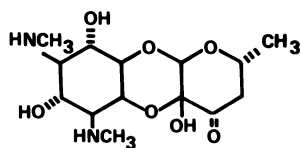
with a modified Collins reagent (14) gave another α,β -unsaturated ketone, which was identified as the $\Delta 8,9$ -7-oxo- N,N' -diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide on the basis of its PMR spectrum. Catalytic hydrogenation selectively reduced the $\Delta 8,9$ -double bond with concomitant regeneration of the natural stereochemistry at C-8 to give 7-oxo-9-deoxy- N,N' -diacetyl-4(R)-dihydro-spectinomycin-4,4a-acetonide. Sodium borohydride reduction again proceeded in a stereospecific manner to give only the unnatural epimer at C-7, 7-epi-9-deoxy- N,N' -diacetyl-4(R)-dihydro-spectinomycin-4,4a-acetonide. The acetyl blocks were removed with base and the acetonide block with dilute acid to provide 7-epi-9-deoxy-4(R)-dihydro-spectinomycin sulfate. The structure and stereochemistry were assigned from a complete analysis of the 270 MHz PMR spectrum in pyridine- d_5 solution at 110°.

All of the spectinomycin analogs described (Figure 10), 7-epi-spectinomycin and 7-epi-4(R)-dihydro-spectinomycin, 7-deoxy-4(R)-dihydro-spectinomycin and 7-epi-9-deoxy-4(R)-dihydro-spectinomycin, are devoid of antibiotic activity as measured by the agar dilution method on pH 8 nutrient agar at 500 μ g/ml versus a variety of organisms.

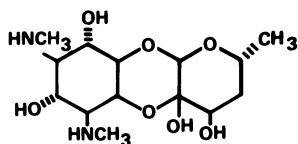
At this point, it is suffice to say that our modification rationale, derived from the various aminocyclitol antibiotics, is not applicable to spectinomycin. Both the stereochemistry and the presence of the C-7 hydroxyl group is important to antibiotic activity. The question of modification at C-9, however, was unanswered since the 7-epi-analogs proved to be inactive.

In an effort to deoxygenate the 9-position, the 9-*o*-toluene-sulfonate ester of N,N' -di-*Z*-4(R)-dihydro-spectinomycin-4,4a-acetonide (Figure 11) was prepared by standard procedures. Also prepared were the 9-mesylate and the 7,9-dimesylate as well as the corresponding triflates. We were unable to introduce a tosyl group into the 7-position. None of these sulfonate esters could be displaced or reductively cleaved by a variety of reagents. After removal of the carbobenzoxy groups, however, the 9-tosylate readily cyclised (15) to give the 8,9-epimino-4(R)-dihydro-spectinomycin-4,4a-acetonide. High pressure hydrogenation of this epimine, as well as its 6-acetyl derivative, under a variety of conditions (16), failed to give a ring opened analog. The ring was readily opened with NH_4Cl and with HCl (17) to give, respectively, and after removal of the acetonide block, 9-chloro-9-deoxy-4(R)-dihydro-spectinomycin and 8-epi-chloro-8-des-(methyl-amino)-9-epi-(methylamino)-9-deoxy-4(R) dihydro-spectinomycin. 8,9-Epimino-4(R)-dihydro-spectinomycin itself was obtained by removal of the acetonide block with sulfuric acid.

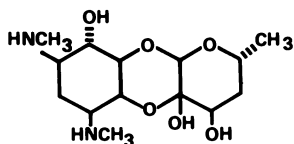
9-Epi-chloro-9-deoxy-4(R)-dihydro-spectinomycin and 9-epi-chloro-9-deoxyspectinomycin were prepared directly (Figure 12) from suitably blocked intermediates by chlorination, with inversion, using the *N*-chlorosuccinimide-triphenylphosphine method of Hanessian, et. al. (18).



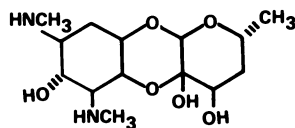
7-Epispectinomycin



7-Epi-4(R)-Dihydrorespectinomycin



7-Deoxy-4(R)-Dihydrorespectinomycin



7-Epi-9-Deoxy-4(R)-Dihydrorespectinomycin

Figure 10.

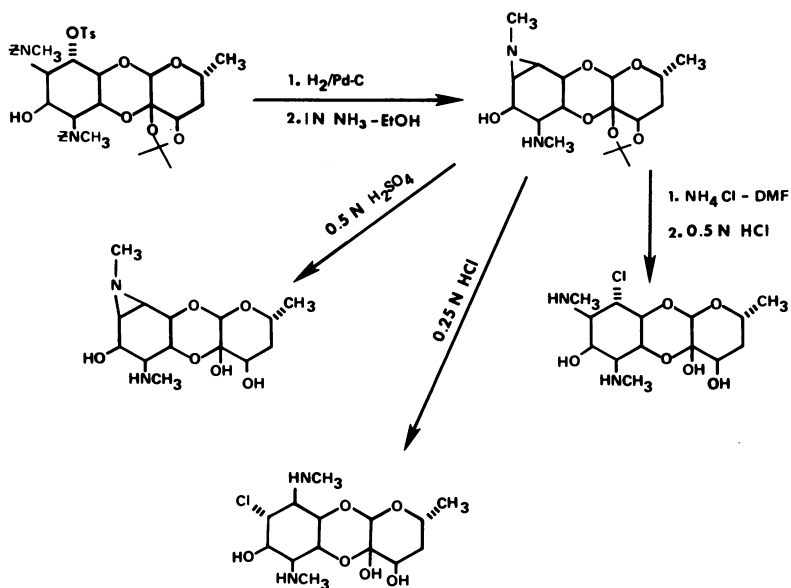


Figure 11.

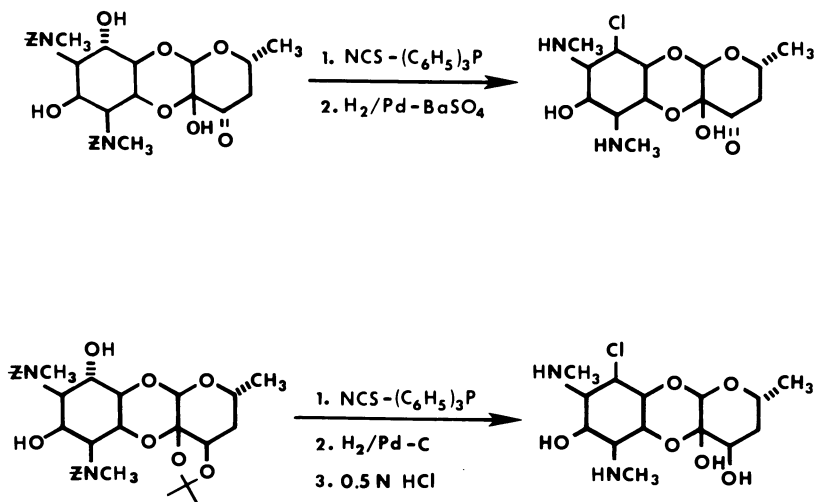


Figure 12.

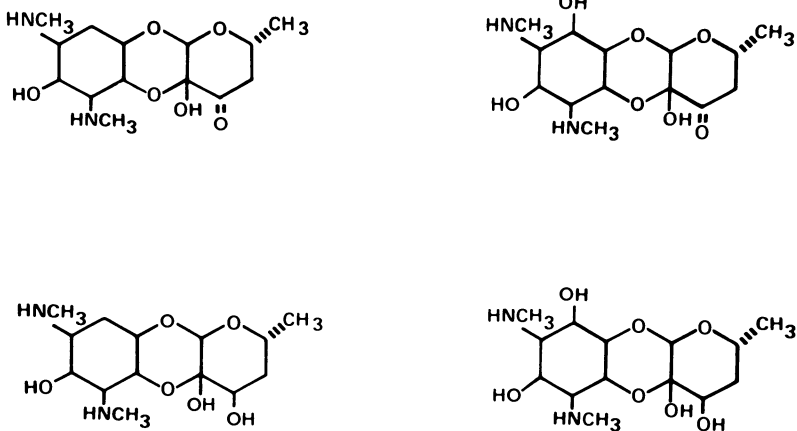


Figure 13.

Again, all of these analogs failed to exhibit antibiotic activity and the effect of C-9 deoxygenation and epimerization remained unknown.

Recently, however, Foley, Lin, and Weigle of Hoffmann-La Roche (19,20) prepared the 9-deoxy- and 9-epi-analogs of both spectinomycin and 4(R)-dihydrospectinomycin (Figure 13). All four compounds were found to be devoid of antibiotic activity.

All of the modifications thus far made in the cyclitol portion of spectinomycin and 4(R)-dihydrospectinomycin, including such changes as N-demethylation, N-alkylation, N-acylation, and 9-O-acylation (21), result in the complete loss of antibiotic activity. We have managed to confirm that spectinomycin is indeed an atypical aminocyclitol antibiotic.

Acknowledgement

We wish to acknowledge the contributions of M. Cirovic, D. A. Dunnigan, R. S. Egan, R. J. Mauritz, S. L. Mueller and R. S. Stanaszek of Abbott Laboratories and K. Mochida, Y. Mori and T. Nishinaga of the Tokyo Research Laboratory, Kyowa Hakko Kogyo Company, Ltd.

Literature Cited

1. Wiley, P. F., Argoudelis, A. D., and Hoeksema, H., J. Amer. Chem. Soc. (1963) 85, 2652~2659.
2. Cochran, T. G., Abraham, D. J., and Martin, L. L., J.C.S. Chem. Comm. (1972) 494~495.
3. Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., and Waring, M. J., in "The Molecular Basis of Antibiotic Action", pp. 306~307, John Wiley and Sons, London, 1972.
4. Novak, E., Schlagel, C. A., LeZotte, L. A., and Pfeifer, R. T., J. Clin. Pharm. (1974) 442~447.
5. Davies, J., Antimicro. Agents and Chemoth. (1968) 1967, 297~303.
6. Shier, W. T., Rinehart, K. L., and Gottlieb, D., Proc. Natl. Acad. Sci. U.S. (1969) 63, 198~204.
7. Benveniste, R., Yamade, T., and Davies, J., Infect. Immun. (1970) 1, 109~119.
8. Kawabe, H., Inoue, M., and Mitsuhashi, S., Antimicro. Agents and Chemoth. (1974) 5, 553~557.

9. Knight, J. C. and Hoeksema, H., J. Antibiot. (1975) 28, 136~142.
10. Rosenbrook, Jr., W. and Carney, R. E., J. Antibiot. (1975) 28, 953~959.
11. Rosenbrook, Jr., W. Carney, R. E., Egan, R. S. Stanaszek, R. S., Cirovic, M., Nishinaga, T., Mochida, K., and Mori, Y., J. Antibiot. (1975) 28, 960~964.
12. Pfitzner, K. E. and Moffatt, J. G., J. Amer. Chem. Soc. (1965) 87, 5670~5678.
13. Rosenbrook, Jr., W. Carney, R. E., Egan, R. S., Stanaszek, R. S., Cirovic, M., Nishinaga, T., Mochida, K., and Mori, Y., J. Antibiot. (1978) 31, 451~455.
14. Ratcliffe, R. and Rodehorst, R., J. Org. Chem. (1970) 35, 4000~4002.
15. Carney, R. E. and Rosenbrook, Jr., W. J. Antibiot. (1977) 30, 960~964.
16. Daniels, P.J.L., Weinstein, J., Tkach, R. W., and Morton, J., J. Antibiot. (1974) 27, 150~154.
17. Ali, Y., Richardson, A. C., Gibbs, C. F., and Hough, L., Carbohyd. Res. (1968) 7, 225~271.
18. Hanessian, S., Ponpipom, M. M., and Levallee, P., Carbohyd. Res. (1972) 24, 45~56.
19. Foley, L., Lin, J.T.S., and Weigle, M., J. Antibiot. (1978) 31, 979~984.
20. Foley, L., Lin, J.T.S., and Weigle, M., J. Antibiot. (1978) 31, 985~990.
21. Rosenbrook, Jr., W., Carney, R. E., Nishimaga, T., Mochida, K., and Mori, Y., unpublished work.

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The Structures of Diastereomers of Dihydrospectinomycins

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Spectinomycin (1) (1,2) as well as the dihydrospectinomycins 2 and 3 (3) (Figure 1) represent unusual structures among the aminocyclitol antibiotics. Most antibiotics of this class contain streptomycin or 2-deoxystreptomycin linked to at least one α -pyranose unit. Spectinomycin, however, contains 2-epi-N,N'-dimethylstreptomycin linked to a single sugar unit by a β -glycosidic bond. Another interesting feature of structures 1, 2 and 3 is the selective formation of the hemiketal linkage between the C-4 hydroxyl group and the C-2' carbonyl carbon.

As depicted in Figure 2, opening of the hemiketal bond in the spectinomycin hydrate (4a) would give the hydrated α -diketone 5a. The symmetrical cyclitol unit in structure 5a has available for the generation of an intramolecular hemiketal linkage two diastereotopic equatorial alcohols at C-4 and C-6 which could, in theory, generate four different isomers.

Hemiketal formation using the C-4 hydroxyl group either regenerates the spectinomycin skeleton 4a, having a cis ring fusion between the 1,4-dioxin ring and the sugar ring, or the isomer 6a, with a trans ring fusion. Clearly, the spectinomycin skeleton 4a is expected to be more stable than the isomeric skeleton due to the presence in 6a of a high energy boat conformation in the 1,4-dioxin ring. Rotation about the glycoside bond in 5a allows hemiketal formation using the C-6 hydroxyl group leading to the two diastereomers of spectinomycin 7a and 8a. The linear diastereomer 7a has a trans ring fusion between the 1,4-dioxin ring and the pyranose ring, while 8a possesses a cis ring fusion.

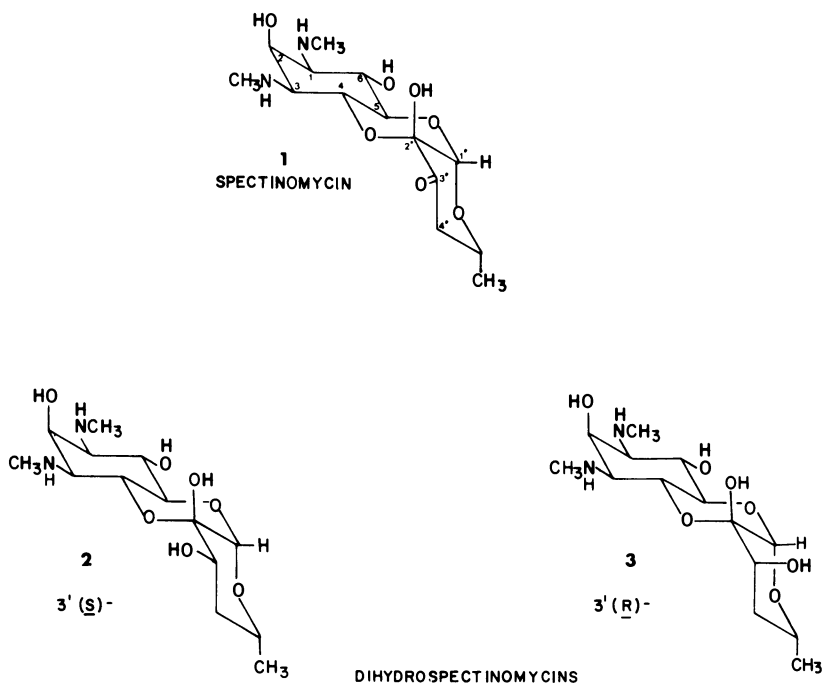


Figure 1.

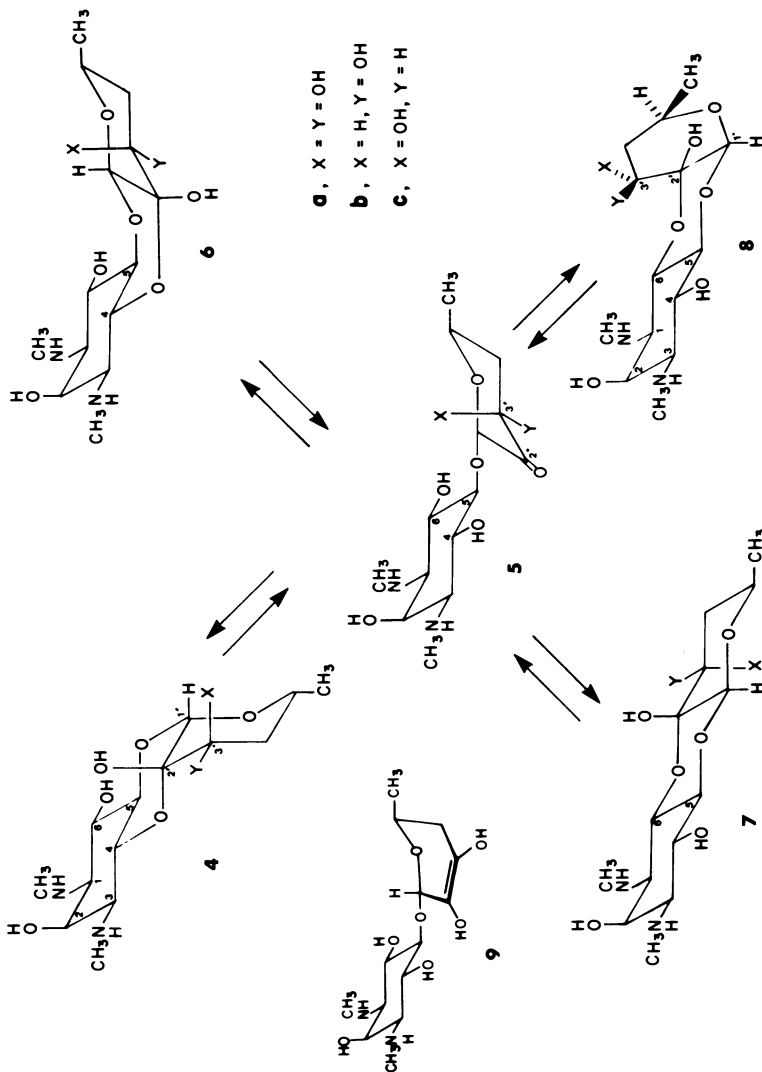


Figure 2.

This opening-reclosure sequence is also conceivable for the 3'(R)- and 3'(S)-dihydrospectinomycins 4c and 4b, respectively. In addition, opening of the ketal bond in the 3'(R)-dihydrospectinomycin (4c) generates the α -hydroxy ketone 5c, which might be expected to be in equilibrium with the enediol 9. Tautomerization of 9 would then allow the epimerization of the 3'(R), axial alcohol, into the 3'(S) isomer 5b, equatorial alcohol. (The reverse would be expected if, in the unlikely case, the 3'(R) was the most stable configuration.)

Intrigued by the possible existence of diastereomers derived from the skeletons 7 and 8, we investigated two side products formed during the preparation of the acetonide derivatives of 3'(R)- and 3'(S)-dihydrospectinomycins. The structures of these two compounds, and the implications that their isolations have concerning the configurations and conformations possible in these molecules are the subjects of this report.

Due to the instability of the α -keto hemiketal functionalities in spectinomycin (1) we and others (4-10) chose to carry out modification work on a protected dihydrospectinomycin. The 3'(R)-dihydro-spectinomycin derivative 10 was chosen because the 2',3'-cis diol system readily allowed its protection as the acetonide derivative 11 (see Figure 3). The conditions we employed, identical to those reported by Rosenbrook, et. al. (5), allowed the preparation of not only the desired derivative 11 but also a previously unreported isomeric acetonide, albeit in low yield. The chemistry used to prove 12 as the structure of this isomeric acetonide has been previously reported by us (11).

Treatment of the isomeric acetonide with acid resulted in its conversion back to the 3'(R)-dihydro-spectinomycin 10. This simple reaction, indicating that no epimerization had occurred at the 3' position, allowed us to rule out the linear structure 16 as well as an isomer of the spectinomycin skeleton derived from structure 6c, because in both the 3'(R) configuration required the 2',3'-diols to have a trans diaxial orientation. Additionally, this reaction demonstrated the instability of the diastereomeric skeleton 12 (or 8) relative to the spectinomycin skeleton 10 (or 4).

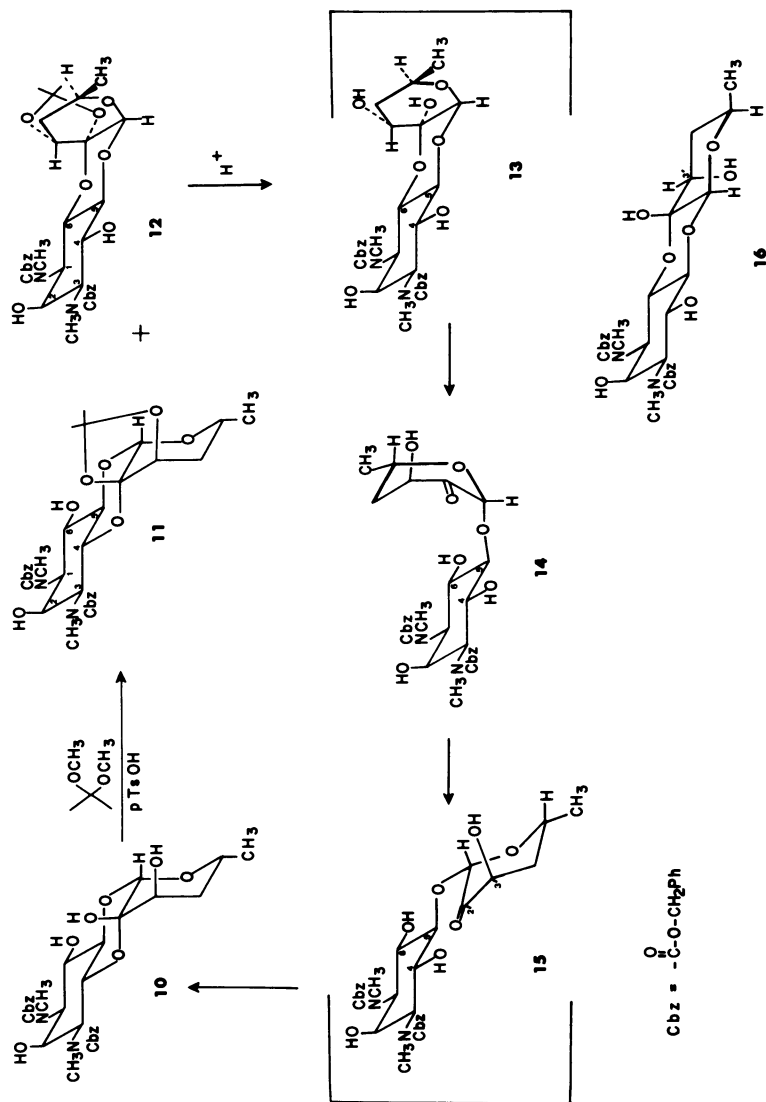
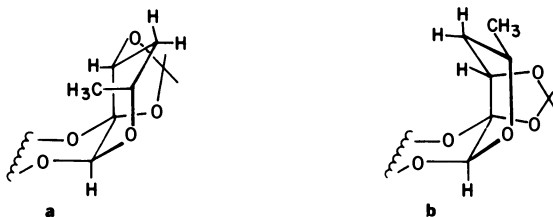


Figure 3.

In the diastereomeric structure 12 the hemiketal linkage is formed using the C-6 hydroxyl, whereas in the spectinomycin skeleton it is the C-4 hydroxyl group which is involved. The chemistry outlined in Figure 4, made use of the instability of the diastereomeric skeleton relative to that of spectinomycin to prove the structure of 12 (11). Information regarding the preferred conformation of 12 (partial structures a or b below) was readily available from the PMR spectra of this compound and its derivatives. The chair conformer a would be expected to exhibit a splitting pattern for the 3' proton compatible with axial-axial and axial-equatorial couplings, while the boat conformer b should have only the small coupling constants of equatorial-axial and equatorial-equatorial couplings. As noted in our earlier report (11), the narrow triplet ($J = 3$ Hz) observed for the 3' proton is only consistent with the pyranose ring having a boat conformation. This conformation allows the 5' methyl substituent to assume the more stable equatorial orientation.



During the preparations of the cyclic carbamates 17a and 18a, we noted the formation of small amounts of two side products subsequently shown to be the bis cyclic carbamate 23 and the unsaturated cyclic carbamate 24. These derivatives are only obtainable from the 1,2-cyclic carbamate 17a (and not from the 2,3-cyclic carbamate 18a); 23 on further heating with K_2CO_3 in DMF was converted into the unsaturated cyclic carbamate 24, see Figure 5. The X-ray structure of 24 shown in Figure 6 established its structure and also confirmed the proposed structure 12 (11) for the diastereomeric acetonide.

Earlier an enediol, see structure 9 in Figure 2, was suggested as an intermediate allowing the epimerization of the hydroxyl group at the 3' position.

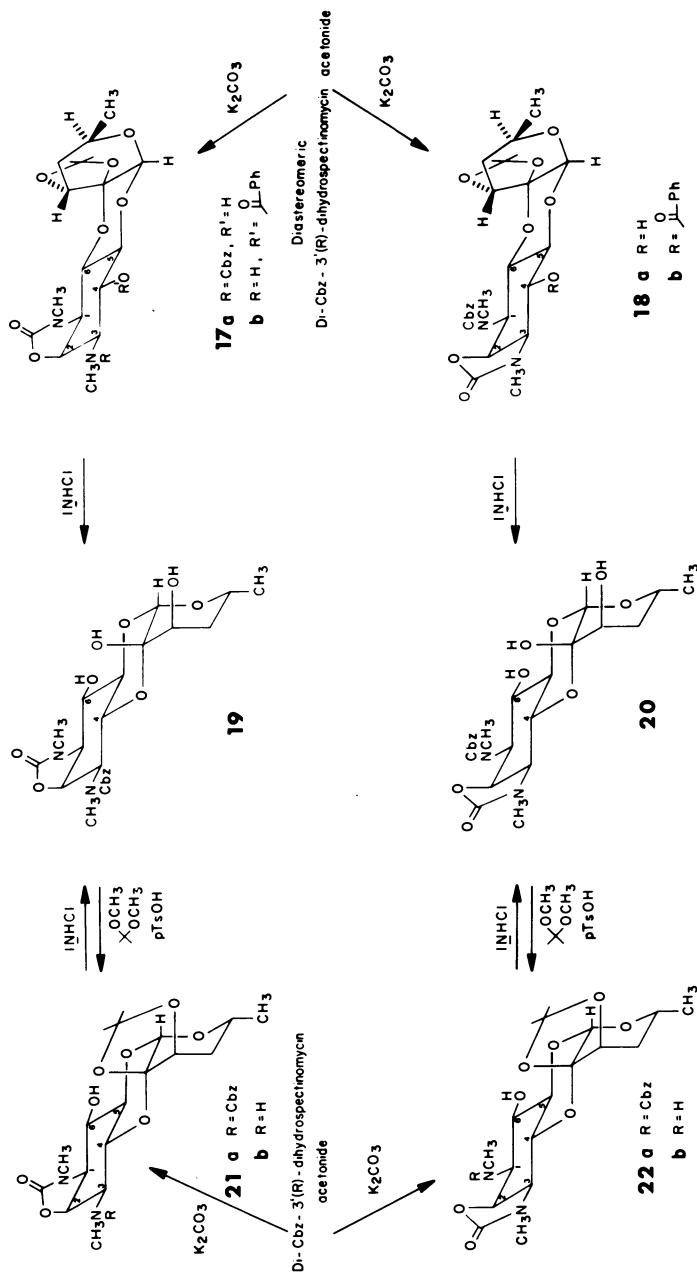


Figure 4.

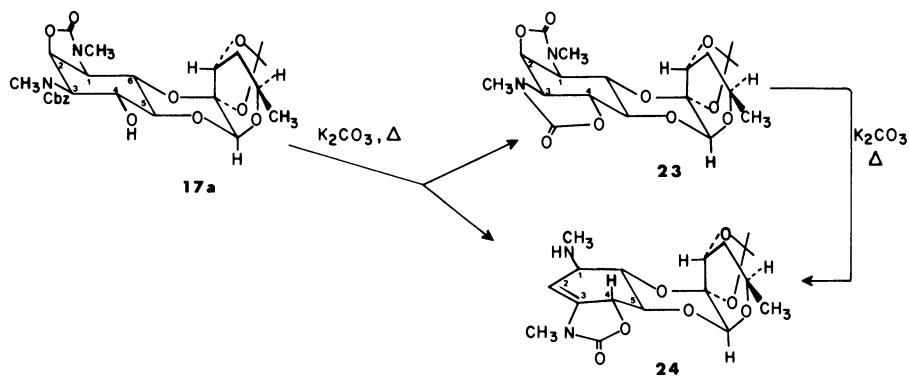
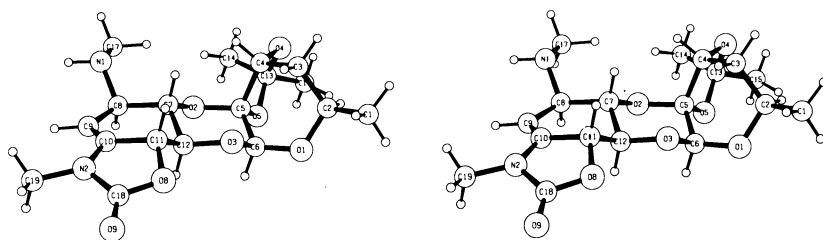


Figure 5.

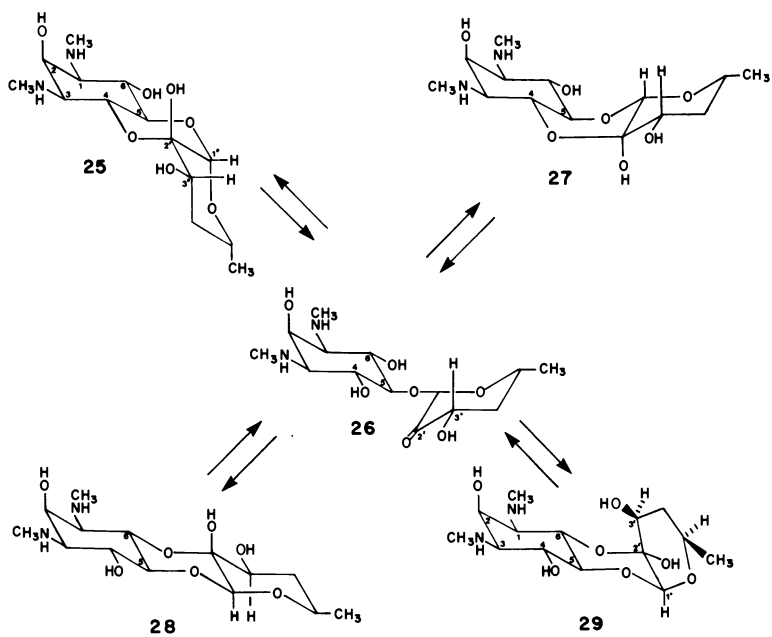
Figure 6. stereodrawing showing the structure and conformation of the unsaturated cyclic carbamate **24**

Our experimental results, showing that the 3'(R) configuration was maintained, do not support such a possibility and thus interconversion between the 3'(R)- and 3'(S)-dihydrospectinomycins is not possible. We were, however, still intrigued by the possible existence of the linear diastereomeric structure 28 and felt that, as shown in Figure 7, it might be trapped as its acetonide derivative starting from the 3'(S)-dihydrospectinomycin 25. Formation of an acetonide derivative from the diastereomer having the same skeleton as found in the 3'(R) series is not possible because of the trans diaxial orientation of the 2',3'-diols in this conformation, see structure 29.

Treatment of the 3'(S)-dihydrospectinomycin derivative 30, as shown in Figure 8, with 2,2-dimethoxypropane in DMF containing *p*-toluenesulfonic acid at 80° C for 48 hours gave the trans acetonide derivative 31 and a trace of an isomeric acetonide. This isomeric acetonide on reaction with aqueous 1N hydrochloric acid regenerated the starting 3'(S)-dihydrospectinomycin derivative 30; again no epimerization of the 3' position was observed.

There are two possible diastereomeric structures for this compound--the linear structure 33 or the angular structure 34, which is a conformational isomer of the 3'(R) diastereomeric skeleton. [As noted earlier, the 3'(S)-epimer 29, having the same skeleton as the 3'(R)-diastereomer, cannot form an acetonide. However, by changing the conformation in both the 1,4-dioxin and sugar rings, the 2',3'-diol system assumes a trans diequatorial orientation and thus can form the acetonide derivative 34.] A third possibility that this material was the isomeric compound 35 could be excluded using the chemistry outlined in Figure 9.

Again, as in the case of the 3'(R)-diastereomer 12, the instability of the unknown acetonide's skeleton relative to that of spectinomycin allowed us to show that this isomeric compound was a diastereomer of 3'(S)-dihydrospectinomycin. The 1,2-cyclic carbamate 36a and 2,3-cyclic carbamate 37a were prepared by heating the 3'(S)-dihydrospectinomycin acetonide 31 with K₂CO₃ in DMF at 95° C for four hours. In order to carry out PMR decoupling experiments, 36a was converted into its benzoate 36b by reaction with benzoyl chloride in pyridine followed by hydrogenolysis using a palladium on carbon catalyst. Irradiation of H-6 at δ 5.47 [H-C-O-C(O)Ph]

*Figure 7.*

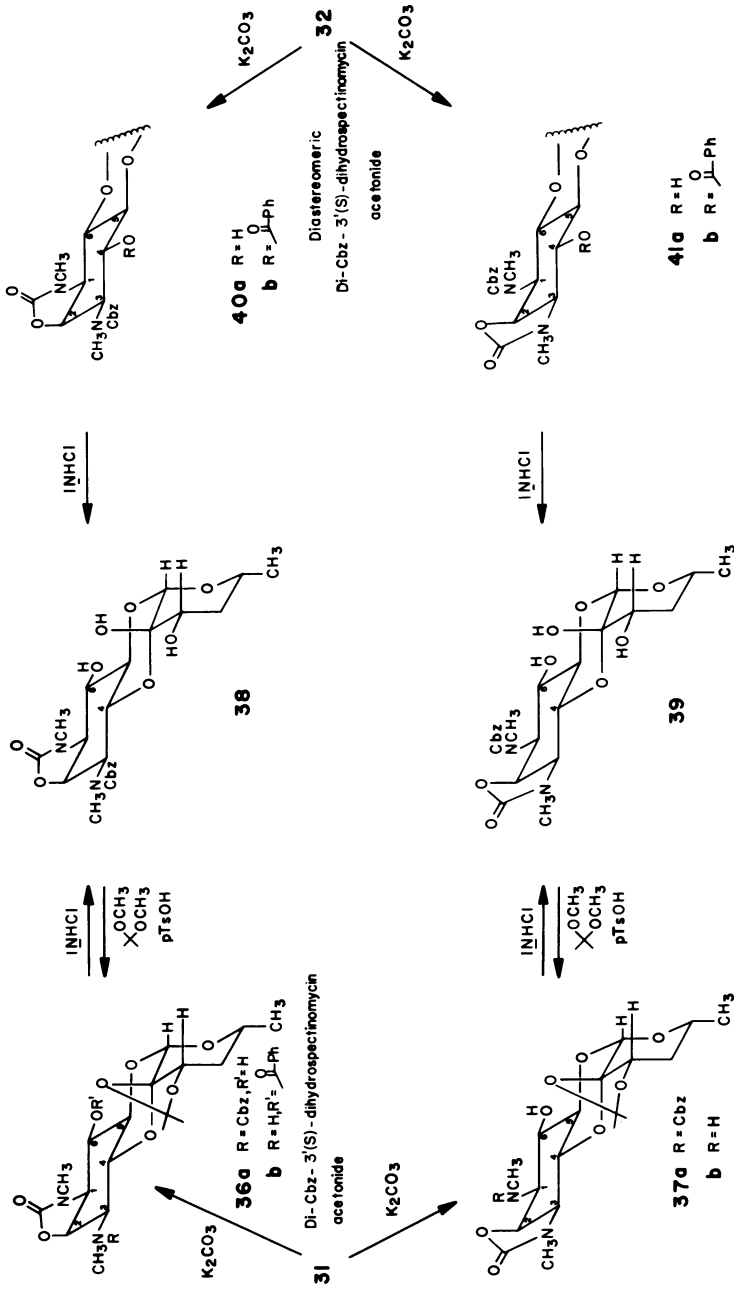


Figure 9.

in 36b caused the collapse of the triplet at δ 3.83 [$\text{H-C-N}(\text{CH}_3)\text{-CO}_2^-$, H-1] to a doublet and of the distorted doublet of doublets at δ 4.07 (H-5) to a doublet. This decoupling experiment provided confirmation that the carbon containing the methylamino moiety of the cyclic carbamate was adjacent to the benzoate methine as required by the structure shown in 36b and thus confirmed the 1,2-cyclic carbamate structure of 36a. Similar decoupling experiments carried out on the derivative 37b confirmed its structure and that of 37a.

Reaction of the isomeric acetonide 32 with K_2CO_3 in DMF at 95°C for four hours gave two new isomeric cyclic carbamates, 40a and 41a. Treatment of the less polar cyclic carbamate with benzoyl chloride in pyridine gave a benzoate derivative for PMR decoupling experiments. Irradiation of the benzoate methine proton at δ 5.67 caused the collapse of the doublet of doublets for the Cbz methylamino methine proton at δ 4.88 to a doublet. Simultaneously, the doublet of doublets assigned to the proton on the carbon carrying the ether of the glycoside linkage at δ 4.25 collapsed to a doublet. Since the doublet of doublets at δ 3.56, assigned to the proton on the carbon containing the methylamino group of the cyclic carbamate moiety, was unaffected, the partial structure 40b was assigned to this derivative and thus 40a to the less polar cyclic carbamate. Again, similar decoupling experiments carried out on 41b confirmed 41a as the structure of the more polar cyclic carbamate.

Acid hydrolysis of the acetonide 40a resulted in its conversion, after the reintroduction of the acetonide group, into the 1,2-cyclic carbamate 36a and the identical treatment of 41a gave, as expected, the 2,3-cyclic carbamate 37a. These results fully confirmed that the isomeric acetonide was one of the dihydrospectinomycin diastereomers 33 or 34 and not the isomeric acetonide 35. (If the unknown acetonide had been 35 then the isomeric cyclic carbamate having the carbon containing the methylamino group of the cyclic carbamate adjacent to the methine carrying the ether of the hemiketal linkage, on opening and reclosure to form the more stable spectinomycin skeleton would have formed 37a and not, as observed, 36a. This is because in both 35 and 31 it is the same hydroxyl group, at C-4, which is involved in the formation of the hemiketal bond.) Also, it should be noted that throughout these transformations no

epimerization at the 3' position was observed, again ruling out the intermediacy of an enediol, such as 9 in Figure 2.

The crystalline bis cyclic carbamate 42, together with minor amounts of the unsaturated cyclic carbamate 43, were prepared by treating the 1,2-cyclic carbamate 40a with K_2CO_3 in DMF at $90^\circ C$ for four hours, see Figure 10. The X-ray structure of 42 shown in Figure 11 established 34 as the structure of the 3'(S) diastereomeric acetonide.

Since the 3'(S)-diastereomer 34 was isolated in only trace quantities, the possibility existed that the other diastereomer, 33, was also present but had been missed. The chemistry outlined in Figure 12 was designed to give information as to the possible existence of the linear diastereomer 33. Protection of the C-4 hydroxyl as the methyl ether prevents the rearrangement of the diastereomeric skeleton back to the more stable spectinomycin skeleton. The O-methyl ether 44, prepared by reacting the 2,3-cyclic carbamate 41a with sodium hydride followed by methyl iodide, on aqueous acid treatment generated the diol 45, which would be expected to be in equilibrium with the α -hydroxy ketone 46. This α -hydroxy ketone has only two modes of cyclization available to it--reclosure to the diol 45 giving back the starting material 44, after reintroduction of the acetonide ring, or closure to form the linear structure 48, which might then be trapped as the acetonide 47. When this reaction was carried out the only acetonide derivative isolated was identical to the starting material 44; no evidence was obtained for the existence of the linear diastereomer 47.

This work clearly demonstrates (see Figure 13) that under mildly basic or acidic conditions the 3'(R)-dihydro-spectinomycin is in equilibrium with the α -hydroxy ketone 49, and also that the 3'(S)-dihydro-spectinomycin is in equilibrium with the α -hydroxy ketone 51. Isolation of the diastereomers indicates that in each case this equilibrium mixture contains a small amount of the diastereomer which is trapped as the acetonide derivatives 12 and 34. The fact that no epimerization of the 3' position has been observed rules out equilibration of the two α -hydroxy ketones 49 and 51 via the enediol 50. The failure to observe the formation of the 3'-keto derivative 52 also militates against the existence of an enediol intermediate. The rearrangement of 2-keto uloses into 3-keto uloses via an enediol intermediate, similar to

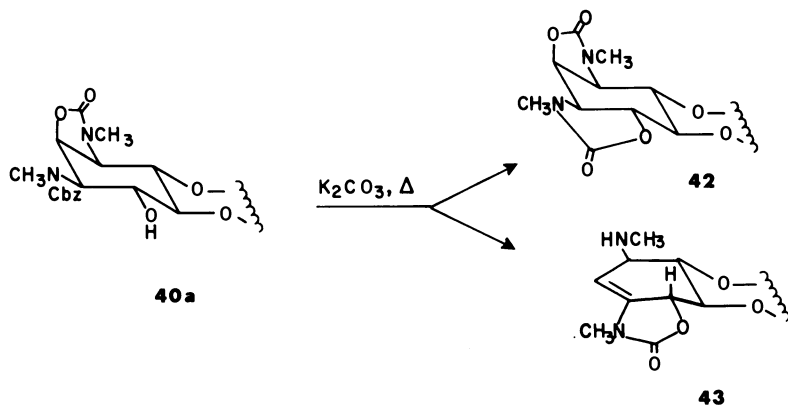
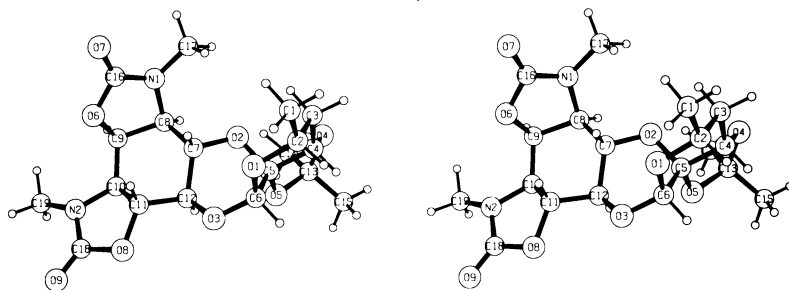


Figure 10.

Figure 11. Stereodrawing showing the structure and conformation of the bicyclic carbamate **42**

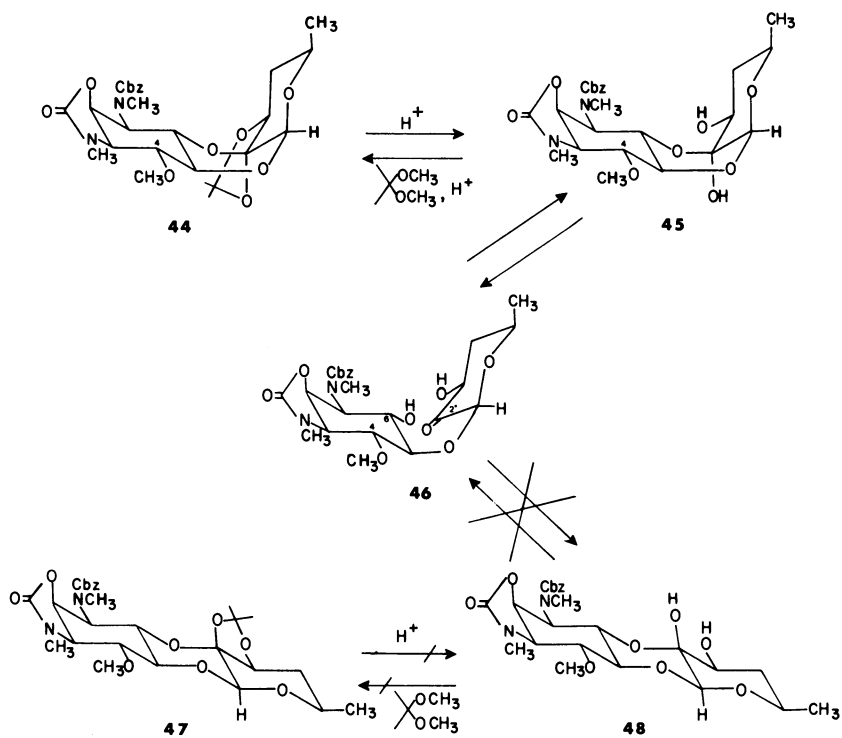


Figure 12.

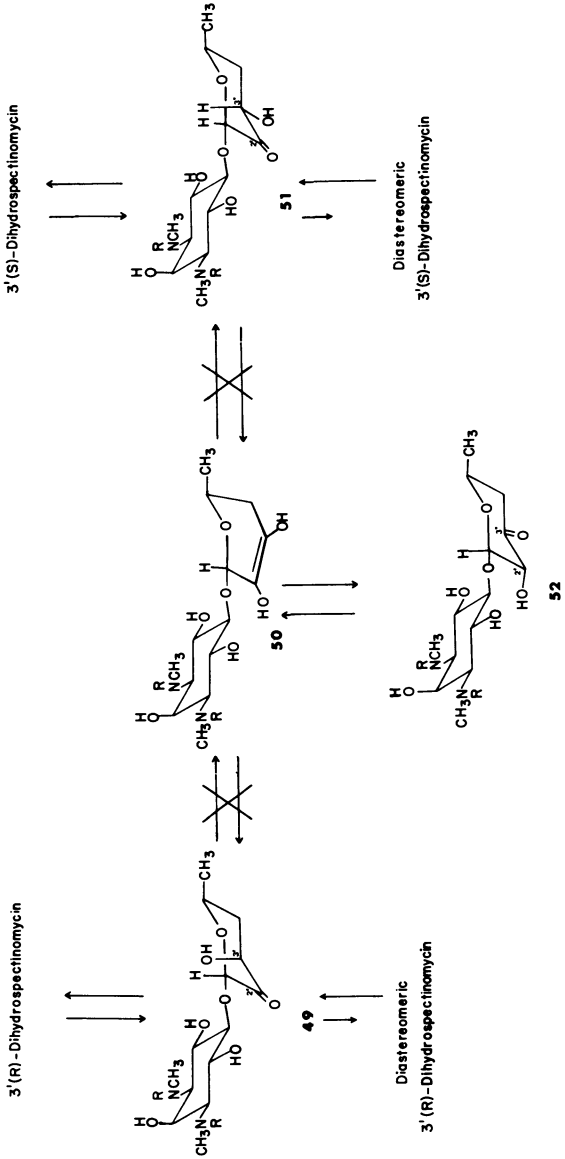


Figure 13.

50, is well documented (12,13). The failure of the enediol 50 to play a role in the chemistry of spectinomycin is also evident in the work of Knight and Hoeksema (3), see Figure 14. These workers reported that reduction of either dihydrospectinomycin with sodium borohydride gave two tetrahydrospectinomycins epimeric at only the 2' position; the 3' configuration of the starting material was maintained.

Under strongly basic conditions, however, we have found indirect evidence for the intermediacy of an enediol. Reaction of either the 3'(S)- or 3'(R)-dihydrospectinomycins or their derivatives, without the acetamide protective group, with barium hydroxide in refluxing methanol-water gave the cyclitol 54. The proposed mechanism, outlined in Figure 15 for the 3'(R)-dihydrospectinomycin 53, involves the opening of the hemiketal linkage to give the α -hydroxy ketone 49 which on enolization forms the enediol 50. The cyclitol moiety 54 is eliminated from the enediol 50 by a retro Michael reaction. While the opening of 53 to give 49 is reversible, as we have shown, the enolization of 49 to give 50 appears to be irreversible. Elimination of the cyclitol unit takes place rather than tautomerization to give either the 3'(R) or 3'(S) derivatives 49 or 51 or the 3'-keto derivative 52. The irreversible nature of the last two steps could be demonstrated by interrupting the reaction and isolating unreacted material. In each case, the recovered spectinomycin derivative was shown to have the 3' configuration of the starting dihydrospectinomycin, no evidence for epimerization at the 3' position could be detected.

Figure 16 shows the structures of the two diastereomers, the 3'(R) 12 and the 3'(S) 34, which are simply conformational isomers of the same basic skeleton containing a cis ring fusion between the 1,4-dioxin and sugar rings. We have considered three possible factors which may contribute to the preferential formation of the spectinomycin skeleton 57 (11 and 31) over its isomeric skeleton 58 and the two diastereomeric skeletons 55 and 56, and also for the preference for the diastereomeric structure 55 (12 and 34) over the linear structure 56. (While these arguments are presented in Figure 16 for 3'(S)-dihydrospectinomycin, they are equally applicable to the 3'(R)-dihydrospectinomycins as well as to spectinomycin.)

As shown in Figure 16, the observed products are always the result of axial attack of the hydroxyl

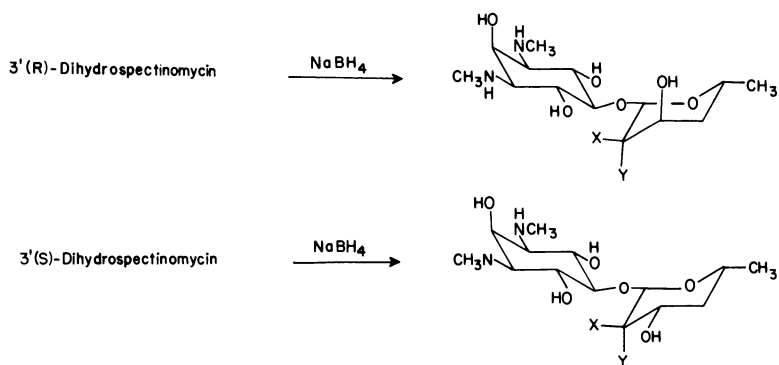


Figure 14. Reduction of dihydrospectinomycins giving tetrahydrospectinomycins (3). (top) $X = H, Y = OH$; (bottom) $X = OH, Y = H$

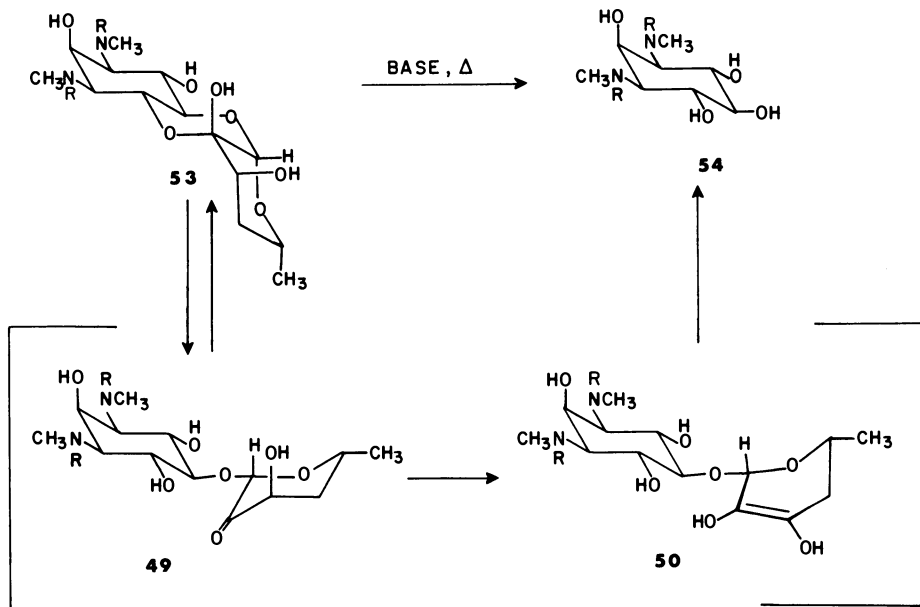


Figure 15.

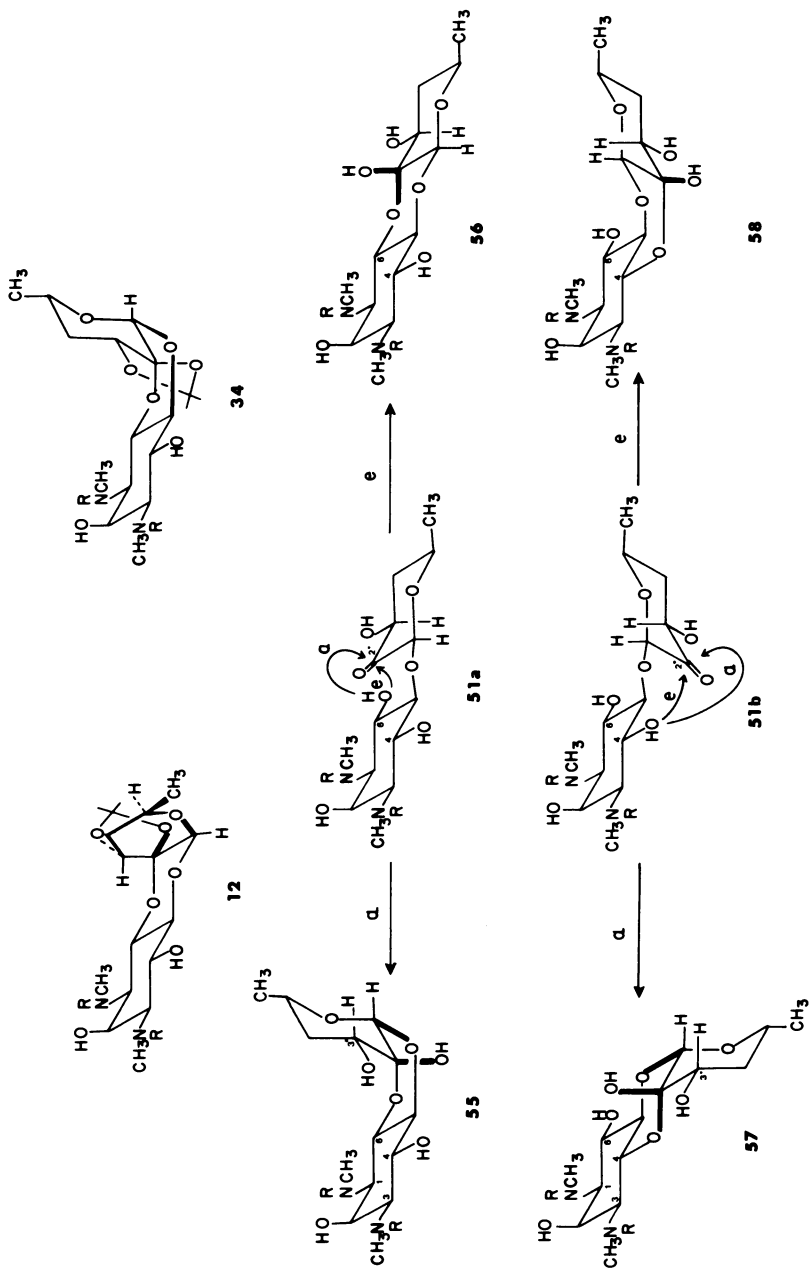


Figure 16.

groups at C-4 or C-6 on the 2' carbonyl carbon. A possible explanation may be found in the known preference for axial nucleophilic attack on the cyclohexanone carbonyl (14). In equatorial attack on the carbonyl carbon, the incoming nucleophile encounters torsional strain from the axial substituents on the α -carbons, while in axial attack there is steric strain between the nucleophile and the axial groups on the β -carbons. When the substituents on the α and β atoms are the same, the steric strain encountered in axial attack is less severe than the torsional strain involved in equatorial attack and the axial mode predominates (14,15). In the present case, axial attack would be further favored by the presence of only one axial substituent on a β atom. In addition, it has been demonstrated that the trajectory for nucleophilic attack on carbonyls has an angle of 100° to 110° to the plane of the carbonyl (16,17). In the formation of the hemiketal from either 51a or 51b, the attacking nucleophile is constrained from achieving the desired trajectory for equatorial attack by the glycoside linkage. An examination of molecular models indicates that in axial attack the oxygen atom of the hydroxyl group can more nearly achieve the desired trajectory.

The preferential formation of 55 and 57 may also reflect the Reeves " Δ^2 effect" (18,19), in which the presence of an axial hydroxyl group on a carbon adjacent to an anomeric center destabilizes the equatorial anomer relative to the axial anomer. Thus, one would expect the Reeves " Δ^2 effect" to destabilize 56 relative to the observed diastereomer 55, and to destabilize the unobserved spectinomycin isomer 58 relative to the spectinomycin skeleton 57.

The third factor accounting for these preferences involves the influence of the anomeric effect and, in particular, its interpretation in terms of the stabilization resulting from an overlap of the lone-pair electrons on oxygen with the antibonding orbital of the adjacent carbon oxygen bond (20, 21, 22, 23, 24). As shown by the heavy lined bonds in structures 55, 56, 57 and 58 (Figure 16), the spectinomycin skeleton 57 has three such stabilizing interactions while the isomer 58 has only one. Both diastereomeric structures 55 and 56 each possess two. Thus, while the anomeric effect does not explain the preference for the diastereomeric structure 55 over structure 56, it does contribute to the stability of the spectinomycin

skeleton 57 over its isomer 58 and either diastereomer 55 or 56.

In conclusion, the isolation of only the diastereomer having the structure 55, with the exclusion of the linear diastereomeric structure 56, can be explained by the " Δ^2 effect" and preferred axial attack on the carbonyl. The stability of the spectinomycin skeleton 57 over the isomer 58 can be explained by contributions from the " Δ^2 effect" and the anomeric effect. Preferred axial attack on the 2' carbonyl, as well as the presence of a high energy boat conformation in the 1,4-dioxin ring of 58 further favor 57. Finally, the stability of the spectinomycin skeleton 57 over the diastereomeric skeleton 55 can be rationalized by the operation of the anomeric effect and the presence of a high energy boat conformation in both diastereomeric conformations, 12 and 34.

As noted earlier, the findings presented here "... cast doubt on a proposed biosynthetic scheme for spectinomycin involving the rearrangement of a 2-hydroxy-3-ulose to a 3-hydroxy-2-ulose via an enediol similar to 9. (25) Additionally, the observation that a symmetrical cyclitol intermediate, such as 5, will preferentially cyclize to form the spectinomycin skeleton greatly simplifies any synthetic approach to this molecule" (11).

Abstract

The structures and chemistry of the diastereomers 12 and 34 of 3'(R)- and 3'(S)-dihydrospectinomycins, which establish the existence of the equilibrium $4 \rightleftharpoons 5 \rightleftharpoons 8$, are described. The X-ray structures of derivatives of these diastereomers, which gave final proof of the structure of 34 and confirmed the proposed structure 12, are also given. Finally, we present an explanation, involving the anomeric and " Δ^2 " effects and the known preference for axial attack on a carbonyl, for the configurational preference and diastereoselectivity observed in the cyclization of 5.

Acknowledgement

We wish to thank Dr. John F. Blount for carrying out the X-ray analysis of structures 24 and 42.

Literature Cited

1. Wiley, P.F.; Argoudelis, A.D.; Hoeksema, H. J.Amer.Chem.Soc. 1963, 85, 2652-9.
2. Cochran, T.G.; Abraham, D.J.; Martin, L.L. J.Chem.Soc., Chem.Comm. 1972, 494-5.
3. Knight, J.C.; Hoeksema, H. J.Antibiot. 1975, 28, 136-42.
4. Rosenbrook, W., Jr.; Carney, R.E. J.Antibiot. 1975, 28, 953-9.
5. Rosenbrook, W., Jr.; Carney, R.E.; Egan, R.S.; Stanaszek, R.S.; Cirovic, M.; Nishinaga, T.; Mochida, K.; Mori, Y. ibid. 1975, 28, 960-4.
6. Carney, R.E.; Rosenbrook, W., Jr. ibid. 1977, 30, 960-4.
7. Rosenbrook, W., Jr.; Carney, R.E.; Egan, R.S.; Stanaszek, R.S.; Cirovic, M.; Nishinaga, T.; Mochida, K.; Mori, Y. ibid. 1978, 31, 451-55.
8. Foley, L.; Lin, J.T.S.; Weigele, M. ibid. 1978, 31, 979-84.
9. Foley, L.; Lin, J.T.S.; Weigele, M. ibid. 1978, 31, 985-90.
10. Foley, L.; Lin, J.T.S.; Weigele, M. ibid. 1979, 32, 418-9.
11. Foley, L.; Weigele, M. J.Org.Chem. 1978, 43, 4355-9.
12. Defaye, J.; Gadelle, Q. Carbohydr.Res. 1977, 56, 411-4.
13. Theander, O. Adv.Carbohydr.Chem. 1962, 17, 284-9.
14. Cherest, M.; Felkin, H. Tetrahedron Lett. 1968, 2205-8.
15. Kobayashi, Y.M.; Lambrecht, J.; Jochims, J.C.; Burkert, U. Chem.Ber. 1978, 111, 3442-59.
16. Burgi, H.B.; Dunitz, J.D.; Lehn, J.M.; Wipff, G. Tetrahedron 1974, 30, 1563-72.
17. Schweizer, W.B.; Procter, G.; Kaftory, M.; Dunitz, J.D. Helv.Chim.Acta 1978, 61, 2783-2808.
18. Reeves, R.E. J.Amer.Chem.Soc. 1950, 72, 1499-1506.
19. Wick, A.E.; Blount, J.F.; Leimgruber, W. Tetrahedron 1976, 32, 2057-65.
20. Romers, C.; Altona, C.; Buys, H.R.; Havinga, E. Top.Stereochem. 1969, 4, 73-7.
21. David, S. In "Anomeric Effect, Origin and Consequences," Szarek, W.A.; Horton, D., Eds.; ACS Symp. Ser. #87; American Chemical Society: Washington, D.C., 1979; pp. 1-16.

22. Lemieux, R.U.; Koto, S.; Voisin, D. ibid;
pp. 17-29.
23. Jeffrey, G.A. ibid; pp. 50-62.
24. Wolfe, S.; Whangbo, M-H.; Mitchell, D.J.
Carbohydr.Res. 1979, 69, 1-26.
25. Hoeksema, H.; Knight, J.C. J.Antibiot. 1975,
28, 240-1.

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Chemical Modification of Aminoglycosides: A Novel Synthesis of 6-Deoxyaminoglycosides¹

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As part of a program to study structure-activity relationships among the semisynthetic aminoglycoside antibiotics, we elected to prepare a family of 6-deoxyaminoglycosides. 6-Deoxyneomycin and related compounds have been described in the literature (2, 3, 4, 5). The starting point in our synthesis was neamine which is readily obtained by methanolysis of neomycin. As shown in Figure 1 neamine (1) was blocked on nitrogen by the trifluoroacetyl group giving tetrakis-amide (2) in high yield. The trifluoroacetyl blocking group proved to be quite desirable in this situation since it not only could be readily removed with dilute alkali, but also conferred good solvent solubility on the intermediates. Then too, the trifluoroacetyl intermediates were sufficiently volatile to permit satisfactory vpc-mass spectrum evaluation. The trifluoroacetyl derivative (2) when treated with 2,2-dimethoxypropane under mild conditions gave a high yield of monoketal (3) with varying amounts of diketal (4). This diketal was readily converted to monoketal (3) in the presence of dilute acid. Carbon-13 nuclear magnetic resonance definitely established that the ketal group in monoketal (3) was at O-5,6 as shown. This is in agreement with the findings of ketalization of neamines blocked by other groups on nitrogen.

The next two steps proceeded smoothly and in high yield (Figure 2). The hydroxyls at C-3' and 4' were acylated either with acetyl, or in cases where a UV visible group was desired for TLC referencing, with p-nitrobenzoyl, to form (5a) or (5b). Mild acid hydrolysis gave blocked neamine derivatives (6a) or (6b) in almost quantitative yield. The question now was which of these two equatorial hydroxyls would be more reactive. The pioneering work of Umezawa in the synthesis of the kanamycins indicated that the 6-hydroxyl would be more available for glycosylation by the Koenigs-Knorr reaction than the 5-hydroxyl (6). He pointed out that this selectivity was in keeping with the observation that hydroxyls adjacent to a glycosidic bond show diminished

¹ This is the second part of a series on the modification of aminoglycosides. See Ref. 1 for Part I.

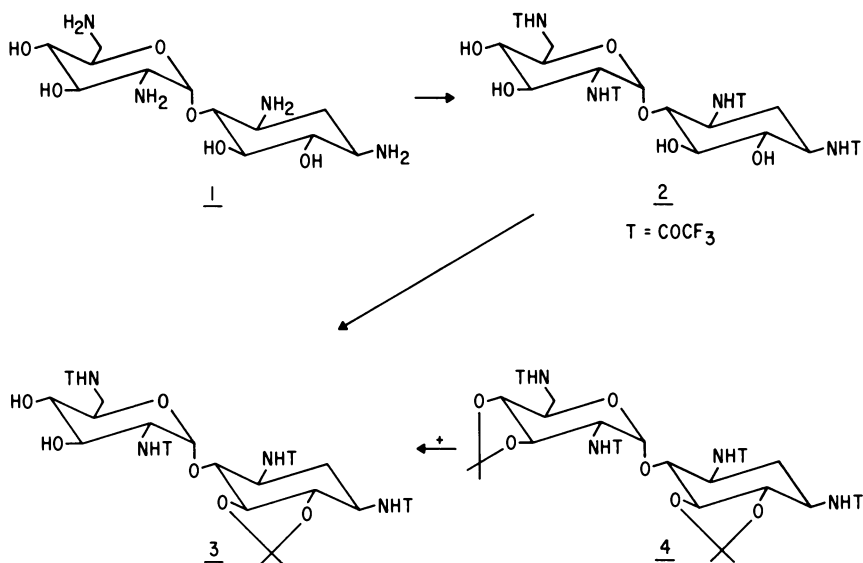


Figure 1.

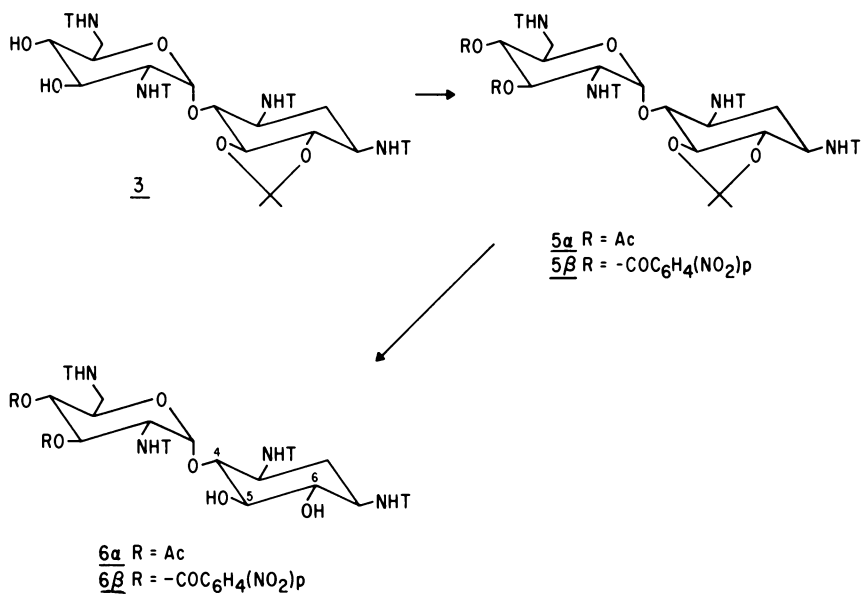


Figure 2.

reactivity. This observation has been repeatedly confirmed by other investigators (7, 8, 9).

We have examined the glycosylation of this diol using both the Koenigs-Knorr and the glycal glycosylation procedures. Substitution occurred selectively at 0-6. In no case did we isolate a pure fraction which could be assigned an 0-5 substituted structure. Thus 5-0-substituted-neamines are not readily available from this intermediate. One facet of our program, however, was the preparation of just such analogs. One possible way to prepare such compounds from the available intermediates would be to remove the more reactive 6-hydroxyl, leaving only the 5-hydroxyl as a site for glycosylation.

From some of our related work, as well as reports in the literature, we know that the various hydroxyls on the neamine moiety contribute little to *in vitro* antibacterial potency (10, 11). A 5-0-substituted 6-deoxy-neamine (7) may be expected to be as potent as a 5-0-substituted-neamine (8) but less difficult to prepare (Figure 3). The removal of the 6-hydroxyl was therefore of interest and was accomplished by what we believe to be an unique reaction. Tosylation of diol (6a) was quite selective even in the presence of a large excess of tosyl chloride to give 6-tosylate (9) (Figure 4). In addition to the 6-tosylate, about 5% of the 5-tosylate could be isolated. CMR clearly indicated that the major product was the expected 6-0-tosylate (9). When treated with potassium iodide in DMF, the replacement of iodide for tosyl was not in evidence, but ketone (10) was isolated in 60% yield. A clue as to how this transformation takes place may be gained in noting that treatment of tosylate (9) with LiCl in DMF resulted in facile displacement of chloride for tosyl. This suggests that the first step in ketone formation is replacement to iodide (11) which loses HI in the presence of DMF to yield ketone (10). In addition to the major product of this reaction, several minor products were also isolated. One of these products, formed by the loss of a trifluoroacetamido group, is unsaturated ketone (12) (Figure 5). When the ketone forming step was carried out under more vigorous conditions or in hexamethylphosphoramide, the major reaction product was substituted catechol (13).

Reduction of ketone (10) with sodium cyanoborohydride gave chiefly the 5-equatorial alcohol (14) as would be predicted by Barton's rule (Figure 6). In addition, a few percent of the isomeric 5-axial alcohol (15) was also isolated. Degradation of 5-alcohol (14) with concentrated hydrobromic acid followed by chromatography over an ion exchange resin resulted in the isolation of 2,6-dideoxy-D-streptamine (16). This material proved to be identical with a known sample prepared by independent synthesis and obtained from Dr. S. D. Gero of the Institut de Chimie des Substances Naturelles (2).

Thus, the configuration of the 5-hydroxyl in (14) is unequivocally equatorial or β as shown. Hydrogenation of ketone

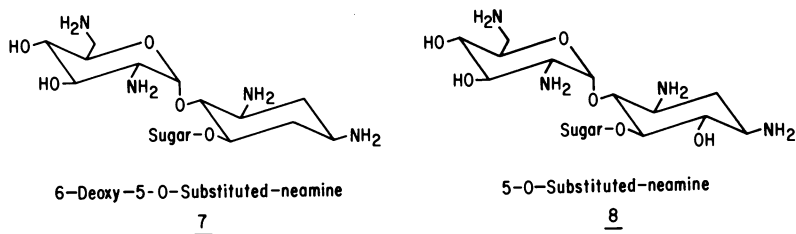


Figure 3.

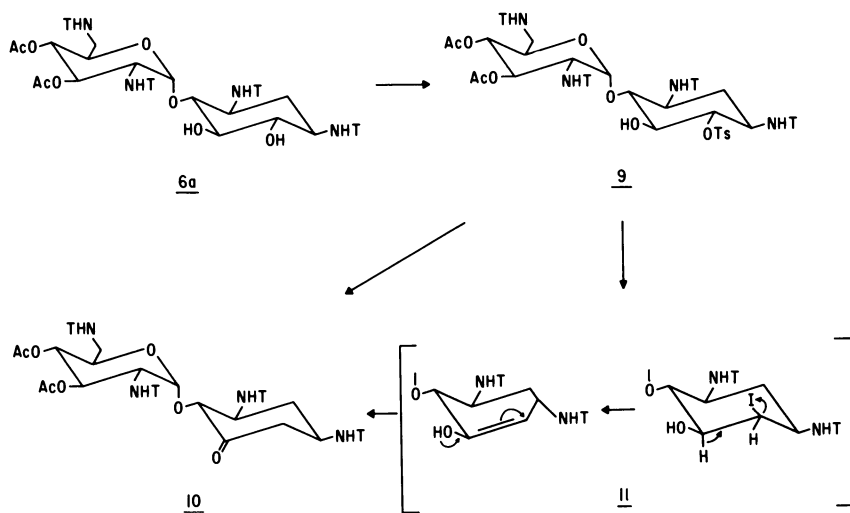


Figure 4.

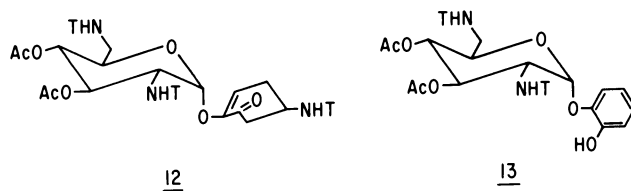


Figure 5.

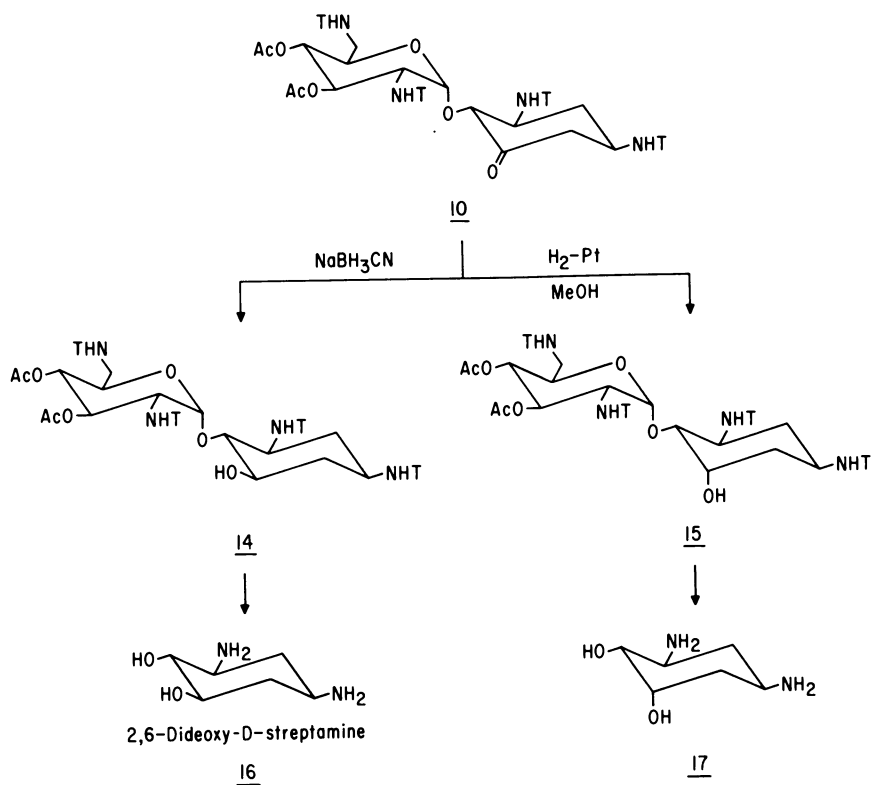


Figure 6.

(10) over platinum in methanol solution gave primarily the axial alcohol (15). This hydrogenation was highly stereospecific though trace amounts of the 5- β -alcohol were isolated on chromatography. Degradation of axial alcohol (15) with hydrobromic acid afforded a diamino-tetradeoxyinositol (17) which was similar, but different, from its isomer (16).

The blocking groups on oxygen and nitrogen of compounds (14) and (15) were readily removed with alkali to give the epimeric pair of 6-deoxyneamines (18) and (19) (Figure 7). The antibacterial spectrum and potency of these two neamine derivatives were almost identical with that of neamine. This indicated that the presence of the 6-hydroxyl is not critical to antibacterial activity, nor must the configuration of the hydroxy at C-5 be equatorial as in neamine.

Maximum potency of neamine analogs is only realized when substitution is present at either O-5 or O-6 (11). Therefore, the 5-equatorial or natural alcohol (14) was glycosylated with 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide in the Koenigs-Knorr reaction (Figure 8). The β epimer (β at 1") (20 β) was isolated in 35% yield, while only 8% of the less desirable α epimer (20 α) was obtained. The blocking groups were readily removed with dilute alkali to afford 6-deoxyribostamycin (21 β) and its epimer (21 α) (Figure 9).

A similar pair of epimeric aminoglycosides (22 β) and (22 α) were prepared from the unnatural 5-axial alcohol (15) by glycosylation followed by hydrolysis. Thus we have the 4-isomeric 6-deoxyribostamycins to evaluate by *in vitro* antibacterial assay.

The *in vitro* antibacterial testing data for these aminoglycosides is tabulated in Figure 10. In the first column are listed the bacteria against which the compounds were tested. This spectrum contains a few Gram-positive bacteria, but it is weighted in favor of the more difficult gram-negative bacteria. The results of the assay are expressed as MIC values, the minimum concentration of the drug expressed in micrograms per ml which will completely inhibit growth of the given bacteria under conditions of the assay. In general, 6-deoxyribostamycin was the most potent. Its 5-epimeric analog, rather surprisingly, also showed significant activity. The 1"- α -epimers, given in the two columns on the right, were generally less active. This difference is less in the C-5 unnatural series than in the C-5 β or natural series.

One of our main objectives of this program was the preparation of aminoglycosides which possess useful activity *versus* pseudomonads. While 6-deoxyribostamycin has significant antibacterial potency, its MIC *vs Pseudomonas aeruginosa* is something less than satisfactory. The ineffectiveness of many aminoglycosides to pseudomonads is due to the production of inactivating enzymes by these bacteria (10). The 3'-hydroxyl group is one of the prime sites for attack by phosphorylating enzymes. Aminoglycosides which lack a 3'-hydroxyl or in which the 3'-hydroxyl

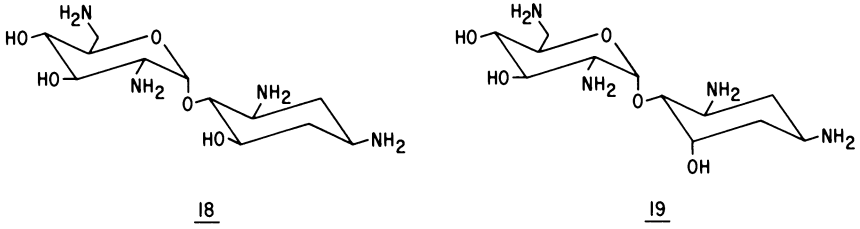


Figure 7.

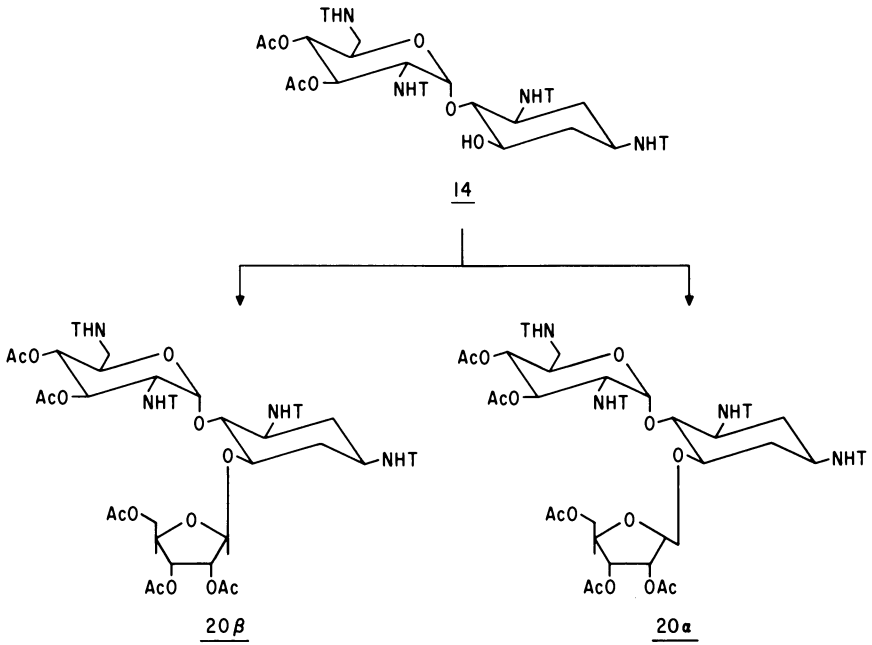


Figure 8.

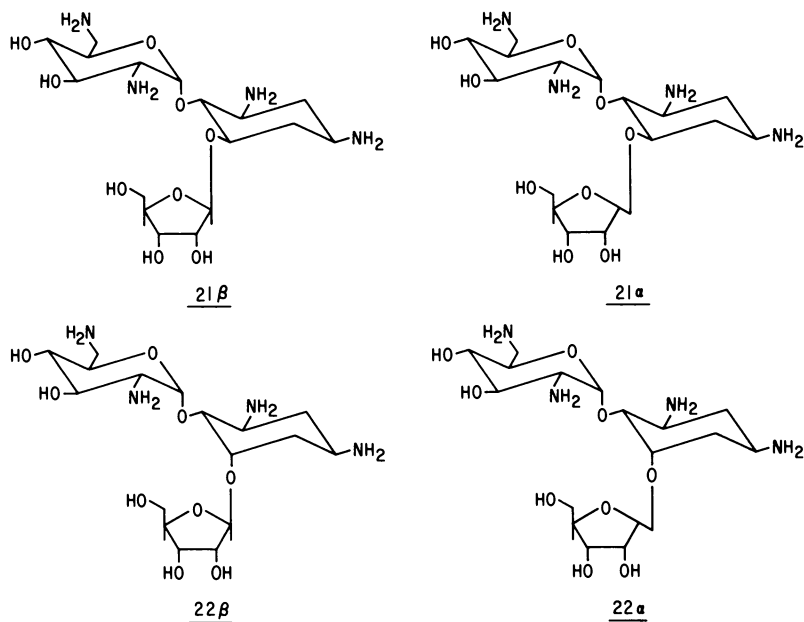


Figure 9. Isomeric 6-deoxyribostamycins

Organism	6-Deoxy-ribostamycin	6-Deoxy-5-epi-ribostamycin	6-Deoxy- α -ribostamycin	6-Deoxy-5-epi- α -ribostamycin
<i>S. aureus</i> UC 76	31.2	250	125	31.2
<i>S. pyogenes</i> UC 152	3.9	31.2	31.2	31.2
<i>S. faecalis</i> UC 694	500	1000	>500	1000
<i>S. pneumoniae</i> UC 41	15.6	31.2	125	31.2
<i>E. coli</i> UC 45	31.2	125	250	31.2
<i>K. pneumoniae</i> UC 58	2.0	15.6	15.6	15.6
<i>S. schottmuelleri</i> UC 126	7.8	62.5	125	31.2
<i>Ps. aeruginosa</i> UC 95	>500	1000	>500	1000
<i>P. vulgaris</i> UC 93	31.2	62.5	500	500
<i>P. mirabilis</i> A-63	125	500	>500	1000
<i>S. marcescens</i> UC 131	62.5	62.5	500	31.2
<i>S. flexneri</i> UC 143	31.2	125	500	125
<i>S. typhi</i> TG-3	15.6	31.2	62.5	31.2

Figure 10. Antibacterial activities of 6-deoxyribostamycins (minimum inhibitory concentration mcg/mL)

has been chemically removed, possess much greater potency to pseudomonads than those containing this group. Therefore, removal of the 3'-hydroxyl, or more simply the removal of both the 3'- and 4'-hydroxyls, was an attractive means to increase potency *vs* pseudomonads (11). The steps for removal of the 3',4'-hydroxyl using well-documented chemistry are outlined in Figure 11 (12, 13). Diol (23), whose preparation was described earlier, was converted to dimesylate (24) in high yield. When treated with potassium iodide and zinc in DMF, unsaturated compound (25) was obtained. This material was catalytically hydrogenated to form diol (26). In the manner previously described, this diol was selectively monotosylated to the 6-tosylate (27), (Figure 12). Conversion to ketone (28) and reduction proceeded smoothly, though the reduction was less stereospecific than in the 3',4'-diacetoxy series. The isomeric alcohols (28 β) and (29 α) were then glycosylated and saponified to complete the preparation of the two pairs of epimeric 3',4',6-trideoxyribostamycins shown in Figure 13. *In vitro* antibacterial testing data for these compounds using the same spectrum of organisms as shown before are outlined in Figure 14. Once again the most active is the 5 β -1'' β or natural isomer, 3',4',6-trideoxyribostamycin. Note particularly the MIC value *vs Pseudomonas aeruginosa* which is 2 mcg/ml compared with a value of greater than 500 in the 3',4'-dihydroxy series. A comparison of *in vitro* activity of 3',4',6-trideoxyribostamycin and 6-deoxyribostamycin with ribostamycin and kanamycin is shown in Figure 15.

Note that MIC values for 3',4',6-trideoxyribostamycin are competitive with those for ribostamycin and kanamycin. Against one strain of *Pseudomonas aeruginosa* shown here, 3',4',6-trideoxyribostamycin is much more potent than the older antibiotics. The MIC values for 3',4',6-trideoxyribostamycin *vs* a group of resistant pseudomonads and *Staphylococcus aureus* clinical isolates are shown in Figure 16. 3',4',6-Trideoxyribostamycin is more effective *vs* various pseudomonads and some staphylococci than kanamycin, but less effective than gentamicin.

In vivo testing data for 3',4',6-trideoxyribostamycin against several organisms when administered subcutaneously in the mouse is given in Figure 17. Each value is a CD₅₀, the dose of compound, expressed in mg/mg, which protects 50% of the mice from a lethal infection of the given organism. Whereas 3',4',6-trideoxyribostamycin was somewhat less effective than ribostamycin *vs K. pneumoniae* it was much more potent *vs Ps. aeruginosa*. Trideoxyribostamycin was only about 1/4 or 1/6 as potent as gentamicin against *Ps. aeruginosa* in this assay and also less effective against *E. coli*. However, as shown in Figure 18, 3',4',6-trideoxyribostamycin possesses only about 1/5 the acute toxicity of gentamicin when assayed in the mouse. Thus the therapeutic index for 3',4',6-trideoxyribostamycin against

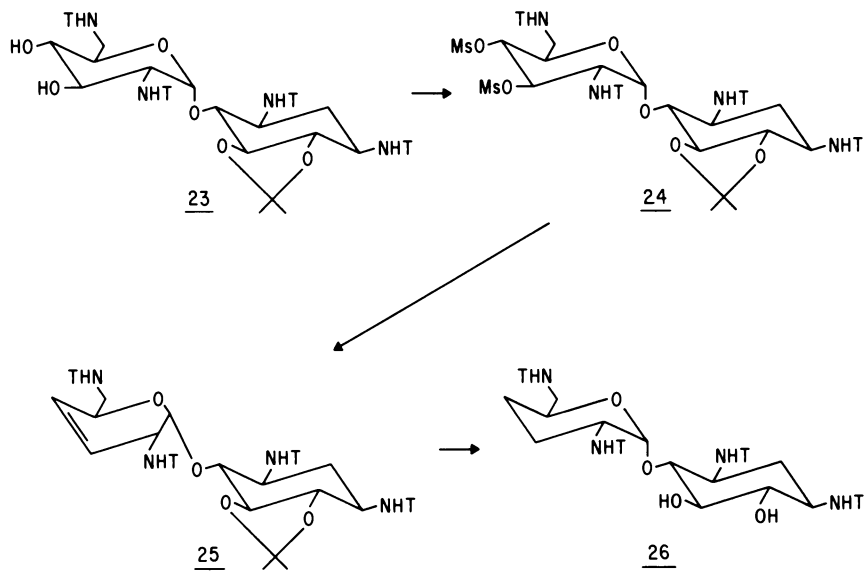


Figure 11.

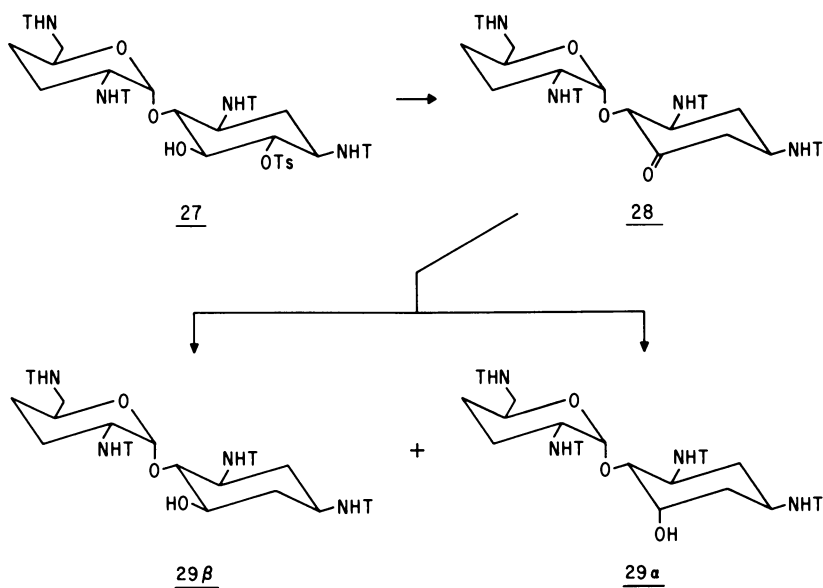


Figure 12.

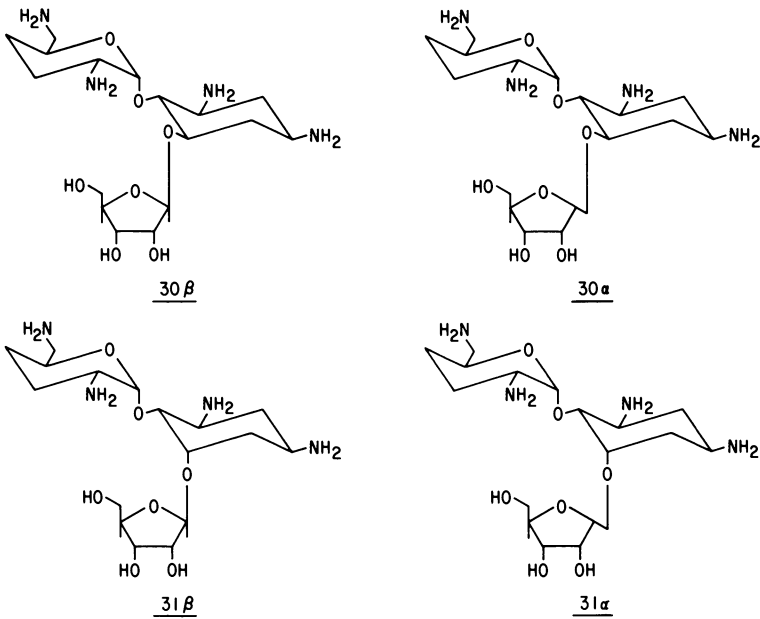


Figure 13. Isomeric 3',4',6'-trideoxyribostamycins

Organism	3',4',6-Tri-deoxyribo-stamycin	3',4',6-Tri-deoxy-5-epi ribo-stamycin	3',4',6-Tri-deoxy- α -ribo-stamycin	3',4',6-Tri-deoxy-5-epi- α ribo-stamycin
<i>S. aureus</i> UC 76	15.6	31.2	7.8	62.5
<i>S. pyogenes</i> UC 152	2.0	15.6	7.8	3.9
<i>S. faecalis</i> UC 694	250	>500	>500	>250
<i>S. pneumoniae</i> UC 41	15.6	125	31.2	
<i>E. coli</i> UC 45	31.2	125	125	>250
<i>K. pneumoniae</i> UC 58	2.0	15.6	15.6	15.6
<i>S. schottmuelleri</i> UC 126	3.9	62.5	125	62.5
<i>Ps. aeruginosa</i> UC 95	2.0	31.2	62.5	31.2
<i>P. vulgaris</i> UC 93	7.8	62.5	125	125
<i>P. mirabilis</i> A-63	125	>500	500	>250
<i>S. marcescens</i> UC 131	7.8	62.5	31.2	62.5
<i>S. flexneri</i> UC 143	31.2	250	125	125
<i>S. typhi</i> TG-3	7.8	125	62.5	31.2

Figure 14. Antibacterial activities of 3'4'6-trideoxyribostamycins (minimum inhibitory concentration mcg/mL)

Organism	3',4',6-Tri-deoxyribo-stamycin	6-Deoxyribo-stamycin	Ribo-stamycin	Kanamycin
<i>S. aureus</i> UC 76	15.6	31.2	15.6	7.8
<i>S. pyogenes</i> UC 152	2.0	3.9	7.8	15.6
<i>S. faecalis</i> UC 694	250	500	250	250
<i>S. pneumoniae</i> UC 51	15.6	15.6	7.8	125
<i>E. coli</i> UC 45	31.2	31.2	7.8	3.9
<i>K. pneumoniae</i> UC 58	2.0	2.0	1.0	1.0
<i>S. schottmuelleri</i> UC 126	3.9	7.8	3.9	2.0
<i>Ps. aeruginosa</i> UC 95	2.0	500	500	62.5
<i>P. vulgaris</i> UC 93	7.8	31.2	15.6	15.6
<i>P. mirabilis</i> A-63	125	125	62.5	62.5
<i>S. marcescens</i> UC 131	7.8	62.5	31.2	7.8
<i>S. flexneri</i> UC 143	31.2	31.2	15.6	15.6
<i>S. typhi</i> TG-3	7.8	15.6	7.8	2.0

Figure 15. *In vitro* comparison of 3',4',6-trideoxyribostamycins and 6-deoxyribo-stamycin with ribostamycin and kanamycin (minimum inhibitory concentration mcg/mL)

Organism (Resistant)	3',4',6-Tri-deoxyribostamycin	Gentamicin	Kanamycin
<i>Ps. aeruginosa</i> 6436	31.2		>250
6437	250		15.6
3680	1.0		31.2
3681	2.0		15.6
3682	3.9		62.5
3683	2.0		15.6
<i>S. aureus</i> 6686	250	125	>250
6687	2.0	0.25	>250
6688	31.2	3.9	250
6691	>250	3.9	>250
6695	>250	3.9	>250

Figure 16. *In vitro* testing vs. clinical isolates (minimum inhibitory concentration mcg/mL)

<u>Compound</u>	
3',4',6-Trideoxyribostamycin Sulfate	381 mg/kg
Gentamicin Sulfate	85 mg/kg

Figure 17. *In vitro* antibacterial testing (mouse protection assay (CD_{50} mg/kg · kg, SQ, mice))

Organism	3',4',6-Trideoxy- ribostamycin Sulfate	Ribostamycin Sulfate	Gentamicin Sulfate
<i>K. pneumoniae</i> UC 58	28.3 (19.6-40.7)	10.7 (8.2-14.0)	-
<i>Ps. aeruginosa</i> UC 231	82 (54-136) 121 (79-187)	-	19 (13-27)
<i>S. aureus</i> UC 76	23 (16.5-32)	-	0.51 (0.35-0.72)
<i>E. Coli</i> UC 311 UC 45	21 (14-32) 20 (15-26)	-	2.5 1.09 (0.88-1.34)

Figure 18. Acute toxicity (LD_{50} , IV-mouse)

Ps. aeruginosa in the mouse is not too far removed from that of gentamicin.

The synthesis of a family of 6-deoxyribostamycins has been described and *in vitro* and *in vivo* antibacterial testing data have been reviewed. The key step in the preparation of these semisynthetic antibiotics is the unique preparation of 3',4'-di-O-acetyl-5,6-dideoxy-5-oxo-1,2',3,6'-tetrakis-N-(trifluoroacetyl)neamine (10) from 3',4'-di-O-acetyl-6-O-tosyl-1,2',3,6'-tetrakis-N-(trifluoroacetyl)neamine (9). This intermediate has potential use for the preparation of other 5-substituted-6-deoxy-neamines.

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Literature Cited

1. Reid, R. J., Mizsak, S. A., Reineke, L. M., Zurenko, G. E., and Magerlein, B. J., Abstracts of Papers, 176th ACS National Meeting, Miami Beach, Florida, September 11-14, 1978, Medi 18.
2. Cleophax, J., Gero, S. D., Leboul, J., Akhtar, M., Barnett, J. E. G., and Pearce, C. J., J. Amer. Chem. Soc. (1976) **98**, 7110.
3. Suami, T., Nishiyama, S., Ishikana, Y., and Katsura, S., Carbohyd. Res. (1977) **53**, 239.
4. Cleophax, J., Delaumeny, J. M. Gero, S. D., Rolland, A., and Rolland, N., J. Chem. Soc., Chem. Commun. (1978) 773.
5. Hayashi, T., Iwaoka, T., Takeda, N., and Ohki, E., Chem. Pharm. Bull. (1978) **26**, 1786.
6. Umezawa, S., Tsuchiya, T., Jihihara, T., and Umezawa, H., J. Antibiot. (1971) **24**, 711.
7. Hasegawa, A., Nishimura, D., and Nakajima, M., Carbohyd. Res. (1973) **30**, 319.
8. Suami, T., Nishiyama, S., Ishikawa, Y., and Katsura, S., Carbohydr. Res. (1976) **52**, 187.
9. Tanabe, M., Yasuda, D. M., and Detre, G., Tetrahedron Lett. (1977) 3607.
10. Benveniste, R., and Davies, J., Antimicrobial Agents and Chem. (1973) **4**, 402.
11. Price, K. E., Godfrey, J. C., and Kauaguchi, in D. Perlman, Ed., "Structure-Activity Relationships Among the Semisynthetic Antibiotics," pp. 239-395, Academic Press, New York (1977).
12. Jikihara, T., Tsuchiya, T., Umezawa, S., and Umezawa, H., Bull. Chem. Soc., Japan (1973) **46**, 3507.
13. Umezawa, S., Umezawa, H., Okazaki, T., and Tsuchiya, T., Bull. Chem. Soc., Japan (1972) **45**, 3624.

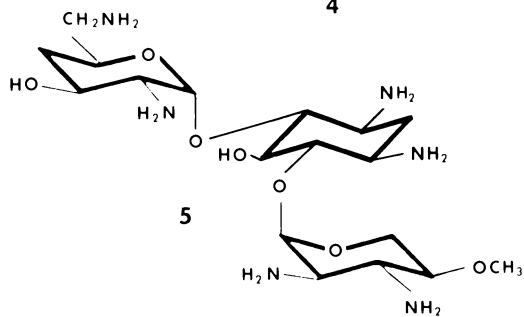
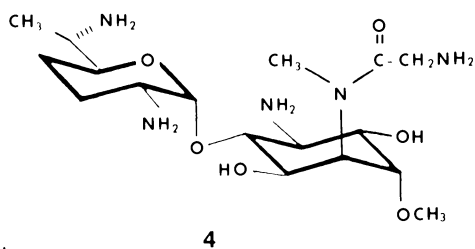
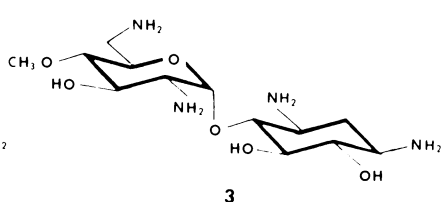
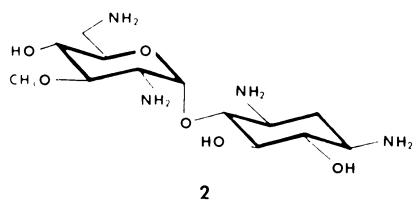
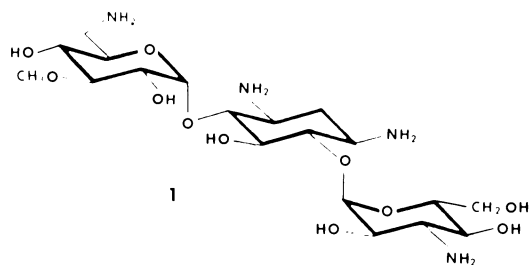
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The Effect of *O*-Methylation on the Activity of Aminoglycosides

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Following the discovery by Hamao Umezawa (1) and co-workers that certain aminoglycoside resistant organisms of clinical origin owed their resistance to an R-factor mediated ability to phosphorylate kanamycin and neomycin at the 3'-hydroxyl group chemical modification of these antibiotics was directed at possible ways of overcoming this inactivation. The 3'-*O*-methyl and 3'-deoxy derivatives of kanamycin A were prepared by glycosidation of a suitably protected pseudodisaccharide with appropriately substituted glucopyranosyl chlorides (2). The 3'-*O*-methylkanamycin 1 obtained was a 1:1 mixture of anomers at the 1' position and was found to be essentially devoid of antibacterial activity whereas the 3'-deoxy derivative, obtained as the α -glycoside was found to have strong antibacterial activity against strains of *Escherichia coli* and *Pseudomonas aeruginosa* resistant to the parent antibiotic. Similar preparations of 3'-*O*-methylneamine 2 and 4'-*O*-methylneamine 3 (3) by the same group led to the same disappointing lack of antibacterial activity. Subsequently the wide variety of chemical modifications of aminoglycoside antibiotics carried out in many laboratories have tended to avoid *O*-alkylations and to emphasize deoxygenation. It was therefore somewhat surprising when two different families of aminoglycosides discovered under a joint research project between Abbott Laboratories and the Kyowa Hakko Kogyo Company should have highly active components each of which contain an *O*-methyl group in their structures. Fortimicin A 4 is the most active of a group of atypical pseudo-disaccharides (4) many of the members of which carry an *O*-methyl substituent on the cyclitol. Seldomycin factor 5 5 (5) is the most active of a family of aminoglycosides produced by *Streptomyces hofunensis* and possesses an *O*-methyl group at the 4" position. This antibiotic is immediately recognized as a close relative of the kanamycin-gentamicin group of aminoglycosides and it was decided to investigate the effect of a 4"-*O*-methyl group on the activity of this family of antibiotics.



The gentamicins $\delta\alpha\text{-}\zeta$ and sagamicin $\delta\alpha$ were a particularly attractive subgroup for \underline{O} -methylation at this position. They possess two secondary hydroxyl groups at C-5 and C-2" and a tertiary hydroxyl at the C-4" position albeit in the epimeric stereochemistry to that of the methoxy group in seldomycin factor 5 ζ .

The general approach shown in Scheme 1 was adopted. The amino groups and the two secondary hydroxyl were to be protected and the product treated with the Goldman-Albright oxidation reagent to convert the tertiary hydroxyl into a methylthiomethyl ether (6). Desulfurization of this would result in the desired 4"- \underline{O} -methyl derivative which on deprotection would give the required product. This scheme was executed using gentamicin Cla $\delta\alpha$ as substrate and acetyl as the \underline{O} and \underline{N} protecting group. Selectivity in preparing the required hepta-acetyl derivative was less than expected. The tertiary hydroxyl was relatively easily acetylated and an octa-acetyl and a non-acetyl product, the other, in which the 6'-amine had been converted into an imide nitrogen bearing two acetyl groups, were major contaminants in the initial product. These were separated by silica gel chromatography. The hepta-acetyl gentamicin Cla was converted in good yields to 4"- \underline{O} -methylgentamicin Cla by the steps outlined. The deprotection was by a simple barium hydroxide hydrolysis. The 4"- \underline{O} -methylgentamicin Cla was characterized by an OCH_3 singlet at $\delta 3.67$ in the proton magnetic spectrum. PMR spectrum determined in deuterium oxide with tetramethylsilane as external reference quoted unadjusted.

The C-5 hydroxyl groups of 4,6-di- \underline{O} -substituted 2-deoxystreptamine antibiotics is known to be sterically hindered and under milder conditions of acetylation it was possible to prepare a hexa-acetyl derivative. Following the same reactions led to the sequence in Scheme 2. This substrate gave the 5-oxo compound which was stereoselectively reduced during the Raney Nickel reduction to give a 5-epi-hydroxy 4"- \underline{O} -methyl derivative. Barium hydroxide hydrolysis of this led to 5-epi-4"- \underline{O} -methylgentamicin Cla.

The chemical shifts from the carbon magnetic resonance spectra of these compounds and their parent antibiotic are shown in Table 1. The resonances assigned to the carbons of the purpurosamine ring are virtually identical for all three compounds as are the resonances of the carbons of the 2-deoxystreptamine ring of gentamicin Cla and the 4"- \underline{O} -methyl derivative. The three carbons, C-3", C-5", and the \underline{C} -methyl carbon show the expected upfield β shift following alkylation of the 4"hydroxyl while the 4"-carbons show a downfield β shift. The methoxyl carbon resonance occurs at 49.7 ppm downfield from tetramethylsilane. The resonances of the carbons of the garosamine ring of 4"- \underline{O} -methylgentamicin Cla are indistinguished from those of its 5-epimer. The carbon resonances for the 2-deoxystreptamine ring of 5-epi-4"- \underline{O} -methylgentamicin Cla

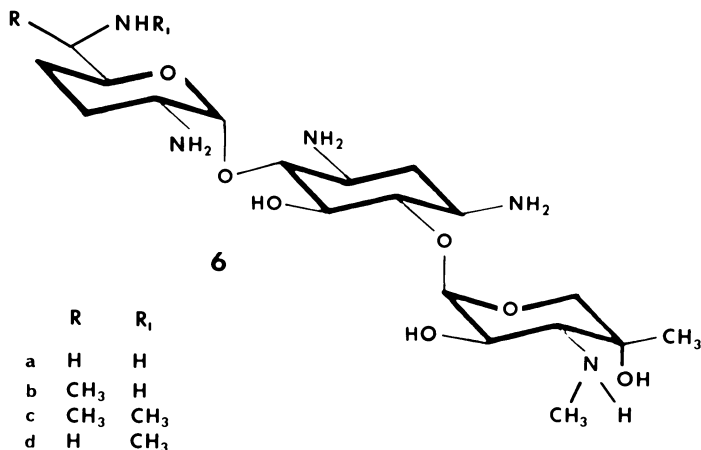
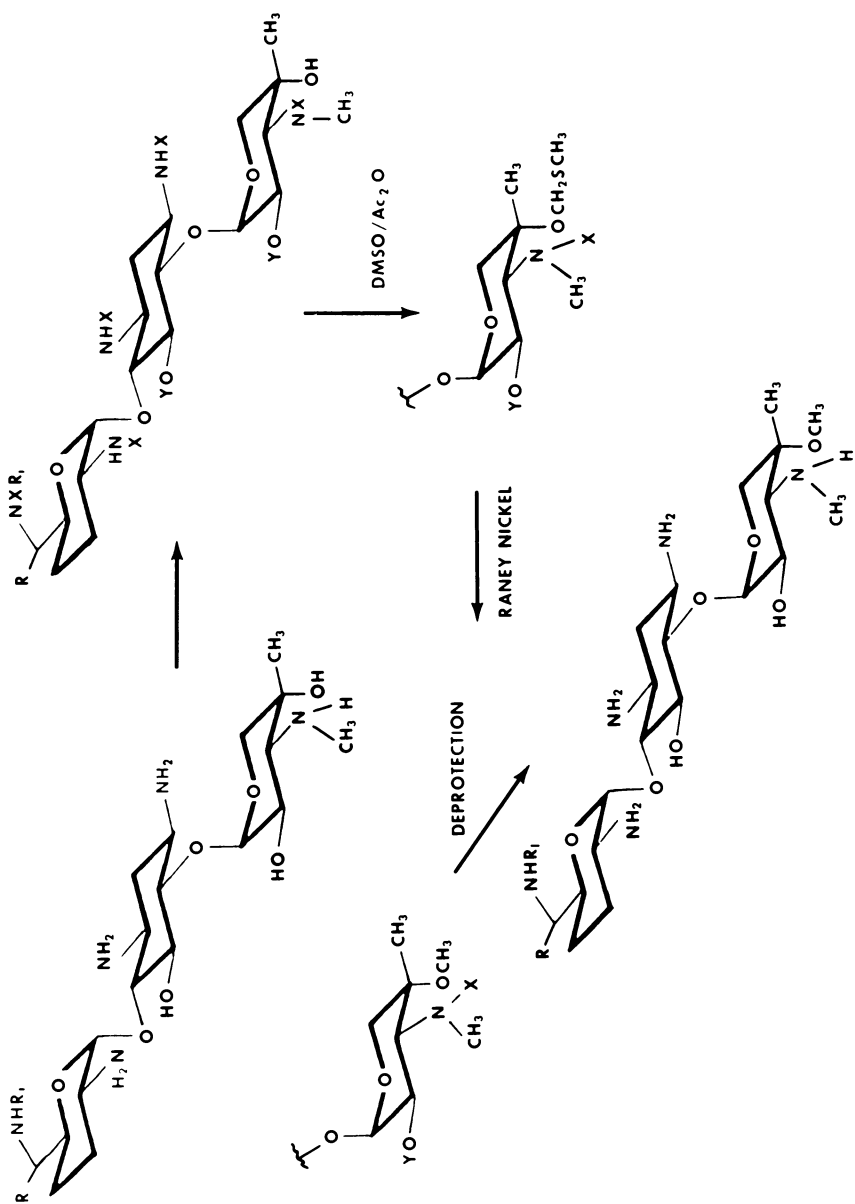


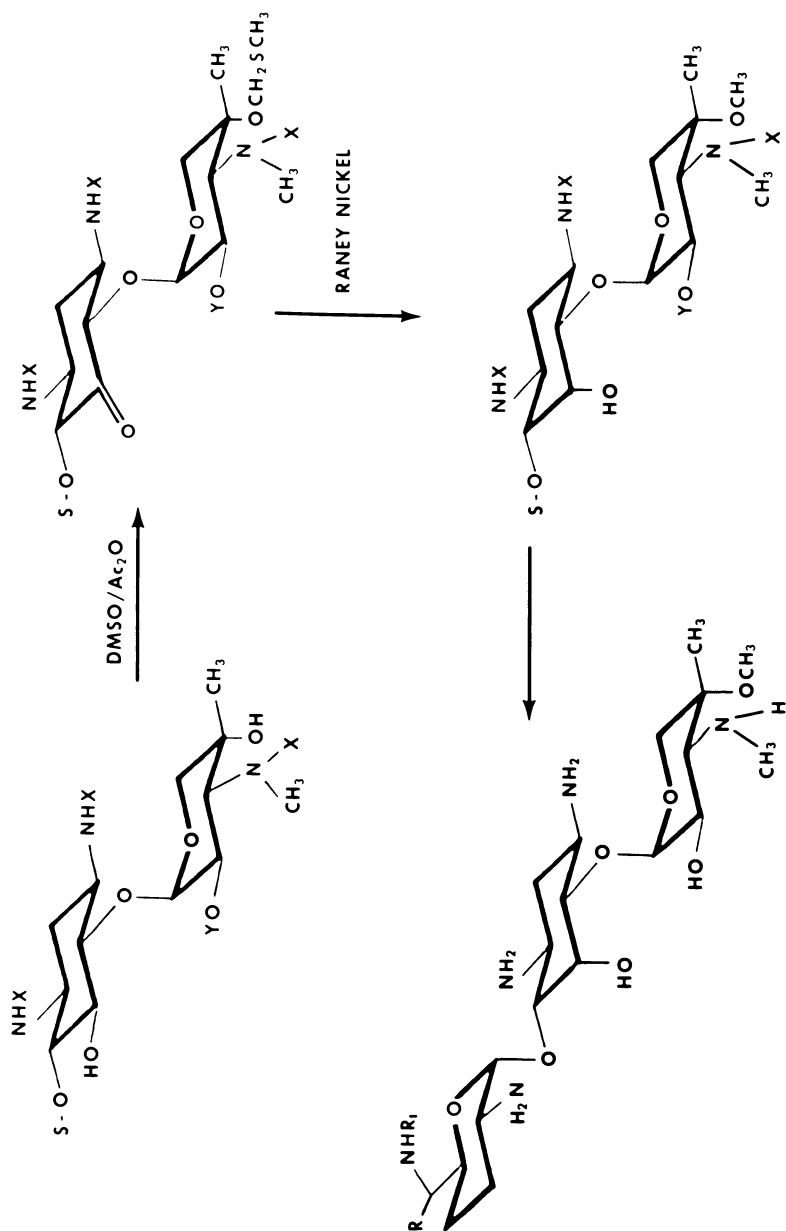
TABLE 1

Carbon Magnetic Resonance Spectra

	<u>Gentamicin Cla</u>	<u>4"-O-Methyl-gentamicin Cla</u>	<u>5-Epi-4"-O-methyl-gentamicin Cla</u>
C-1'	102.2	101.9	96.2
C-2'	51.0	50.8	50.3
C-3'	27.1	26.9	27.2
C-4'	28.5	28.3	28.3
C-5'	71.5	71.2	70.7
C-6'	46.1	45.9	45.7
C-1	51.7	51.5	48.2
C-2	36.7	36.6	36.7
C-3	50.6	50.4	47.5
C-4	88.3	87.9	85.8
C-5	75.4	75.3	68.7
C-6	87.8	86.6	79.6
C-1"	101.3	101.0	102.3
C-2"	70.2	70.0	70.1
C-3"	64.4	62.2	62.0
C-4"	73.3	77.7	77.7
C-5"	68.7	65.3	65.1
CCH ₃	23.0	17.4	17.3
NCH ₃	38.0	38.0	38.0
OCH ₃	---	49.7	49.7



Scheme I



Scheme 2

show an upfield shift from those of other normal isomer as would be associated with inversion from an equatorial to an axial substituent. In an attempt to improve the selectivity of the *O*-protection step, carboethoxy groups were chosen as the *N*-protecting group in the synthesis starting with sagamicin 6d. The per-*N*-carboethoxy derivative was prepared quantitatively and *O*-acetylated to give the desired 5,2"-di-*O*-acetyl-penta-*N*-carboethoxysagamicin. This was converted in good yield to the 4"-*O*-methyl derivative by treatment with dimethylsulfoxide/acetic anhydride followed by Raney Nickel desulfurization. Barium hydroxide removal of the protecting groups gave the desired 4"-*O*-methylsagamicin and a major by-product 4"-*O*-methylsagamicin-1*N*,3*N*-cyclic ureide **7**.

The carbon magnetic resonance spectral data of these compounds and the parent antibiotic are shown in Table 2. Again no significant difference occurs in the resonances assigned to the *N*-methylpurpurosamine ring, and the resonances of the carbons of the garosamine ring of 4"-*O*-methylsagamicin shows the shifts from their position in the spectrum of the parent which would be expected from 4"-*O*-alkylation. The spectrum of the 2-deoxystreptamine ring of the cyclic ureide shows a considerable upfield shift of all carbons in comparison to their position in the spectrum of the parent. This transformation has flipped the chair form of this ring from that conformation with all five substituents equatorial to that with all five axial. The multiplicity of the C-2 resonance at 18.2 ppm was confirmed as a triplet by ORSFD experiments.

The antibacterial activity of these compounds is shown in Table 3. This assay is run under conditions, (sensitive organisms, low nutrients, and high pH) which maximize the apparent activity of the antibiotics. The data show that the 4"-*O*-methyl derivatives of gentamicin Cla and sagamicin are from 2 to 4 fold less active than the parent antibiotics. Epimerization at the 5-position, as has been shown in the case of sisomicin (**7**), is beneficial. This change restored activity lost by 4"-*O*-methylation and 5-*epi*-4"-*O*-methylgentamicin Cla is as active as the parent antibiotic. Thus, methylation of a 4"-axial hydroxyl group of the gentamicins is detrimental to activity albeit much less so than methylation of the 2' and 3' equatorial hydroxyl groups of neamine and kanamycin. It is interesting to note that deoxygenation of gentamicin Cl **8**_a at the 4" position has been reported by Mallams and co-workers (**8**) to give a compound inactive except for some very weak activity against sensitive Gram positive strains. Thus at this particular position, *O*-methylation would appear to be much less detrimental than deoxygenation. The 4"-equatorial methoxy group of seldomycin factor 5 **8**_a was removed by the Monneret reaction (**9**) using lithium in ethylamine to give *O*-demethylseldomycin factor 5. The same reaction was carried out on 3'-deoxyseldomycin factor 5 **8**_b, a semisynthetic

TABLE 2
Carbon Magnetic Resonance Spectra

	<u>Sagamicin</u>	<u>4''-O-Methyl-</u> <u>sagamicin</u>	<u>4''-O-Methylsaga-</u> <u>micin</u> <u>1N,3N-cyclic ureide</u>
C-1'	101.2	101.3	100.4
C-2'	50.5	50.5	50.3
C-3'	26.5	26.5	27.0
C-4'	28.5	28.6	28.5
C-5'	68.1	68.3	68.2
C-6'	55.1	55.1	54.9
NCH ₃	35.3	35.3	35.2
C-1	51.5	51.5	47.9
c-2	36.5	36.5	18.2
C-3	50.3	50.3	45.9
C-4	87.7	87.6	78.7
C-5	75.4	75.3	69.3
C-6	86.9	87.0	77.9
C-0	----	----	159.2
C-1''	101.2	101.0	98.8
C-2''	70.1	69.9	70.5
C-3''	64.2	62.0	62.1
C-4''	73.1	77.7	77.5
C-5''	68.5	65.2	65.6
CCH ₃	22.5	17.3	17.4
NCH ₃	37.7	37.9	38.3
OCH ₃	----	49.7	49.7

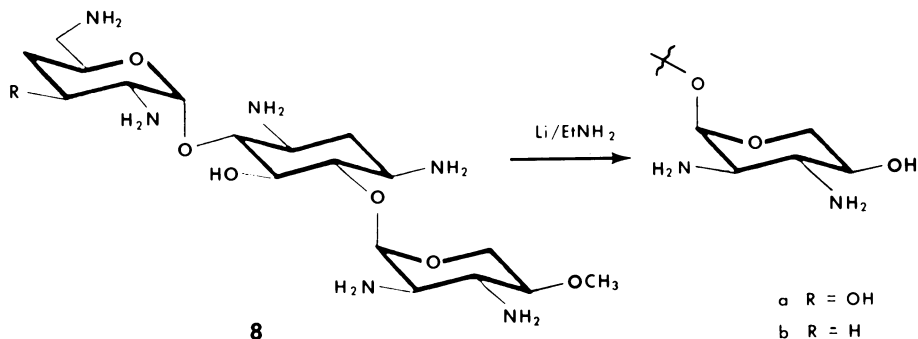


TABLE 3

		Antibacterial Activities (MICs, ug/ml)			
		Genta micin Cla	4"-O-Methyl- gentamicin CLA	5-epi-4"- O-Methyl- gentamicin Cla	Saga- micin Methyl- sagamicin
<u>Staphylococcus aureus</u> ATCC 6538P		<0.005	<0.005	<0.005	<0.005
<u>Escherichia coli</u> ATCC 26		0.01	0.01	0.02	0.01
<u>Escherichia coli</u> R3		3	10	1.6	10
<u>Pseudomonas aeruginosa</u> BMH #1		0.2	0.6	0.2	1.2
<u>Proteus vulgaris</u> ATCC 6897		0.04	0.08	0.04	0.04
<u>Shigella sonnei</u> ATCC 9290		0.04	0.08	0.04	0.04
<u>Salmonella typhi</u> ATCC 9992		0.02	0.02	0.01	0.08
<u>Klebsiella pneumoniae</u> ATCC 10031		0.01	0.04	0.005	0.08

TABLE 4

Carbon Magnetic Resonance Spectra

	<u>Seldomycin Factor 5</u>	<u>O-Demethyl seldomycin Factor 5</u>	<u>3'-Deoxy seldomycin Factor 5</u>	<u>O-Demethyl-3'- deoxyseldomycin Factor 5</u>
C-1'	102.3	101.8	102.2	101.0
C-2'	57.7	57.6	50.8	51.1
C-3'	69.1	68.7	27.0	26.2
C-4'	36.4	36.8	28.4	28.0
C-5'	71.3	70.7	71.6	70.6
C-6'	45.7	45.2	45.9	45.3
C-1	51.1	51.1	51.2	50.6
C-2	36.5	36.3	36.7	36.4
C-3	50.1	50.1	59.3	50.2
C-4	88.1	87.0	88.2	86.9
C-5	75.1	75.2	75.2	75.2
C-6	87.0	86.9	87.2	87.1
C-1''	100.1	100.3	100.2	100.3
C-2''	56.2	56.1	56.3	56.1
C-3''	54.8	56.1	54.9	56.1
C-4''	80.3	69.9	80.3	70.0
C-5''	60.8	63.2	60.9	63.3
OCH ₃	58.7	----	58.8	----

TABLE 5

MIC's (ug/ml) of O-Demethylselodomycin Factor 5 and the 3'-Deoxy Derivative

	3'-OH-4''- OCH ₃	3'-OH- 4''-OH	3'-H-4''- OCH ₃	3'-H-4''- OH
<u>Bacillus subtilis</u> U. of Ill. 10707	<0.01	<0.01	<0.01	<0.01
<u>Staphylococcus aureus</u> ATCC 6538P	0.04	0.04	0.04	0.02
<u>Streptococcus faecalis</u> ATCC 10541	10	20	20	>20
<u>Escherichia coli</u> ATCC-26 R3	0.31	0.31	0.16	0.16
R5	>20	>20	1.25	2.5
R16	>20	>20	0.08	0.8
R19(AAC-3-I)	2.5	5	0.04	0.4
76-2	2.5	0.16	2.5	0.16
NR-79	0.63	2.5	0.16	0.16
	1.6	0.31	0.16	0.16
<u>Klebsiella pneumoniae</u> ATCC 10031	0.04	0.04	0.04	0.04
<u>Proteus vulgaris</u> ATCC 6897	0.31	0.31	0.31	0.31
<u>Providencia stuartii</u> ATCC 25825 164	1.25 10	1.25 20	2.5 >20	2.5 20
<u>Pseudomonas aeruginosa</u> BMH #1 KY-8512 PST	5 10 >20	5 5 >20	1.25 2.5 >20	0.63 1.25 >20

derivative of seldomycin factor 5. The products are characterized by the absence of a methoxyl peak in their proton magnetic resonance spectra as shown in Table 4. The resonances assigned to the carbons of the hexose and the 2-deoxystreptamine ring are essentially the same in the spectra of each O-demethyl derivative and its corresponding parent. Those assigned to the carbons of the pentose moiety of the O-demethyl compounds show the expected large upfield shift at C-4" and small downfield β shifts at C-3" and C-5" associated with dealkylation at the 4" oxygen.

The antibacterial activity of these compounds is shown in Table 5. No significant difference is seen between the activities of the parents and their O-demethyl derivatives with the exception of that against the R-19 strain of Escherichia coli. This organism is resistant to gentamicins, sagamicin, seldomycin factor 5 and 3'-deoxyseldomycin factor 5, but sensitive to kanamycin, tobramycin, O-demethylseldomycin factor 5 and 3'-deoxy-O-demethylseldomycin factor 5. This organism is known to possess an AAC-3-type I enzyme which, these results suggest, is unable to function in the presence of an equatorial hydroxyl group at the 4"-position.

Thus O-methylation is shown to be somewhat detrimental at the axial hydroxyl group and of little consequence at the equatorial hydroxyl group at the C-4" position in this class of antibiotics.

Acknowledgement

The authors gratefully acknowledge Ms. Marianna Jackson and staff for antibacterial assays.

Literature Cited

1. Umezawa, H., Okanishi, M., Kondo, S., Hamana, K., Utahara, R., Malda, K., and Mitsuhashi, S., Science, **157** 1559-61 (1967).
2. Umezawa, S., Tsuchiya, T., Muto, R., Nishimura, Y., and Umezawa, H., J. Antibiotics, **24**, 274-5 (1971).
3. Umezawa, S., Tikhara, T., Tsuchiya, T., and Umezawa, H., J. Antibiotics, **25**, 322-4 (1972).
4. Egan, R. S., Stanaszek, R. S., Cirovic, M., Mueller, S. L., Tadanier, J., Martin, J. R., Collum, P., Goldstein, A. W., DeVault, R. L., Sinclair, A. C., Fager, E. E., and Mitscher, L. A., J. Antibiotics, **30**, 552-63 (1977).

5. McAlpine, J. B., Sinclair, A. C., Egan, R. S., DeVault, R. L., Stanaszek, R. S., Cirovic, M., Mueller, S. L., Goodley, P. C., Mauritz, R. J., Wideburg, N. E., Mitscher, L. A., Shirahata, K., Matsushima, H., Sato, S., and Iida, T., J. Antibiotics, 30, 39-49 (1977).
6. Albright, J. D., and Goldman, L., J. Amer. Chem. Soc., 87, 4214-6 (1965).
7. Waitz, J. A., Miller, G. H., Moss, Jr., E., and Chiu, P.J.S., Antimicrobial Agents and Chemotherapy, 13, 41-48 (1978).
8. Mallams, A. K., Vernay, H. F., Crowe, D. F., Detre, G., Tanabe, M., Yasuda, D. M., J. Antibiotics, 26, 782-783 (1973).
9. Monneret, C., Florent, J. C., Kabore, I., and Khuong-Huu, Q., J. Carb., Nucleosides and Nucleotides, 1, 161-168 (1974).

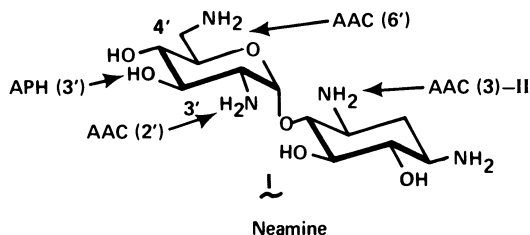
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The Synthesis and Biological Properties of 3'- and 4'-Thiodeoxyneamines and 4'-Thiodeoxykanamycin B

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The aminoglycosides are a clinically important class of antibiotics with broad activity against many strains of gram-negative bacteria. Concurrent with the extensive use of aminoglycosides, resistant organisms, many of which contain transferable R-factors, have become more prevalent. The plasmids in the resistant strains code for enzymes which inactivate various aminoglycosides by phosphorylation, acetylation or adenylation (1,2). The antibiotics can be rendered resistant to inactivation through appropriate structural modifications (1,2). For our purposes the pseudodisaccharide neamine (1), a component of



neomycin and kanamycin B, provided a model substrate for carrying out modifications with this objective. Although 1 is less active against bacteria than typical pseudotrisaccharides such as kanamycin or gentamicin, it is also less toxic (2). Such a

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pseudodisaccharide, suitably modified, might have useful activity of itself, or it might serve as a basis for the construction of appropriate pseudotrisaccharides. The principal mechanism for neamine inactivation is phosphorylation of its 3'-hydroxyl group, a reaction which has an important role in the development of resistance to neomycin and kanamycin. It has been demonstrated that removal of the oxygen function at the 3'-position results in antibiotics with enhanced activity against such resistant organisms (1,2). Along these lines, we have synthesized the 3'- and 4'-thio-3'- and 4'-deoxy- analogs of neamine and 4'-thio-4'-deoxykanamycin B as part of a more general program of modifying aminoglycoside antibiotics. These analogs were obtained by nucleophilic opening of the appropriate epoxy precursors using benzyl mercaptide.

Preparation of Epoxide Intermediates

Selective tosylation (3) of the 3'-hydroxyl group of 5,6-0-cyclohexylidene-tetracarboxymethoxy neamine, 2 (4), followed by treatment with sodium methoxide yielded the previously described (5) crystalline *allo*-epoxide 4 (Figure 1). Although the isomeric *galacto*-epoxide 7 (Figure 2) could be obtained by methoxide treatment of the 4'-tosylate, which in turn was isolated as a minor product from the tosylation of 2 (5), a more efficient route was needed for its large scale preparation. Reaction of 2 with excess benzoyl chloride in pyridine at low temperature yielded, along with some dibenzoate, the 3'-mono-benzoate 5[†]: yield, 68%; $[\alpha]_D^{25} +67.4^\circ$ (c 1, CHCl₃). The monoester was readily separated from the more soluble diester by precipitation from ether-petroleum ether. Since only one monoester could be detected in the product, any 4'-monobenzoate formed in the reaction mixture must have been benzoylated to the diester. Mesylation of 5 using methanesulfonyl chloride and triethylamine in methylene chloride at -10° gave 6[†]: yield, 86%; $[\alpha]_D^{25} +32.5^\circ$ (c 1, CHCl₃); nmr, 7.2-8.2 ppm (5H, m, aromatic), 2.9 ppm (3H, s, mesylate). On treatment with sodium methoxide, 6 gave *galacto*-epoxide 7[†]: yield, 81%; $[\alpha]_D^{25} +2.5^\circ$ (c 1, CHCl₃). Epoxide 7 was readily distinguishable from the isomer 4 by tlc (silica, acetonitrile-ether, 1:1) and HPLC (Microporasil[®], CHCl₃-MeOH, 95:5). Although none of these intermediates were crystalline, product 7, obtained by precipitation, was chromatographically homogeneous and its preparation was amenable to large scale work.

Opening of Epoxides with Benzyl Mercaptan

Galacto-epoxide 7 (Figure 3) was treated under nitrogen for 3 hr with two equivalents of benzyl mercaptide in refluxing

† Satisfactory combustion analyses, nmr and ir spectra were obtained for these compounds.

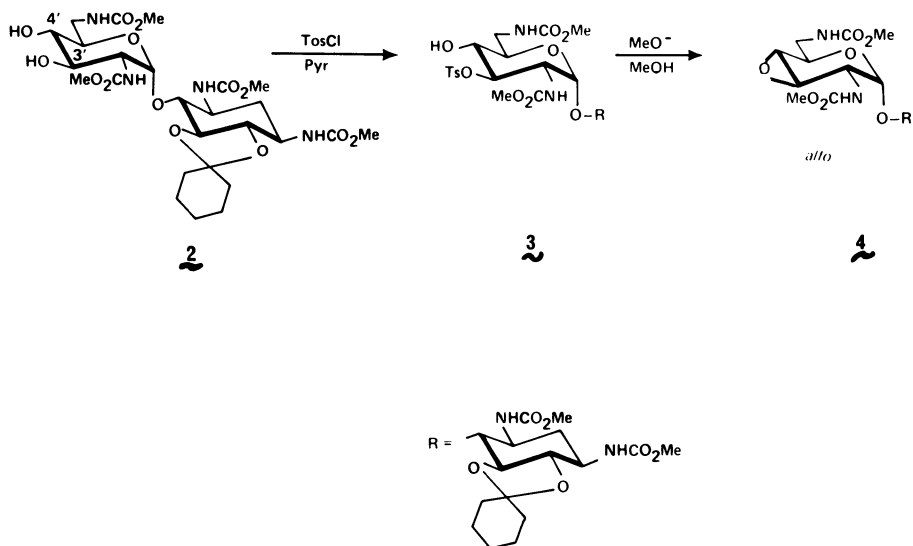


Figure 1.

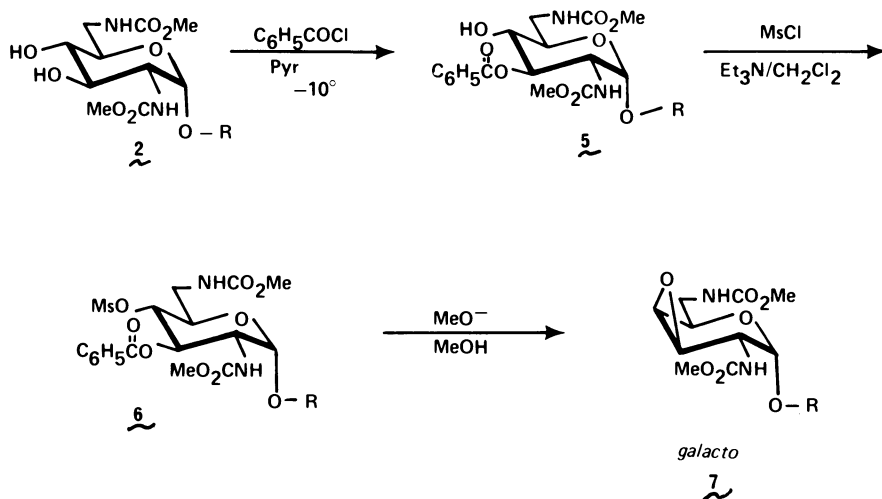


Figure 2.

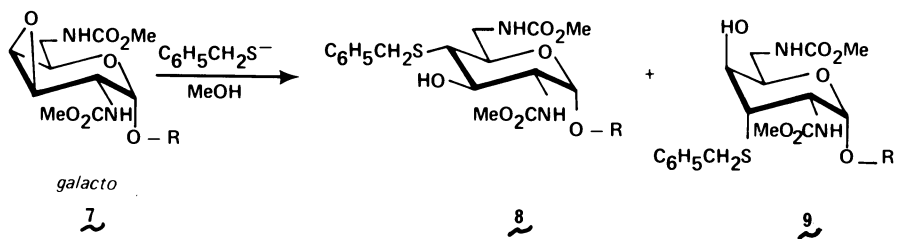


Figure 3.

Table I.
 CHEMICAL SHIFTS FOR ACETYL METHYLS
 (CDCl₃, TMS)

PARTIAL STRUCTURE	CHEMICAL SHIFT	
	3'	4'
<p style="text-align: center;">10</p>	2.00 ppm	2.03
<p style="text-align: center;">11</p>	2.08	2.14
<p style="text-align: center;">12</p>	2.06	
<p style="text-align: center;">13</p>		2.13

methanol to give a mixture of benzylthioethers **8** and **9** (total yield, 87%), from which was obtained crystalline **8**⁺: yield, 57%; m.p. 208–209° (from benzene); $[\alpha]_D^{25} +75.5^\circ$ (c 1, CHCl₃). Chromatography (silica, CHCl₃-MeOH, 99:1) of the mother liquors from the crystallization gave amorphous **9**⁺: yield, 26%; $[\alpha]_D^{25} +29.6^\circ$ (c 1, CHCl₃). The structures of **8** and **9** were assigned on the basis of the nmr chemical shifts of their respective acetates **12** and **13** (Table I). The isomer having the chemical shift for its acetyl methyl protons at higher field (δ 2.06 compared to 2.13) was assigned the di-equatorial structure **12**. As discussed earlier (**5** and references therein), the methyl protons of equatorial acetyl groups such as those in **10** have chemical shifts at higher field than those of axial acetyl groups such as in **11**.

Initial attempts to deblock **8** by reduction with sodium in liquid ammonia followed by hydrolysis failed to yield a satisfactory product. Therefore the benzyl group was left intact and **8** was treated sequentially with acid (3N HCl in MeOH, 1:10; room temperature; 3 hr) and base 5% w/v Ba(OH)₂·8H₂O; reflux overnight). Product **14** (Figure 4) was purified on IRC-50 resin with an ammonium hydroxide gradient (0.1 to 1N; yield, 64%; MS, m/e 428 (M⁺)) and was characterized as its sulfate salt⁺ ($[\alpha]_D^{25} +104.3^\circ$ (c 1, H₂O)) and its amorphous peracetate⁺: $[\alpha]_D^{25} +148.4^\circ$, (c 1, CHCl₃); MS, m/e 722 (M⁺).

Final deblocking of **14** was effected by reduction of its free base using a minimum quantity of sodium in liquid ammonia. Mercaptan **15** was isolated as its sulfate salt ($[\alpha]_D^{25} +42.5^\circ$ (c 1, H₂O); nmr (D₂O), δ 6.0 (1H, d, J=4 Hz); mercaptan content (SH), 68% of theory based on MW 534 (I₂ titration)) and characterized as its amorphous peracetyl derivative⁺: $[\alpha]_D^{25} +123.1^\circ$ (c 1, CHCl₃); MS, m/e 674 (M⁺); nmr (CDCl₃), δ 2.33 (3H, s, SAC) ir (Nujol), 1694 cm⁻¹ (SAC).

Epoxide **4** was also treated with benzyl mercaptide to yield **16** and **18** (Figure 5) which were separated by repeated chromatography [silica, EtOAc-cyclohexane (1:1), CHCl₃-MeOH (99:1), EtOAc-cyclohexane (2:1)]. The major product [yield, 37%; $[\alpha]_D^{25} -81.9^\circ$ (c 1, CHCl₃)] was shown to be the diaxial derivative **16**⁺ by Raney-nickel desulfurization to the reported (**5**) axial alcohol **17**⁺. In addition, the acetate and ketone derived from **17** were identical to previously prepared samples (**5**). The minor product, the diequatorial derivative **18**⁺ [yield, 24%; $[\alpha]_D^{25} +25.4^\circ$ (c 1, CHCl₃)], was deblocked and purified as described before to give the benzyl thioether, characterized as its sulfate salt⁺: $[\alpha]_D^{25} +76.1^\circ$ (c 1, H₂O). Reduction with sodium in liquid ammonia yielded **19** which was isolated as its sulfate salt [$[\alpha]_D^{25} +53.3^\circ$ (c 1, H₂O)]; SH, 70% of theory based on MW 534 (I₂ titration)] and characterized as its peracetate⁺: $[\alpha]_D^{25} +54.2^\circ$ (c 1, CHCl₃), nmr (CDCl₃), δ 2.30 (3H, s, SAC).

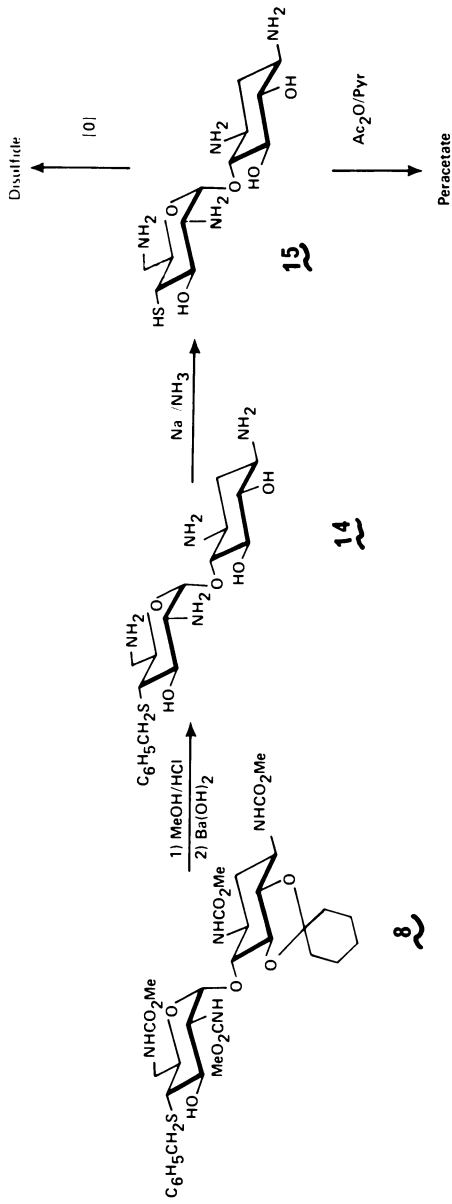


Figure 4.

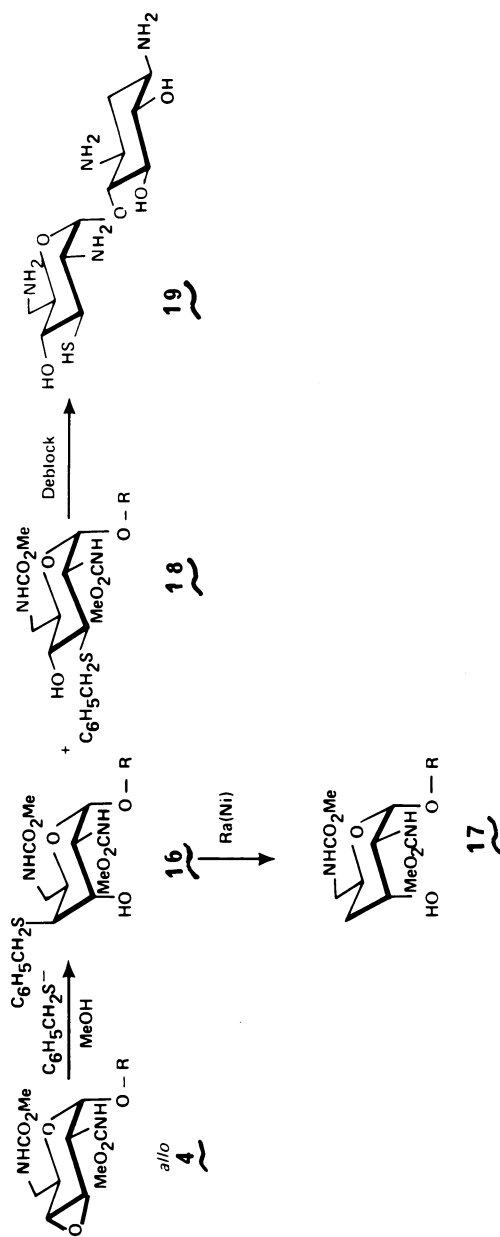


Figure 5.

Antibacterial Activity

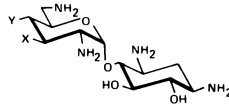
Both 15 and 19 contained approximately 20% ash (combustion analysis) which was presumed to be Na_2SO_4 , resulting from the reduction using sodium metal. Attempts to desalt these products using ion exchange and chromatographic procedures failed and, therefore, they were tested as such for antibacterial activity. Table II shows the *in vitro* activities of 15 and 19 against a series of gram-positive and gram-negative bacteria. Both mercaptans exhibited broad spectrum antibacterial activity similar to, but weaker than neamine (1). Only in the case of the *Pseudomonas aeruginosa* strain was the activity superior to that of 1. The anti-pseudomonas activity was confirmed with nine additional strains against which 15 and 19 exhibited MIC values of 12.5 to 100 $\mu\text{g}/\text{ml}$. All of these clinical isolates were resistant to neamine at 200 $\mu\text{g}/\text{ml}$. When tested against a series of resistant bacterial strains that contain known plasmids (Table III), 19 was active against the APH (3') phosphorylating strains and the AAC (3) acetylating strains whereas 15 was active against the AAC (3) strains but not the APH (3') strains.

Because 15 showed somewhat better *in vitro* activity than 19 and its precursors were available in larger quantities, further work was restricted to the 4'-thio-series. The disulfide of 15, prepared by air oxidation or by treatment with iodine, was readily desalted by chromatography on Sephadex[®] G-10 to give a product[†] $[[\alpha]_D^{25} -12.9^\circ \text{ (c 1, H}_2\text{O)}]$ with satisfactory analytical data. On testing *in vitro* (Tables II and III) it showed essentially the same activity as 15. (The slight improvement in activity can be attributed to a lower ash content.)

Preparation of a Pseudotrisaccharide

In general, pseudotrisaccharides such as kanamycin B (25) or gentamicin exhibit *in vitro* activities that are an order of magnitude better than their corresponding pseudodisaccharides neamine and gentamine [(1, 2, 6) cf. compounds 1 and 25, Table IV]. Consequently, 15 was converted to a pseudotrisaccharide. The acetate 12 was treated with methanolic HCl to yield 20[†] (Figure 6) which was condensed ($\text{AgClO}_4\text{-Ag}_2\text{CO}_3$, Drierite[®], CHCl_3 -dioxane, 6 days) with 2,4,6-tri-O-benzyl-3-acetamido-3-deoxy- α -D-glucosyl chloride, 21 (7), to yield, after chromatography [silica, toluene-methanol (95:5)], a mixture of pseudotrisaccharides (total yield, 18%). The major component, 22[†] [(yield 8%; $[\alpha]_D^{25} +62.7^\circ \text{ (c 0.5, CHCl}_3\text{)}$; lowest R_f (0.13) on tlc (silica, acetone-hexane, 1:1); ir, 1680 and 1730 cm^{-1}], was isolated from the mixture by preparative HPLC (Lichrosorb[®] 10 μ , 10 x 250 mm, acetone-hexane (1:1), refractive index detector). Alkaline hydrolysis under a variety of conditions failed to yield a totally deblocked pseudotrisaccharide. The presence of an acetamido group in the product was indicated by its infrared

Table II.

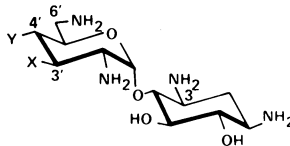


In vitro Antimicrobial Activities ($\mu\text{g/ml}$)

Strain	1	15	19	4' - Thio- neamine disulfide
	Neamine X = OH Y = OH	4' - Thio- neamine X = OH Y = SH	3' - Thio- neamine X = SH Y = OH	X = OH Y = disulfide
<i>Staph. aureus</i> HH127	25	100	200	100
<i>E. coli</i> SK&F 12140	12.5	>200	200	200
<i>Kleb. pneumoniae</i> SK&F 4200	6.3	50	200	50
<i>Sal. paratyphi</i> ATCC 12176	12.5	200	200	100
<i>Shigella</i> <i>paradyseriae</i>	25	200	>200	100
<i>Ps. aeruginosa</i> HH 63	>200	25	25	12.5
<i>Ser. marcescens</i> ATCC 13880	12.5	>200	>200	200
<i>Proteus morgani</i> 179	12.5	100	200	100
<i>Enterobacter</i> <i>aerogenes</i>	12.5	100	200	100

Agar dilution, pH 8.0

Table III.

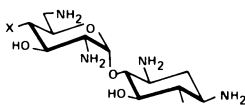


In vitro activities against resistant organisms. ($\mu\text{g/ml}$)

Strain	Enzyme System	1	15	19	4' - Thio- neamine disulfide
		Neamine X = OH Y = OH	4' - Thio- neamine X = OH Y = SH	3' - Thio- neamine X = SH Y = OH	X = OH Y = disulfide
<i>E. coli</i> K802N	—	16	125	500	63
<i>E. coli</i> K802N(pR6)	APH(3') - I	>1000	2000	500	2000
<i>E. coli</i> K802N(pJR214)	APH(3') - I + ANT(2'')	>1000	2000	500	2000
<i>E. coli</i> K802N(pJR67)	APH(3') - II	>1000	1000	500	500
<i>E. coli</i> K802N(pR5)	AAC(6')	250	1000	2000	500
<i>E. coli</i> K802N(pJR88)	AAC(3) - I	31	250	1000	125
<i>Ps. aeruginosa</i> HH63	—	250	63	63	16
<i>Ps. aeruginosa</i> PSI - I	AAC(3) - II	1000	125	125	63
<i>Prov. sp.</i> 64	AAC(2')	>1000	>2000	>2000	>2000

Broth dilution, pH 8.0

Table IV.

In vitro Antimicrobial Activities ($\mu\text{g/ml}$)

Strain	1	25	15	4-Thio-
	Neamine	Kanamycin B	4'-Thio- neamine	Kanamycin B
	X = OH R = H	X = OH R = 3AG	X = SH R = H	X = SH R = 3AG
<i>Staph. aureus</i> HH127	25	1.6	100	6.3
<i>E. coli</i> SK&F 12140	12.5	1.6	>200	25
<i>Kleb. pneumoniae</i> SK&F 4200	6.3	0.4	50	12.5
<i>Sal. paratyphi</i> ATCC 12176	12.5	0.8	200	12.5
<i>Shigella</i> <i>paradysenteriae</i>	25	3.1	200	25
<i>Ps. aeruginosa</i> HH 63	>200	12.5	25	25
<i>Ser. marcescens</i> ATCC 13880	12.5	3.1	>200	50
<i>Proteus morgani</i> 179	12.5	0.8	100	25
<i>Enterobacter</i> <i>aerogenes</i>	12.5	1.6	100	25

Agar dilution, pH 8.0

(1660 cm^{-1}) and field desorption mass spectra (m/e 902, $(M+H)^+$). Apparently, the two benzyl groups flanking the amide inhibit its hydrolysis. Therefore, 22 (Figure 7) was debenzylated with sodium in liquid ammonia, re-alkylated on the mercaptan with benzyl chloride in methanol (8), and the product was isolated as its peracetate, 23⁺: yield, 64%; $[\alpha]_D^{25} +84.7^\circ$ (c 0.5, CHCl_3). Hydrolysis with $\text{Ba}(\text{OH})_2$ (15% w/v in 1:1 MeOH-water, reflux overnight) followed by treatment with n-butyl amine at 150° overnight in a sealed bomb to remove the N-1, N-3 cyclic urea (9, 10) yielded the deblocked benzylthioether which showed two α -anomeric protons in its nmr spectrum: yield 55%; MS, m/e 590 $(M+H)^+$; nmr (D_2O), δ 6.1 (1H, d, $J=4$ Hz), 5.2 (1H, d, $J=4$ Hz), and 7.5 ppm (5H, s, aromatic). Thus, the product is the desired α -glycoside presumably attached to the 0-6 hydroxyl as described earlier (11, 12). Finally, reduction with sodium in liquid ammonia yielded 4-thio-4-deoxykanamycin B (24), isolated as its sulfate salt: yield, 73%; $[\alpha]_D^{25} +65.6^\circ$ (c 0.2, H_2O); SH, 60% of theory based on MW 744 (I_2 titration). On testing *in vitro* (Table IV), the anticipated improvement in activity over pseudodisaccharide 15 was observed against most of the strains of bacteria. However, the activity against *Pseudomonas aeruginosa* remained the same and was, in fact, weaker than that of kanamycin B (25).

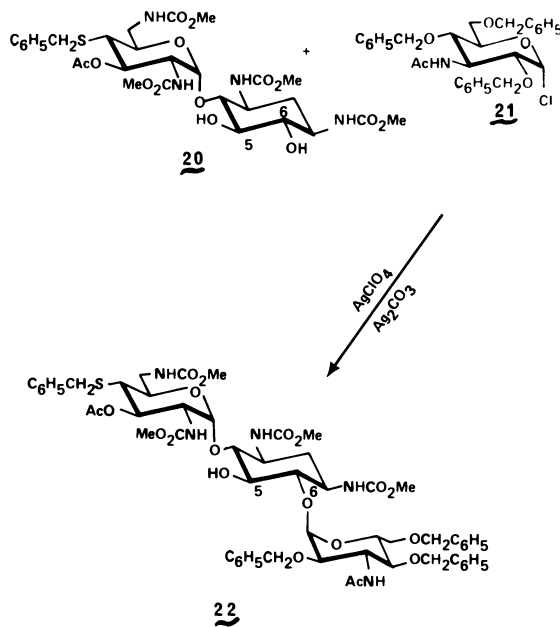
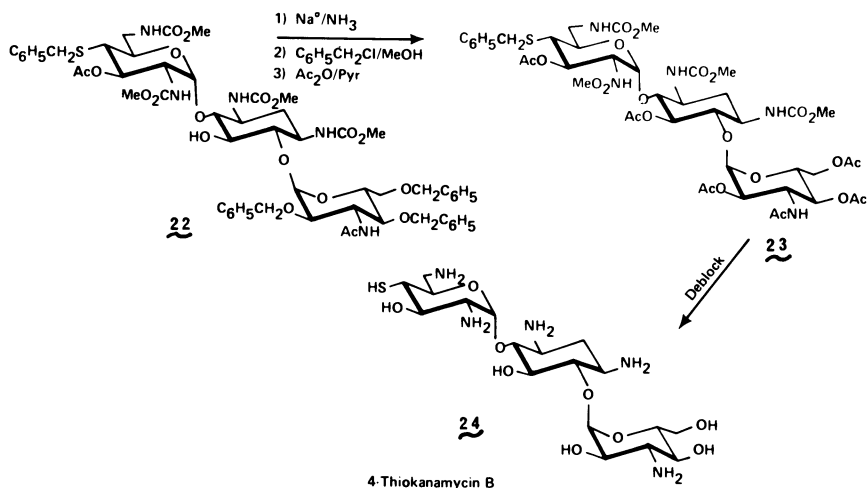


Figure 6.



4-Thiokanamycin B

Figure 7.

Acknowledgements

The authors wish to acknowledge the assistance of Mr. N. Hall Ms. J. Rosenbloom, Dr. G. Wellman and Dr. F. Pfeiffer for the preparation of intermediates, Ms. E. Reich for performing elemental analyses and optical rotations, Mr. G. Roberts for obtaining the mass spectral data, and Dr. J. Uri, Dr. S. Grappel and Mr. J. Guarini for supplying the biological data. The aminoglycoside resistant strains were constructed by Mr. L. Fare.

Literature Cited

1. Umezawa, H., *Adv. in Carbohydr. Chem. and Biochem.* (1974) 30, 183-225.
2. Price, K.E., Godfrey, J.C., and Kawaguchi, H., *Adv. Appl. Microbiol.* (1974) 18, 191-307.
3. Kakagi, Y., Miyake, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., *J. Antibiot.* (1973) 26, 403-406.
4. Jikihara, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., *Bull. Chem. Soc. Jap.* (1973) 46, 3507-3510.
5. Pfeiffer, F.R., Schmidt, S.J., Kinzig, C.M., Hoover, J.R.E. and Weisbach, J.A., *Carbohydr. Res.* (1979) 72, 119-137.
6. Benueniste, R. and Davies, J., *Antimicrob. Agents and Chemother.* (1973) 4, 402-409.
7. Hasegawa, A., Kurihara, N., Nishimura, D. and Nakajima, M., *Agr. Biol. Chem.* (1968) 32, 1123-1129.
8. duVigneaud, V., Audrieth, L.F. and Loring, A.S., *J. Am. Chem. Soc.* (1930) 52, 4500-4504.
9. Carney, R.E., McAlpine, J.B., Jackson, M., Stanaszek, R., Washburn, W.H., Cirovic, M. and Mueller, S.L., *J. Antibiot.* (1978) 31, 441-450.
10. Umezawa, H., Maeda, K., Kondo, S. and Fukatsu, S., *United States Patent* (June 22, 1976), 3,965,089.
11. Sitrin, R.D., Cooper, D.J. and Weisbach, J.A., *J. Org. Chem.* (1978) 43, 3048-3052.
12. Sitrin, R.D., Cooper, D.J. and Weisbach, J.A., *J. Antibiot.* (1977) 30, 836-842.

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Synthesis of Analogs of Kanamycin B

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During recent years, a very large number of analogues of aminocyclitol antibiotics have been prepared in the hope of obtaining safer and more potent agents, while also broadening their spectrum of activity, especially against resistant bacteria.

One approach towards these analogues has been via "mutational biosynthesis," or "mutasynthesis" (1,2), a technique (3) which has permitted the introduction of various modified streptomycin residues into neomycin (1,2,4), ribostamycin (5), neamine (6), sisomicin (7) and gentamicin (8), to name a few. This subject has been reviewed in the papers by Dr. Rinehart and Dr. Daniels during this symposium and needs no further amplification.

The semi-synthetic approach involves either simple chemical modifications of natural antibiotics--dibekacin (9,10), amikacin (11) and netilmicin (12) are successful examples illustrating this method--or a more lengthy synthesis of the desired analogues starting from partially degraded antibiotics (13) such as, for example, neamine and garamine.

In our program, we decided to study various analogues of kanamycin B, using the readily available neamine (1) as a starting material. Kanamycin B is inactivated by resistant bacteria containing aminoglycoside 3'-phosphotransferase [APH(3')] enzymes (14,15) which phosphorylate the 3'-hydroxyl group. At the time we started this project, an aminoglycoside 4'-nucleotidyltransferase [ANT(4')] had just been found (16,17) in *S. aureus*. It was therefore reasonable to avoid inactivation of our analogues by these enzymes by removing the hydroxyls in positions 3' and 4', as had been successfully done in dibekacin (9,10).

Also, various analogues obtained by mutational synthesis, in which the 2-deoxystreptomycin residue had been replaced by a 2,5-dideoxystreptomycin moiety, had shown some increased antibacterial activity (7,18). With this in mind we planned the synthesis of 3',4',5-trideoxykanamycin B (19) and related derivatives.

Elaboration of the neamine moiety (1) requires, first, the protection of its four primary amino groups. Such protecting groups need to be sufficiently stable to withstand further

transformations and yet be removable under as mild conditions as possible at the end of the synthesis. Dimedone derivatization of amine functions has been used previously in the preparation of analogues of kasugamycin (20) and butirosin (21).

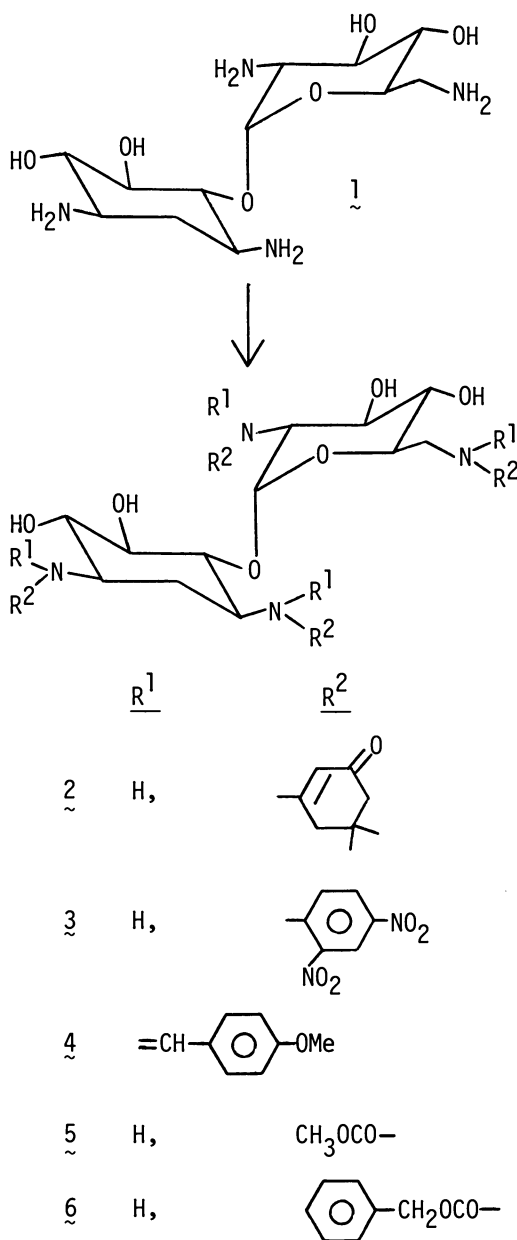
The vinylogous amides resulting from the reaction of dimedone with an amine are stable to acid and base, have an intense UV absorption, and the original amines can be regenerated by treatment with bromine. This protecting group thus appeared suitable for our purpose.

We synthesized the tetrakis(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl) derivative **2** by reaction of **1** with 5,5-dimethyl-1,3-cyclohexanedione in pyridine at reflux and obtained **2** in 32% yield. A trisubstituted derivative was a major by-product (~28%) and the reaction could not be driven to completion.

Since **2** could not be obtained in good yield, we next investigated the 2,4-dinitrophenyl protecting group (22,23). Dinitrophenylamines absorb strongly in the UV spectrum and are stable under acidic and mildly basic conditions; however, in the presence of Amberlite IRA-400 (OH⁻) the original amine is regenerated. This protecting group has been used for the synthesis of paromamine (24) and one of its isomers (25). Reaction of **1** with 1-fluoro-2,4-dinitrobenzene in a mixture of water and acetone in the presence of sodium bicarbonate gave, after purification by chromatography, 46% of the desired tetrakis(2,4-dinitrophenyl) derivative **3** as a bright yellow amorphous solid. Since this derivative required purification by column chromatography, it was also not of general use.

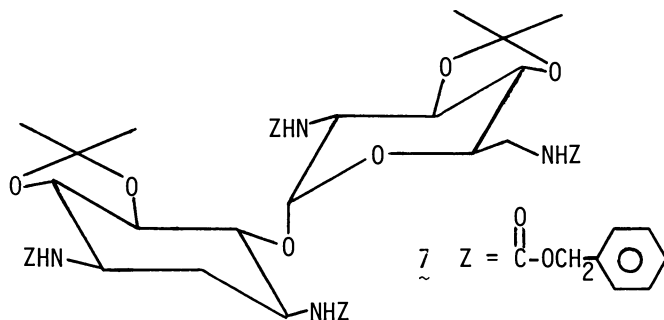
The amine function of 2-amino-2-deoxy-D-glucose has been protected by reacting it with p-anisaldehyde to give the corresponding Schiff base (26). This protecting group has some UV absorption and is stable to base, but it is removed by aqueous acid. It is, however, possible to make the corresponding glycosyl bromide from the peracetylated derivative in the presence of hydrogen bromide and dichloromethane. This method was used for the synthesis of neomycin (27) and an analogue of lividomycin B (28). Reaction of **1** with p-anisaldehyde in ethanol at reflux gave an excellent yield (95%) of **4**; however, this compound was unstable when analyzed by thin layer chromatography and therefore unsuitable for our purpose.

We finally tried the classical methoxycarbonyl and benzyloxycarbonyl protecting groups. The preparation of intermediate **5** has been mentioned without any details for the synthesis of 3',4'-dideoxyneamine (29). In repeating the preparation of **5**, we were not able to achieve the high yield reported in the literature. However, the tetrabenzoyloxycarbonyl derivative **6** (30), used for the synthesis of kanamycin B, was obtained consistently in high yield (~85%) even on a one mole scale. Purification by recrystallization from hot acetic acid gave the analytically pure compound melting at 253-254° C [Lit. (30) mp 259° C dec.].



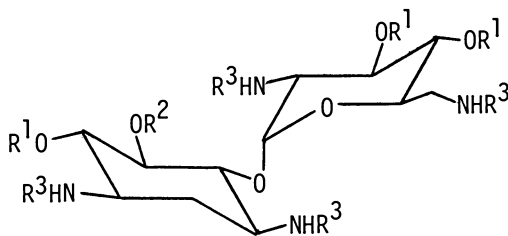
Our first approach towards 3',4',5-trideoxykanamycin B (19) was via a 3',4'-dideoxyneamine intermediate. Umezawa and co-workers (29) have prepared 3',4'-dideoxyneamine by selective

ketalation of **5** with the dimethyl ketal of cyclohexanone, a mixture being obtained, from which the 5,6-*O*-cyclohexylidene derivative was isolated as the major product. When **6** was reacted with benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid, or with benzaldehyde and fused zinc chloride, a mixture of mono-benzylidene derivatives was obtained in 15% yield. On the other hand, when **6** was reacted with dimethoxypropane and *p*-toluenesulfonic acid in DMF, the 3',4'-5,6-di-*O*-isopropylidene derivative **7** was obtained as an analytically pure syrup in 48% yield [^{13}C -NMR, CDCl_3 : $\underline{\text{C}}(\text{Me})_2$ 112.5 and 111.2 ppm].

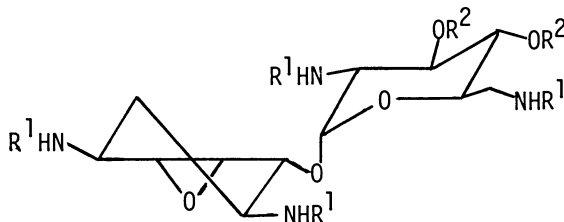


Selective protection of the 3',4'- or 5,6-glycols was abandoned in view of the following development. The purification of the mother liquors from crystallization of **6** was difficult due to the insolubility of the tetrabenzyloxycarbonyl derivative. Acetylation of the mother liquors (acetic anhydride, pyridine, 20° C, 2.5 h), in order to obtain a compound which could be purified by chromatography over silica gel, led in good yield to the formation of a crystalline tri-*O*-acetyl derivative [mp 222-223° C, $[\alpha]_D^{20}$ 50.9° (c 1.0, CHCl_3)]. The structure **8** was first assumed on steric grounds and later proved by degradation of a 5-substituted derivative, *vide infra*. The corresponding tetra-*O*-acetyl derivative **9** was obtained in good yield when the reaction was catalyzed by 4-dimethylaminopyridine and kept at room temperature for 16 h. The crystalline derivative **9** melted sharply at 186° C [$[\alpha]_D^{20}$ 55° (c 0.9, CHCl_3)]. When a similar reaction was carried out with benzoyl chloride and pyridine at room temperature for 3 h, no selectivity was observed, a mixture of the tetra-*O*-benzoyl derivative **10** (28%) and a tri-*O*-benzoyl derivative (34%) being obtained.

Finally, **1** was fully acetylated in the presence of pyridine and acetic anhydride at room temperature for 3 days, the highly crystalline octaacetate **11** being isolated as a solvate (EtOAc) in 79% yield. The above reaction was used as a model for the characterization of final pseudodi- and trisaccharides, which were difficult to obtain in analytically pure form as the free compounds.



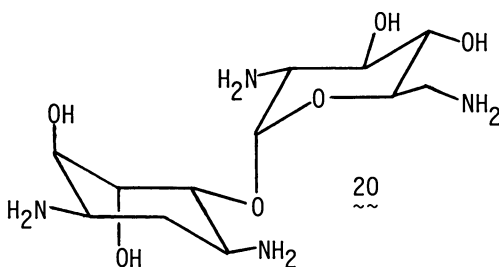
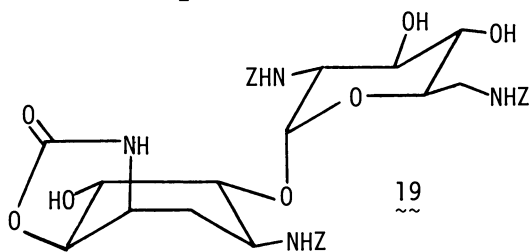
	<u>R¹</u>	<u>R²</u>	<u>R³</u>
8	Ac	H	Z
9	Ac	Ac	Z
10	Bz	Bz	Z
11	Ac	Ac	Ac
12	Ac	Ms	Z
13	H	Ms	Z
14	H	Ms	H



	<u>R¹</u>	<u>R²</u>
15	H	H
16	Z	H
17	Z	Bz
18	Z	Ms

Reaction of 8 with methanesulfonyl chloride in dichloromethane in the presence of triethylamine at 0° C for 15 min gave the corresponding 5-O-mesyl derivative 12 as a foam in 67% yield. Hydrolysis of the acetyl groups of 12 using methanolic ammonia at room temperature for 16 h gave crystalline 13. Hydrogenolysis of the benzyloxycarbonyl groups of 13 with palladium hydroxide on charcoal (31) in glacial acetic acid under 3 kg/cm² of hydrogen for 5 h at room temperature gave the free 5-O-mesyl neamine 14. Treatment of 14 with 0.1 N sodium methoxide in methanol at room temperature for 16 h gave the corresponding 5,6-anhydro derivative

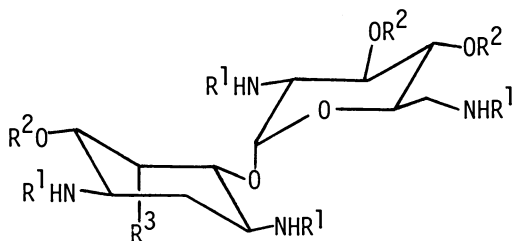
15, which was isolated in 80% yield as the tetrahydrochloride. The same compound was also obtained by treatment of 13 with 0.02 N sodium methoxide in methanol at room temperature for 16 h giving the intermediate 5,6-anhydro derivative 16 in 90% yield as a white solid (mp 257-258° C). Removal of the benzyloxycarbonyl groups of 16 using palladium hydroxide (31) as described above gave 15 as an analytically pure amorphous solid in quantitative yield. Derivatization of the epoxide 16 by reaction with benzoyl chloride or methanesulfonyl chloride in pyridine gave the corresponding 3',4'-di-O-benzoyl derivative 17 in 54% yield and the 3',4'-di-O-mesyl derivative 18 in 39.5% yield, respectively. Hydrolysis of 16 with glacial acetic acid at reflux for 15 min gave the crystalline cyclic carbamate 19, which resulted from participation of the neighboring benzyloxycarbonyl group in the opening of the protonated epoxide. Hydrolysis of the carbamate with barium hydroxide in a mixture of dioxane and water at 100° C for 18 h, followed by reduction of the benzyloxycarbonyl group using palladium hydroxide (31) in glacial acetic acid under 3 kg/cm² of hydrogen for 4 h at room temperature, gave 48% of amorphous 5-epi-6-epineamine 20 (32), isolated as a tetrahydrochloride salt $[[\alpha]_D^{25} 29.1^\circ (c 0.8, H_2O)]$.



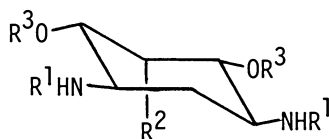
Treatment of 8 with sulfuryl chloride (33,34) in a mixture of dichloromethane and pyridine (3:1) under nitrogen at 0° C for 20 h gave the crystalline 5-chloro-5-deoxy-5-epineamine derivative 21. Crystalline 22 precipitated almost quantitatively when 21 was deacetylated with methanolic ammonia at room temperature for 48 h. Removal of the benzyloxycarbonyl group from 22 with

palladium hydroxide (31) in acetic acid under 3 kg/cm² of hydrogen at room temperature for 16 h gave the free 5-chloro-5-deoxy-5-epineamine 23 as an amorphous solid in 95% yield [$[\alpha]_D^{25}$ 105.3° (c 0.5, H₂O)].

The structures of the above compounds were proved by reduction of the chlorine group of 21 in order to obtain 5-deoxyneamine, 26 (34). Reduction with hydrazine hydrate and Raney nickel (35) in refluxing methanol, both in the presence or absence of barium carbonate, failed. Reduction of 21 with tributylstannane in the presence of α,α -azobisisobutyronitrile (36) in refluxing toluene, however, gave the desired 5-deoxy derivative 24 in 25% yield. This yield could be substantially improved by generating the tributylstannane *in situ* according to the method of Grady and Kuivila (37). When 21 was heated at reflux in toluene in the presence of polymethylsiloxane and hexabutyl-distannoxane, 24 was isolated in 68% yield as a crystalline solid without the need for purification by column chromatography. Removal of the acetyl groups with methanolic ammonia gave crystalline 25 in 90% yield. Finally, reduction of 25 with palladium hydroxide (31) as described above, followed by treatment with methanolic hydrogen chloride and precipitation with ether, gave the tetrahydrochloride salt of 5-deoxyneamine (26) as an analytically pure amorphous powder in 86% yield (an overall yield of 40% from neamine as compared to 9% and 24% by previous synthetic (34) and mutasynthetic (2) routes) [mp >300° C; $[\alpha]_D^{20}$ 96.8° (c 0.5, H₂O); single spot R_f 0.5 on TLC using n-PrOH, AcOH, H₂O, 10:1:9 (v/v)]. Hydrolysis of 26 in 48% hydrobromic acid gave, in 60% yield, 2,5-dideoxystreptomine dihydrobromide (27), which had ¹³C and ¹H-NMR spectra identical to those of a reference sample prepared by unambiguous methods (38,39). (We thank Mr. P. Brock for the preparation of 27 according to the above methods (38,39), and for the characterization of 31 and 32, which were derived from 27.) Hydrolysis of 23 under similar conditions gave 28, which was converted to the tetraacetyl derivative 29. The proton NMR spectrum of 29 showed that the molecule was symmetrical--indeed, only one single acetyl methyl signal was present at 1.98 ppm--while the mass spectrum clearly showed an M⁺ + 2 peak at m/e 350, indicating the presence of four acetyl groups. Peracetylation of 27 also gave a tetraacetyl derivative 30 showing a single acetyl methyl signal at 1.94 δ in the ¹H-NMR spectrum. Further transformations of 27 gave the known N¹,N³-bis(2,4-dinitrophenyl) derivative 31 [mp 261-263° C; mixture mp 261-263° C, $[\alpha]_D^{20}$ 0° (c 1.0, acetone)] and the N¹,N³-dibenzoyloxycarbonyl derivative 32 (mp 201° C, lit. mp (38) 201° C). The melting point and ¹³C- and ¹H-NMR spectra of the compounds were identical to those of samples prepared by an unambiguous method, thus assuring us of the structures of compounds 8 and 12-26.



	$\underline{R^1}$	$\underline{R^2}$	$\underline{R^3}$
21	Z	Ac	Cl
22	Z	H	Cl
23	H	H	Cl
24	Z	Ac	H
25	Z	H	H
26	H	H	H



	$\underline{R^1}$	$\underline{R^2}$	$\underline{R^3}$
27	H	H	H
28	H	Cl	H
29	Ac	Cl	Ac
30	Ac	H	Ac

31		H	H
32	Z	H	H

Examination of the ^{13}C -NMR spectra of 5-chloro-5-deoxy-neamine 23, 5-deoxyneamine 26 and neamine 1 supports the axial configuration for the chlorine atom in 23. Indeed, carbons 1 and 3 of 23 have a 1 ppm upfield shift as compared to neamine (see Table I); although the shift is not of the magnitude expected for an equatorial-to-axial substitution (40), it is in the right direction. Moreover, the magnitude of the shift may be minimized

Table I
 ^{13}C -NMR Chemical Shifts (a)

	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6
1	100.64	55.03	73.43	71.18	72.98	41.45	50.08	35.51	49.07	87.17	75.71	77.34
23	96.01	55.83	75.01	72.24	74.19	42.43	49.10	36.97	48.02	78.26	63.72	74.78
26	96.14	55.76	75.07	72.43	74.03	42.49	54.33	35.96	52.93	78.16	37.17	73.90
56	96.79	50.14	26.93	28.26	71.24	45.75	54.30	36.25	53.00	78.29	37.20	73.93

(a) In parts per million downfield from tetramethylsilane.

Solvent: D_2O -NaOD, pH 12.

by other effects, such as a change of overall conformation possibly due to the absence of hydrogen bonding between the hydroxyl group at position 5 and the amine function at position 2' (see Figure 1). Finally, the axial configuration is in accord with the mechanism proposed by Jones, *et al.* (41) for chlorination of sugars with sulfuryl chloride. Suami, *et al.* (34) have independently reported a synthesis and proof of structure of 5-deoxyneamine 26 following an approach parallel to ours.

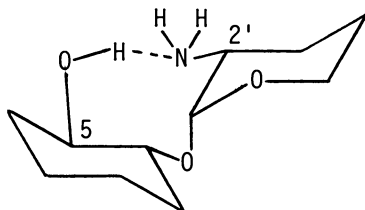


Figure 1.

It is also interesting to note the substantial upfield shift of the 1'-carbon in 23 and 26 relative to neamine (1). This upfield shift could be explained by free rotation around the C₄-C_{1'} bond due to the absence of a hydrogen bond, as proposed above (see Figure 1) and supported by a ¹H-NMR study of various kanamycin derivatives (42).

Having developed a selective and efficient way to synthesize 5-chloro-5-deoxy-5-epineamine (23) and 5-deoxyneamine (26), our next goal was to selectively remove the hydroxyls in positions 3' and 4' in order to obtain a 3',4',5-trideoxynamine derivative as a suitable intermediate for the synthesis of 3',4',5-trideoxykanamycin analogues. Acetalation of 22, either with 2,2-dimethoxypropane or 1,1-diethoxycyclohexane in DMF in the presence of *p*-toluenesulfonic acid, gave the corresponding 3',4'-O-isopropylidene and 3',4'-O-cyclohexylidene derivatives, 33 and 34, in yields of 30% and 72% respectively. In view of the higher yield obtained for compound 34, it was next transformed into the corresponding 6-O-benzoyl derivative 35 and the 6-O-trichloroethoxycarbonyl derivative 36 in yields of 86% and 89% respectively. Hydrolysis of the cyclohexylidene protecting group of 35 and 36 with 80% acetic acid at 80° C for 1 h gave the deprotected pseudodisaccharides 37 and 38 in yields of 95% and 89%.

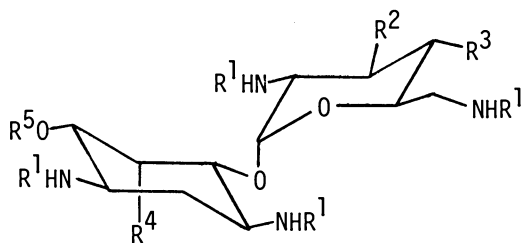
Previous syntheses of 3',4'-dideoxynamine (43) and 3',4'-dideoxykanamycin B (44) have been achieved via the Tipson-Cohen procedure (45), namely by conversion of a 3',4'-di-O-mesylate intermediate into the corresponding 3',4'-unsaturated derivative by treatment with sodium iodide and zinc in hot DMF, followed by catalytic hydrogenation (43,44). Recently, Umezawa, *et al.* (46) have reported the advantage of using benzenesulfonyl rather than methanesulfonyl esters during the introduction of unsaturation

at position 3'-4' in a kanamycin B derivative.

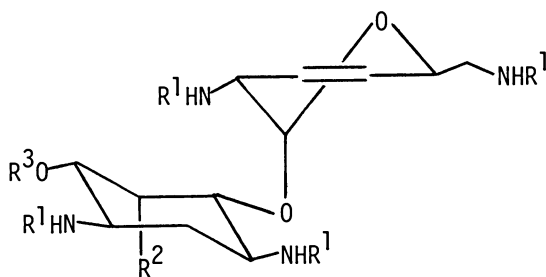
Following the above described methods, 37 was converted to the crystalline 3',4'-di-O-mesylate 39 and 3',4'-di-O-benzylsulfonate 40 in 93% and 70% yields respectively. The chloro derivatives 39 and 40 were then reduced to the corresponding crystalline 5-deoxyneamine intermediates 41 and 42 in yields of 91% and 53% respectively using tributylstannane generated *in situ* (37). Hydrolysis of the benzoyl group at position 6, using a solution of sodium methoxide in a mixture of methanol and chloroform at room temperature for 2 to 19 h, gave crystalline 43 and 44 in 90% yield.

Generation of the 3',4'-olefin 45 through treatment of the 3',4'-di-O-mesyl derivative 43 with zinc and sodium iodide in hot DMF (43,44,45) did not proceed in high yield. The following modifications to the classical method were tried singly or in combination: (a) activation of zinc dust (1) by acid wash, (2) by heating at 110° C under high vacuum, (3) by amalgamation and (4) by coupling with silver, (b) careful drying of sodium iodide, (c) replacement of sodium iodide by potassium iodide, (d) replacement of DMF by (1) hexamethylphosphorotriamide, (2) acetone or (3) dimethylsulfoxide, (e) addition of activated 3 Å molecular sieves, (f) running all operations, including the activation of zinc, under nitrogen. The above conditions were tried at temperatures ranging from 25° C to 124° C from 1 h to 24 h. Unfortunately, none of the above modifications improved the 40% yield obtained using the classical conditions. The low yield in this transformation can partially be explained by the formation of two side products, N¹,N³-dibenzoyloxycarbonyl-2,5-dideoxystreptamine (32) and the glycal (48), which were each isolated in approximately 20% yield. Compound 32 was identical to an authentic sample. The structure of 48 was established by ¹³C- and ¹H-NMR spectroscopy and by elemental analysis. The proton NMR spectrum of 48 in deuteriochloroform showed H-1 as a broad doublet at 6.34 ppm ($J_{1,2} = 5.5$ Hz). Irradiation of this proton led to the collapse of a sharp doublet of doublets centered at 4.69 ppm ($J_{2,3} = 4$ Hz) and assignment of this resonance to H-2. Addition of D₂O simplified three signals: one H-3, centered at 4.51 ppm ($J_{3,4} = 4.5$ Hz, $J_{3,NH} = 8$ Hz), and two others, centered at 3.68 and 3.27 ppm, each showing a large geminal coupling of 14 Hz and therefore assigned to H-6a and H-6b. This clearly indicated that amine functions were present at both C-3 and C-6. The ¹³C-NMR spectrum of 48 showed that C-1 (assigned by single frequency decoupling) resonated at 145.90 ppm while C-2 was at 98.37 ppm. The position of these signals, coupled with the broadening of the proton signals for H-1 and H-3 in the proton NMR spectrum due to long range coupling, assured us of the presence of the glycal structure in 48.

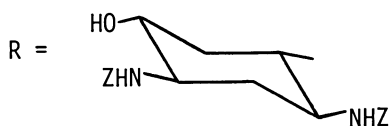
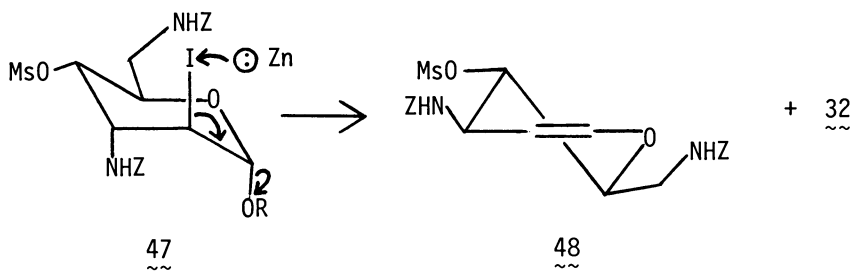
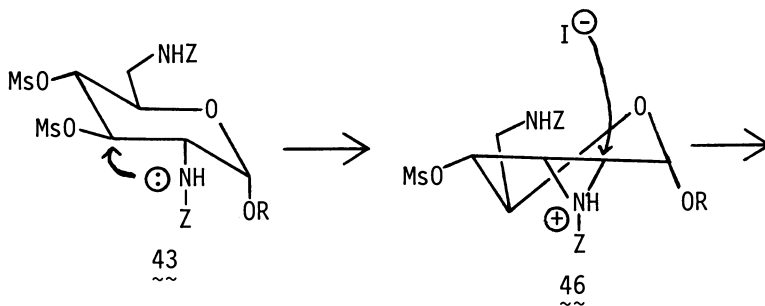
A possible mechanism explaining the formation of these by-products is shown in Scheme I. Participation of the nitrogen of the benzyloxycarbamide at position 2 in the displacement of the



	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>
33	Z			C1	H
34	Z			C1	H
35	Z			C1	Bz
36	Z			C1	
37	Z	OH	OH	C1	Bz
38	Z	OH	OH	C1	
39	Z	OMs	OMs	C1	Bz
40	Z	OSO ₂ CH ₂ C ₆ H ₅	OSO ₂ CH ₂ C ₆ H ₅	C1	Bz
41	Z	OMs	OMs	H	Bz
42	Z	OSO ₂ CH ₂ C ₆ H ₅	OSO ₂ CH ₂ C ₆ H ₅	H	Bz
43	Z	OMs	OMs	H	H
44	Z	OSO ₂ CH ₂ C ₆ H ₅	OSO ₂ CH ₂ C ₆ H ₅	H	H
49	Z	OMs	OMs	C1	H
52	CF ₃ CO	OMs	OMs	H	H
54	EtOCO	OMs	OMs	H	H
56	H	H	H	H	H
57	Ac	H	H	H	Ac



	$\underline{R^1}$	$\underline{R^2}$	$\underline{R^3}$
45	Z	H	H
50	Z	Cl	Bz
51	Z	H	Bz
53	CF ₃ CO	H	H
55	EtOCO	H	H



Scheme 1

3-O-mesyl group of 40 gives the intermediate epiminium ion 46, which is opened diaxially by iodide ion to form 47. Concerted elimination of the anion corresponding to 32 can then arise via attack by zinc or iodide ion on the iodine atom of 47, leading to the glycal 48. While 32 and 48 are consistently the major by-products in these reactions, other degradation products are also observed. Possibly other pathways, such as those mentioned very recently by Umezawa (74) involving N-benzyl-epimine intermediates, could also account for the observed low yields. Similar by-products are detected by TLC when the elimination is done using the 2',3'-di-O-benzylsulfonate 44, and since the isolated yield of 45 was only 23% this approach was not pursued any further. Hydrolysis of the 6-O-benzoyl group of 39 with methanolic sodium methoxide for 15 min at room temperature gave crystalline 49 in 93% yield. Treatment of 49 with sodium iodide and zinc dust in DMF at 100° C for 3 h, however, led to a poor yield of a 1:1 mixture of compounds that have not been characterized. Treatment of the 3',4'-di-O-mesyl derivative 39 with sodium iodide and zinc dust in DMF at 100° C for 2 h gave the corresponding 3',4'-unsaturated derivative 50 in 36% yield as an amorphous powder which was then reduced with tributylstannane generated *in situ* (37) to give 73% of crystalline 51. When 41 was treated with sodium iodide and zinc dust in DMF at 100° C for 24 h, a substantial amount of starting material was still present and the reaction was not investigated any further. Finally, treatment of the 3',4'-dibenzylsulfonate 44 under similar conditions gave only a 23% yield of the unsaturated pseudodisaccharide 45.

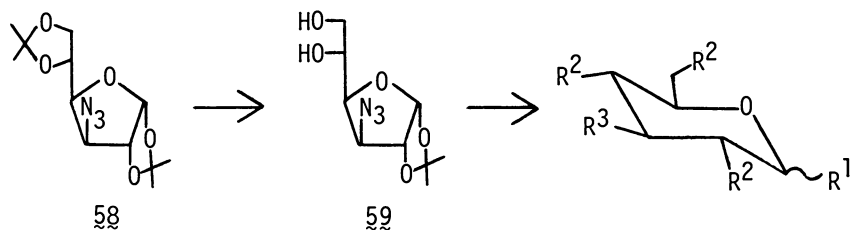
The above series of reactions clearly indicates that the pathway 34 → 35 → 37 → 39 → 41 → 43 gave the best overall yield of the key intermediate 43. In order to find out if the conversion 43 → 45 could be improved by replacing the benzyloxycarbonyl group by another protecting group, 43 was hydrogenolyzed with Pd(OH)₂/C (31) in glacial acetic acid at room temperature under 3.6 kg/cm² of hydrogen for 4 h and the crude product was reacted with trifluoroacetic anhydride in dioxane for 1 h at room temperature, giving the tetrakis-N-trifluoroacetylneamine derivative 52 in 51% overall yield as an analytically pure syrup. Treatment of 52 with sodium iodide and zinc dust in DMF at 110° C for 2 h gave 52% of 53 as a pure syrup after separation by TLC from a slower compound having a mobility expected for the N¹,N³-bis-trifluoroacetyl derivative of 2,5-dideoxystreptamine. Similarly, the crude product resulting from hydrogenolysis of 43 was reacted with ethyl chloroformate and the corresponding crystalline tetra-N-ethoxycarbonyl derivative 54 was obtained in 48% overall yield. The dimesylate 54 was also treated with sodium iodide and zinc dust in DMF at 110° C for 2 h to give the crystalline unsaturated pseudodisaccharide 55 in only 26% yield. Since neither the trifluoroacetyl nor the ethoxycarbonyl protecting group offered any substantial improvement in the conversion of a 3',4'-dimesylate to a 3',4'-unsaturated neamine derivative, our synthetic

effort was pursued using 43 as the key intermediate.

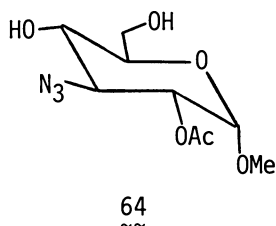
Reduction of 45 with palladium hydroxide on charcoal (31) in acetic acid under 3.6 kg/cm² of hydrogen for 5 h at room temperature gave 3',4',5-trideoxyneamine 56, which was isolated as its tetrahydrochloride salt in 93% yield and characterized as the peracetyl derivative 57 (mp 287-289° C dec.). The ¹³C-NMR spectrum of 56 (see Table I) indicates, as for 23 and 26, an upfield shift for C-1' consistent with a different orientation of the 2,6-diamino-2,6-dideoxyhexose ring due to the lack of hydrogen bonding as postulated above (see Figure 1).

While the synthesis of the key intermediates 43 and 45 was being developed, the preparation of various suitably protected derivatives and analogues of 3-amino-3-deoxyglucose was concurrently achieved. For starting material, we used the readily accessible 3-azido-3-deoxy-1,2:5,6-di-O-isopropylidene- α -D-glucopyranose (58) (47,48), which was obtained by classical procedures from 1,2:5,6-di-O-isopropylidene- α -D-glucopyranose. We improved the yield of 58 from 88% to 95% by using lithium azide instead of sodium azide (47,48) in the displacement of the 1,2:5,6-di-O-isopropylidene-3-O-p-toluenesulfonyl- α -D-allofuranose. Hydrolysis of 58 with 80% acetic acid gave 59 (48) in 97% yield. Methanolysis of 59 using 3% hydrochloric acid in methanol at reflux for 7 h gave, in 96% yield, an α/β mixture of the corresponding methyl 3-azido-3-deoxy-D-glucopyranosides 60 and 61, which could be chromatographically separated as the acetates 62 and 63 in about 50% yield each. The assignment of configurations was based on ¹H-NMR and rotation [62: $[\alpha]_D^{25}$ -21.9°, 63: $[\alpha]_D^{25}$ 122.3° (c 1.0, CHCl₃)] and also on the fact that hydrolysis of the α -anomer 63 with triethylamine in aqueous methanol gave a 1:1 mixture of the known (49) 61 and its 2-O-acetyl derivative 64. Treatment of 59 with a 1% solution of anhydrous hydrogen chloride in allyl alcohol at 65° C for 3 h gave an α/β mixture of the allyl glycosides 65 and 66, which was acetylated and then separated by preparative TLC giving 43% of the pure β -anomer 67 ($[\alpha]_D^{25}$ -28.4°) and 40% of the pure α -anomer 68 ($[\alpha]_D^{25}$ 126.9° (c 1.0, CHCl₃)). In order to ensure that condensation between the 3-amino-3-deoxyglycosyl acetate and the pseudodisaccharides 43 and 45 leads to products with the natural α -configuration, it is crucial that the protecting group at C-2 be non-participating in nature. To this end, we have chosen the benzyl ether and have, in fact, consistently obtained α -glycosides as the major products. Benzylation of the mixture of anomers 65 and 66 with sodium hydride in DMF, followed by addition of benzyl bromide at 0° C and then reaction at room temperature for 1 h, gave 75% of the perbenzylated glucoside. Careful chromatography on a column of silica gel permitted separation of the β -anomer 67 ($[\alpha]_D^{25}$ -7.1°) and the α -anomer 68 ($[\alpha]_D^{25}$ 72.4° (c 1.0, CHCl₃)). Isomerization of the allyl group of 67/68 with potassium *t*-butoxide in DMSO (50) led to an intractable mixture, and therefore this approach was abandoned. Benzylation of the mixture of 60 and 61 as described for the allyl glucosides

65 and 66 gave, after purification by silica gel column chromatography, 42% of the β -anomer 69 ($[\alpha]_D^{25}$ 2.7°) and 47% of the α -anomer 70 ($[\alpha]_D^{25}$ 58.3° (c 1.0, CHCl_3)). When the series of reactions 59 \rightarrow 60/61 \rightarrow 69/70 was repeated on a 20 mmole scale, an overall yield of ~81% was obtained after separation of both isomers by chromatography. Selective acetylation (conc. sulfuric acid 1-2%, acetic anhydride) of the glycosidic bond of 69 could not be achieved without concurrent cleavage of the benzyl group.



	<u>R¹</u>	<u>R²</u>	<u>R³</u>		<u>R¹</u>	<u>R²</u>	<u>R³</u>
60	βOMe	OH	N ₃	70	αOMe	OBn	N ₃
61	αOMe	OH	N ₃	71	βOMe	OBn	N=C(Me) ₂
62	βOMe	OAc	N ₃	72	αOMe	OBn	N=C(Me) ₂
63	βOMe	OAc	N ₃	73	αOMe	OBn	NH ₂
65	$\beta\text{-OH}$	OH	N ₃	74	βOMe	OBn	NHZ
66	$\alpha\text{-OH}$	OH	N ₃	75	αOMe	OBn	NHZ
67	$\beta\text{-OH}$	OBn	N ₃	76	βOMe	OBn	NHAc
68	$\alpha\text{-OH}$	OBn	N ₃	77	αOMe	OBn	NHAc
69	βOMe	OBn	N ₃	78	βOAc	OBn	NHAc



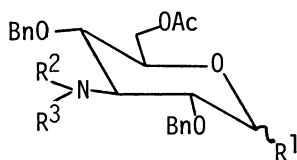
Hydrolysis in the presence of Dowex 50 (H^+) resin in a mixture of dioxane and water at $90^\circ C$ for 18 h left only unreacted 69, and similarly, hydrolysis using a mixture of acetic acid and 6 N hydrochloric acid (88.5:11.5) (51) did not lead to the free glucose derivative in useful yield. Our initial plan was to condense a series of 3-azido-3-deoxyglycopyranosyl acetates with 45. A subsequent single reductive step would then generate the desired unprotected pseudotrisaccharide via reduction of the 3',4'-olefin and azide functions and hydrogenolysis of the benzyloxycarbonyl and benzyl groups. Since, however, the glycoside bond in the 3-azido derivatives 69 and 70 would not undergo acetolysis, we decided to reduce the azide function in order to see if a 3-N-acyl glycoside derivative would be more amenable to acetolysis.

Reduction of a mixture of 69 and 70 with Raney nickel in ethanol under 1 atm of hydrogen gave the Schiff bases 71 and 72 after silica gel column chromatography using a mixture of benzene and acetone (9:1) as the eluant. The α -anomer 72 was treated with acid and the free amine 73 was isolated as an analytically pure syrup $[[\alpha]_D^{25} 58.3^\circ (c 1.0, CHCl_3)]$. When the crude mixture from Raney nickel reduction of 71 and 72 was treated with benzyloxycarbonyl chloride in a mixture of pyridine and chloroform at room temperature for 18 h, the corresponding crystalline 3-N-benzyloxycarbonyl derivatives 74 and 75 were isolated in 13% and 22% yields respectively. Reduction of a mixture of 69 and 70 with Raney nickel in the presence of acetic anhydride and ethyl acetate under 1 atm of hydrogen at room temperature for 3 h gave 82% of the corresponding anomeric mixture of N-acetyl glucoside derivatives 76 and 77, which could be separated into the known (52) β -anomer 76 and the α -anomer 77 by preparative TLC. Hydrolysis of 76 with acetic acid and 2 N sulfuric acid at $100^\circ C$, followed by acetylation of the resulting free glucose derivative with pyridine and acetic anhydride, gave 78 (52), albeit in only low yield. Alternatively, the anomeric mixture of 76 and 77 was acetolyzed with an 0.5% mixture of conc. sulfuric acid in acetic anhydride at room temperature for 17 h. The crystalline material obtained in 77% yield after purification by chromatography was shown by NMR to be an anomeric mixture of 3-acetamido-6-O-acetyl-2,4-di-O-benzyl-3-deoxy-D-glucofuranosyl acetate, the α -anomer 79 being the major component. This compound, obtained in 52% overall yield from 58, is suitable for condensation with our protected pseudodisaccharides 43 and 45; before proceeding any further, however, we wanted to check the conditions necessary for the removal of the N-acetyl protecting group. Hydrolysis of the N-acetyl group of 76/77 was not possible using methanolic ammonia, barium hydroxide or hydrazine hydrate while the neighboring benzyl groups were present. Hydrogenolysis of the benzyl groups of 76 with palladium on carbon in a mixture of methanol and acetic acid gave, in 91% yield, the known methyl-3-acetamido-3-deoxy- β -D-glucofuranoside (53), which was hydrolyzed with hydrazine hydrate at $100^\circ C$ in a sealed tube for 18 h to give the desired

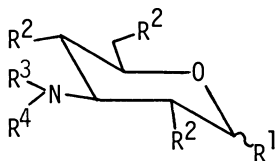
methyl-3-amino-3-deoxy- β -D-glucopyranoside (53) in 88% yield. This high recovery of the free aminoglycoside indicated that an acetyl group was suitable for our purpose.

For the preparation of a suitably protected 3-deoxy-3-methyl-amino-D-glucose derivative, we used the readily available anomeric mixture 69/70, which was converted by reduction to the corresponding N-trifluoroacetamido derivative in the presence of Raney nickel in a mixture of ethyl acetate and trifluoroacetic anhydride for 20 h at room temperature under 1 atm of hydrogen. The resulting anomeric mixture was separated by preparative TLC giving the crystalline β -anomer 80 in 41% yield (mp 189-190° C) and the crystalline α -anomer 81 in 30% yield (mp 173-174° C). It is interesting to note that an α/β mixture can also be crystallized directly from the crude reaction mixture, albeit in low yield. As in the case of 76/77, acetolysis of the anomeric mixture 80/81 gave the corresponding 6-O-acetyl-2,4-di-O-benzyl-3-deoxy-3-trifluoroacetamido- α/β -D-glycopyranosyl acetate (82) in 67% yield. Hydrogenolysis of the anomeric mixture 80/81 in the presence of palladium on charcoal in methanol and acetic acid under 3.6 kg/cm² of hydrogen for 18 h gave a quantitative yield of 83/84. The α -anomer 84 could be crystallized from methanol and ether (mp 264-265° C). Hydrolysis of the N-trifluoroacetyl group was readily achieved in conc. ammonium hydroxide in a sealed tube at 100° C for 18 h to give a mixture of methyl-3-amino-3-deoxy- α/β -D-glucopyranoside, which was characterized by comparison with the previously prepared α -anomer (see above).

Methylation of 81 in a mixture of methyl iodide and DMF in the presence of silver oxide at room temperature was not successful, but at 100° C in a sealed tube for 4 h a quantitative conversion to a single faster moving compound was observed by TLC. Examination of the ¹H-NMR spectrum of the N-methylated derivative, however, did not show a sharp N-methyl peak in the 3 to 3.3 δ region (54), but rather only a broad singlet (<3 H) was seen at 3.2 δ . Since the elemental analysis and the mass spectrum (M⁺-CH₃OH at m/e 541) fitted the structure 86, we assumed that the broad signal in the ¹H-NMR spectrum was due to restricted rotation, a phenomenon often seen in acetamido derivatives (55). When the methylation was carried out on the anomeric mixture 80/81, separation by chromatography permitted the isolation of the β -anomer 85 in 29% yield and the α -anomer 86 in 33% yield. Acetolysis of 86 in a 1:1 mixture of acetic acid and acetic anhydride containing 1% of conc. sulfuric acid at room temperature for 15 min gave, after column chromatography, 60% of the desired 6-O-acetyl-2,4-di-O-benzyl-3-deoxy-3-N-methyl-3-trifluoroacetamido- β -D-glucopyranosyl acetate 87 which is suitable for condensation with our protected pseudodisaccharide intermediates.



	<u>R¹</u>	<u>R²</u>	<u>R³</u>
79	αOAc	Ac	H
82	α/βOAc	TFA	H
87	βOAc	TFA	Me



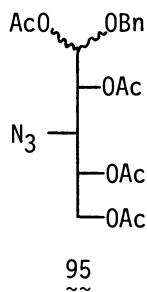
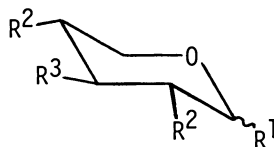
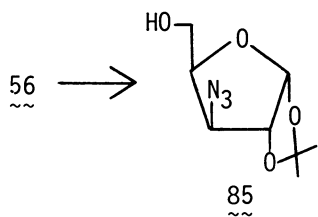
	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>
80	βOMe	OBn	TFA	H
81	αOMe	OBn	TFA	H
83	βOMe	OH	TFA	H
84	αOMe	OH	TFA	H
85	βOMe	OBn	TFA	Me
86	αOMe	OBn	TFA	Me

Sodium metaperiodate oxidation of 3-azido-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (48) (59) followed by reduction of the resulting aldehyde with sodium borohydride in ethanol at 0° C gave 3-azido-3-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (56) (88) in almost quantitative yield. Alcoholysis of 88 with benzyl alcohol or methanol containing anhydrous hydrogen chloride for 10-20 h at 60° C gave the corresponding anomeric mixtures of benzyl- and methyl-D-xylopyranosides 89 and 90 (57) in yields of 82% and 96% respectively. Acetylation of the xylopyranosides gave the corresponding 2,4-di-O-acetyl derivatives 91 and 92 in 57% and 35% yields respectively, and 93 (51) and 94 in 39% and 47% yields respectively. Acetolysis of 91 with 2% conc. sulfuric acid in acetic anhydride at room temperature for 30 min gave 67% of a material which was shown by NMR spectroscopy to have the

acyclic structure 95. Benzylolation of the anomeric mixture 90 using sodium hydride and benzyl bromide in DMF gave the corresponding 2,4-di-O-benzyl derivatives, which were separated by column chromatography giving the β -anomer 96 (51) in 49% yield and the α -anomer 97 in 39% yield. Reduction of the crude anomeric mixture 96/97 with Raney nickel in a mixture of acetic anhydride and ethyl acetate under 1 atm of hydrogen for 1 h at room temperature gave the β -anomer 98 and the α -anomer 99 in 79% overall yield from 90. Acetolysis of 99 in acetic anhydride containing up to 1.5% sulfuric acid at room temperature for 24 h gave an anomeric mixture of the xylopyranosyl acetates 100 and 101 and the methyl- β -D-xylopyranoside derivative 98 in a 6:2:2 ratio; prolongation of the reaction time did not complete the conversion of 98 to the desired glycosyl acetate. The anomeric mixture 98/99 was therefore hydrolyzed with a mixture of acetic acid and 6 N hydrochloric acid (88.5:11.5) (51) and the free xylose derivative 102, isolated in 67% yield, was acetylated to give, in 87% yield, a mixture of 100 and 101 in a 2:1 ratio. This anomeric mixture provides another useful intermediate to generate analogues of aminocyclitol antibiotics. Hydrogenolysis of 99 in the presence of palladium on charcoal under 3.6 kg/cm² of hydrogen for 16 h at room temperature gave methyl-3-acetamido-3-deoxy- α -D-xylopyranoside (103) in 81% yield $[[\alpha]_D^{25} 138^\circ$ (c 1.0, MeOH)]. Hydrolysis of the N-acetyl of 103 with hydrazine hydrate at 100° C for 16 h gave the known (54) methyl-3-amino-3-deoxy- α -D-xylopyranoside (104). Methylation of 98 with sodium hydride and methyl iodide in tetrahydrofuran at room temperature for 18 h (58) gave a good yield of the desired N-methyl derivative 105. This derivative once again shows restricted rotation in the ¹H-NMR spectrum, but in this case distinct signals for each rotamer (N-CH₃ 2.07 and 2.47; N-COCH₃ 1.85 and 2.13; 100 MHz, CDCl₃) are apparent. Similarly, when 105 is examined by TLC at -18° C (CHCl₃-acetone, 95:5) two distinct spots can be seen. Methylation of the anomeric mixture 98/99 gave, in 91% yield, the corresponding N-methyl derivatives 105/106, which were acetolyzed in a mixture of acetic acid, acetic anhydride and conc. sulfuric acid (50:50:2) at room temperature for 15 min to give the desired xylopyranosyl acetates 107 in 57% yield. This anomeric mixture is also useful for condensation with our protected neamine intermediates (vide infra).

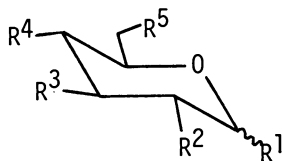
A recent paper (59) describes the preparation of various highly active analogues of kanamycin in which 3-amino-3-deoxy-D-glucose has been replaced by a 3,4,6-trideoxy-3-methylamino-D-xylo-hexopyranose moiety. We felt it would be interesting to condense a similar derivative with our intermediates 43 and 45.

In order to obtain a suitable monosaccharide for condensation, we started our synthesis from the anomeric mixture 60/61, which was treated with benzaldehyde dimethyl acetal in DMF in the presence of a trace of perchloric acid at 70° C for several hours to give the corresponding 4,6-O-benzylidene derivatives 108 in 85% yield. Benzylolation of 108 permitted chromatographic resolution of the anomeric mixture giving the β -anomer 109 (32%) and

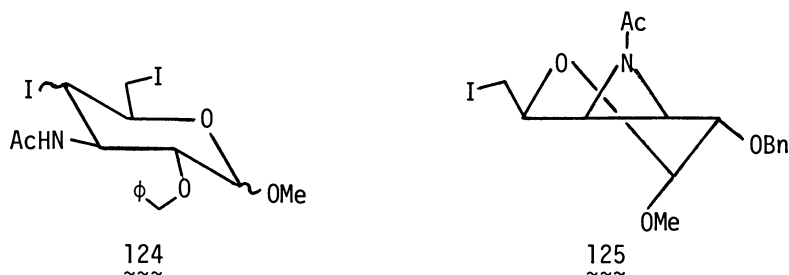


	<u>R¹</u>	<u>R²</u>	<u>R³</u>
89	α/β OBn	OH	N ₃
90	α/β OMe	OH	N ₃
91	β OBn	OAc	N ₃
92	α OBn	OAc	N ₃
93	β OMe	OAc	N ₃
94	α OMe	OAc	N ₃
96	β OMe	OBn	N ₃
97	α OMe	OBn	N ₃
98	β OMe	OBn	NHAc
99	α OMe	OBn	NHAc
100	β OAc	OBn	NHAc
101	α OAc	OBn	NHAc
102	α/β OH	OBn	NHAc
103	α OMe	OH	NHAc
104	α OMe	OH	NH ₂
105	β OMe	OBn	MeNAc
106	α OMe	OBn	MeNAc
107	α/β OAc	OBn	MeNAc

the α -anomer 110 (54.5%), both in crystalline form. The possibility of using the 2,4-dinitrophenyl group as a protecting group for the amine function was explored on the β -anomer 111, obtained by reduction of 109 with Raney nickel. Reaction of 111 with 2,4-dinitrofluorobenzene in a mixture of pyridine and dichloromethane at room temperature for 15 min gave the protected derivative 112 in 78% yield (mp 194-195° C). This approach was abandoned, however, in favor of the following one. Hydrolysis of 110 in 80% acetic acid at 80° C for 15 min gave crystalline 113 in quantitative yield. Mesylation of the latter gave the corresponding 4,6-di-O-mesyl derivative 114 in 98% yield. Similarly, hydrolysis of 109 gave crude 115, which was directly treated with mesyl chloride in pyridine to give a mixture of compounds from which the 4,6-di-O-mesyl derivative 116 was isolated in low yield. The other major product was the 4-O-acetyl-6-O-mesyl derivative 117, which presumably results from traces of acetic acid left in crude 115. Combined with mesyl chloride, acetic acid must act as an acetylating agent and lead to 117. Hydrolysis of the acetyl group gave 118, which was mesylated to give 116 identical to that above. Displacement of the mesyl groups from 116 with sodium iodide in butanone gave the 6-iodo derivative 119 in 83% yield and only a trace of the desired 4,6-diiodo glycoside. Reduction of 114 with Raney nickel in the presence of acetic anhydride and ethyl acetate under 3.6 kg/cm² of hydrogen for 18 h gave the corresponding 3-N-acetyl derivative 120, which was isolated as a crystalline solid in 79% yield. A similar reaction was performed on the anomeric mixture 114/116 and gave the corresponding 3-N-acetyl derivative 120/121 in good yield. Displacement of the 4,6-di-O-mesyl derivative 120 with sodium iodide in DMF at 80° C for 12 h gave, surprisingly, the 6-iodo-4-O-mesyl derivative 122 as the only product of the reaction. Reduction of 122 with Raney nickel produced the 6-deoxy derivative 123, which gave a complex mixture when reacted with sodium iodide in DMF or in butanone. On the other hand, displacement of 120 or the anomeric mixture 120/121 with sodium iodide in butanone under reflux for up to 48 h gave various amounts of the 4,6-diiodo gluco- and galactoside isomers 124 and, in one case, a small amount of the N-acetyl-3,4-imino derivative 125. These results indicate that neighboring group participation at C-3 is necessary for successful displacement of the C-4 mesylate. A similar result has been observed by Richardson (60). Reduction of the mixture of isomers 124 with Raney nickel in methanol under one atmosphere of hydrogen at room temperature for 2 days gave a 57% yield of the crystalline 4,6-dideoxy α -anomer 126, which was acetylated in the presence of acetic acid and acetic anhydride (1:1) containing 2% conc. sulfuric acid for 35 min at room temperature to give an anomeric mixture of glycosyl acetates. The major α -anomer 127 was isolated as a crystalline solid in approximately 55% yield and was used for condensation with a pseudodisaccharide intermediate (see below). Methylation of 126 with methyl iodide and sodium hydride



	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>
108 ~~~~	α/βOMe	OH	N ₃		
109 ~~~~	βOMe	OBn	N ₃		
110 ~~~~	αOMe	OBn	N ₃		
111 ~~~~	βOMe	OBn	NH ₂		
112 ~~~~	βOMe	OBn	NHDNP		
113 ~~~~	αOMe	OBn	N ₃	OH	OH
114 ~~~~	αOMe	OBn	N ₃	OMs	OMs
115 ~~~~	βOMe	OBn	N ₃	OH	OH
116 ~~~~	βOMe	OBn	N ₃	OMs	OMs
117 ~~~~	βOMe	OBn	N ₃	OAc	OMs
118 ~~~~	βOMe	OBn	N ₃	OH	OMs
119 ~~~~	βOMe	OBn	N ₃	OMs	I
120 ~~~~	αOMe	OBn	NHAc	OMs	OMs
121 ~~~~	βOMe	OBn	NHAc	OMs	OMs
122 ~~~~	αOMe	OBn	NHAc	OMs	I
123 ~~~~	αOMe	OBn	NHAc	OMs	H
126 ~~~~	αOMe	OBn	NHAc	H	H
127 ~~~~	αOAc	OBn	NHAc	H	H
128 ~~~~	αOMe	OBn	MeNAc	H	H
129 ~~~~	α/βOAc	OBn	MeNAc	H	H



in tetrahydrofuran for 1 h at room temperature gave the corresponding N-methylacetamido derivative 128, which underwent acetolysis under the same conditions used with 126 to give 84% of the desired glycosyl acetate 129, another suitable intermediate for condensation with our protected pseudodisaccharides 43 or 45 (*vide infra*).

Having a series of suitably protected 3-amino-3-deoxy glycosyl acetates available, we first attempted to condense the 3-acetamido-6-acetyl-2,4-di-O-benzyl-3-deoxy- α -D-glucopyranosyl acetate 79 with the 3',4'-unsaturated neamine intermediate 45 in the presence of stannic chloride (61,62,63) in boiling acetonitrile. This led to a complicated mixture from which a pseudo-trisaccharide was isolated in only 6% yield. In view of the poor yield of condensation with 45, probably due to its low solubility, the more soluble 3',4'-di-O-mesylnamine intermediate 43 was investigated next. Conversion of 79 into the corresponding glycosyl bromide 130 was accomplished by slowly passing dry hydrogen bromide into a solution of 79 in dichloromethane at 0° C. After removal of the solvent, the crude glycosyl bromide 130 was condensed with 43 in the presence of mercuric cyanide, mercuric bromide (64) and calcium sulfate in nitromethane at room temperature for 5 days. Chromatography of the resulting mixture led to the isolation of the desired α -anomer 131 in 50-56% yield and the corresponding β -anomer in 2% yield. Using mercuric cyanide alone gave 131 in lower yield (40%). Mercuric bromide alone did not catalyze the glycosidation, nor did a mixture of silver carbonate and silver perchlorate (65,66). Using silver triflate (67) in dichloromethane at -22° C for 4 days followed by room temperature for 2 days gave 9% of 131 and 7% of the corresponding β -anomer. Finally, when the glycosidation was done in the presence of tetraethylammonium bromide (68) and molecular sieves (4 Å) in dichloromethane at room temperature for 8 days, only 14% of 131 was isolated.

Conversion of the 3-trifluoroacetamido-6-O-acetyl-2,4-di-O-benzyl-3-deoxy-D-glucopyranosyl acetates 82 into the crystalline α -glycosyl bromide 132 was effected as described above.

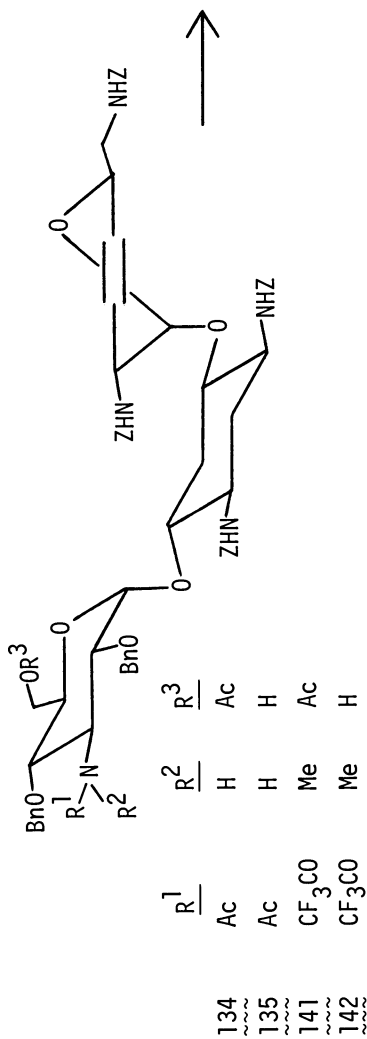
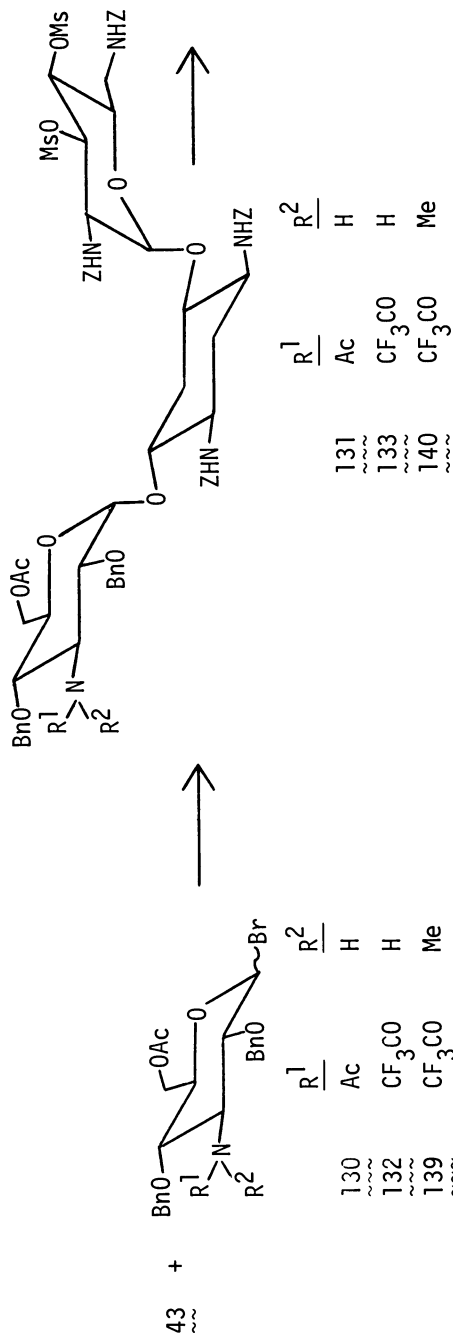
Condensation of 43 and 132 in the presence of mercuric cyanide, mercuric bromide and calcium sulfate in nitromethane proceeded very quickly, the α -glycoside 133 being isolated in 35% yield after only 3 h reaction at room temperature. A substantial amount (18%) of β -glycoside was also formed under these conditions.

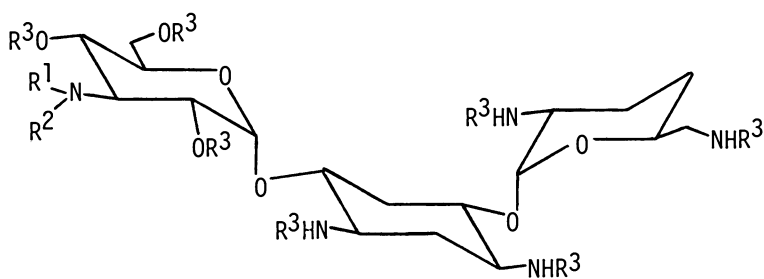
It is interesting to note the remarkable increase in reactivity of the glycosyl bromide 132 over 130. It is unfortunate, however, that this is accompanied by the formation of an increased amount of the undesired β -glycoside, since the trifluoroacetyl group can be readily removed under fairly mild conditions.

Treatment of 131 with sodium iodide and zinc in DMF at 110° C for 90 min gave 134 in 35% yield after column chromatography. Hydrolysis of the 6"-O-acetyl group of 134 with methanolic ammonia at room temperature for 16 h gave 135 in 82% yield. Hydrogenolysis of the benzyl and benzyloxycarbonyl groups of 135 was effected using palladium hydroxide on carbon (31) and gave, after chromatography over IRC-50 (NH₄⁺) resin, 3"-N-acetyl-3',4',5-trideoxykanamycin B (136) in 80% yield. Hydrazinolysis of 136 at 100° C in a sealed tube for 19 h then gave the desired 3',4',5-trideoxykanamycin B (137), which was isolated as its pentahydrochloride salt in 43% yield and was characterized as the peracetyl derivative 138. A recent paper (19) has reported the preparation of 137 by a completely different route using Barton's deoxygenation process (69).

Treatment of 87 with anhydrous hydrogen bromide in dichloromethane gave the corresponding glycosyl bromide 139 which, without any purification, was reacted with 43 in the presence of mercuric cyanide, mercuric bromide and calcium sulfate in anhydrous nitromethane for 18 h at room temperature giving the α -glycoside 140 in 96% yield after purification by preparative TLC. The structure of 140 was established by comparison of the ¹³C-NMR spectra (DMSO) of 85 (C-1: 104.71 ppm) and 86 (C-1: 96.36 ppm) with that of 140 (C-1: 95.77 ppm). The slightly lower reactivity of 139 as compared to 132 is not as surprising as the remarkable increase in yield and the exclusive formation of the α -anomer 140. Elimination of the 3'- and 4'-mesyl groups from 140 by treatment with sodium iodide and zinc in DMF for 1 h at 110° C gave the unsaturated derivative 141 in 43% yield. Removal of the 6"-O-acetyl group from 141 with methanolic ammonia gave, in 75% yield, the crystalline derivative 142, which was reduced with palladium hydroxide on carbon (31) in acetic acid at room temperature for 24 h under 3.6 kg/cm² of hydrogen to give 143 in 44% yield.

Condensation of the bromo sugar 144, obtained from 107 in the usual manner, with the protected pseudodisaccharide 43 in the presence of tetraethylammonium bromide and activated molecular sieves (4 Å) in dichloromethane at room temperature for 7 days gave the α -glycoside 145 in 47% yield. The Lemieux method of condensation (68) was chosen over that using mercuric salts (64) because, in this particular case, it gave a very much improved yield. The purification of 145 was especially laborious due to





	<u>R¹</u>	<u>R²</u>	<u>R³</u>
136	Ac	H	H
137	H	H	H
138	Ac	H	Ac
143	H	Me	H

the presence of stable rotamers, which rendered TLC analysis very difficult. Treatment of 145 with sodium iodide and zinc in DMF at 110° C for 3 h had to be repeated once in order to bring the reaction to completion. Purification of 146 was also laborious but did permit the isolation of an analytically pure and crystalline material, albeit in low yield (18.5%). Hydrogenolysis followed by hydrazinolysis then gave, in 21% overall yield, the desired aminocyclitol analogue 147.

The glycosyl bromide 148 was prepared from 127 as described above. Condensation of 148 with 43 was done according to the Lemieux method (68) and gave, after 5 days at room temperature, a mixture of α - and β -glycosides in 62% yield. The mercuric salts procedure (64) gave only a 44% yield of anomeric pseudotrisaccharides, the desired α -anomer 149 being isolated after chromatography as a crystalline solid in about 30% yield. Treatment with sodium iodide and zinc in DMF at 110° C for 30 min then gave the unsaturated derivative 150 in 26% yield. The desired aminocyclitol derivative 151 was then obtained in 39% yield by hydrogenolysis of 150 followed by hydrazinolysis.

Treatment of the glycosyl acetates 129 with anhydrous hydrogen bromide in dichloromethane at 0° C for 15 min gave the glycosyl bromide 152, which was condensed with 43 using Lemieux's method (68) (35% yield of 153) and the mercuric salts procedure (64) (30% of 153). In the latter procedure we were able to recover 65% of unreacted 43, bringing the effective yield of 153 to 85%. In none of the previous condensations were we able to recover any unreacted 43, even when glycoside formation did not proceed satisfactorily. The usual treatment of 153 with sodium iodide and zinc in DMF at 110° C for 30 min gave the crystalline unsaturated derivative 154 in 27% yield. Hydrogenolysis in glacial acetic acid under 4 kg/cm² of hydrogen for 14 h at room temperature followed by hydrazinolysis at 100° C for 20 h gave, after purification over an IRC-50 (NH₄⁺) resin column, the desired aminocyclitol 155 in 50% overall yield. The final product 155 was isolated as its pentahydrochloride salt and characterized as the perbenzyloxycarbonyl derivative 156. The aminocyclitol antibiotic 155 has recently been reported in the patent literature (70).

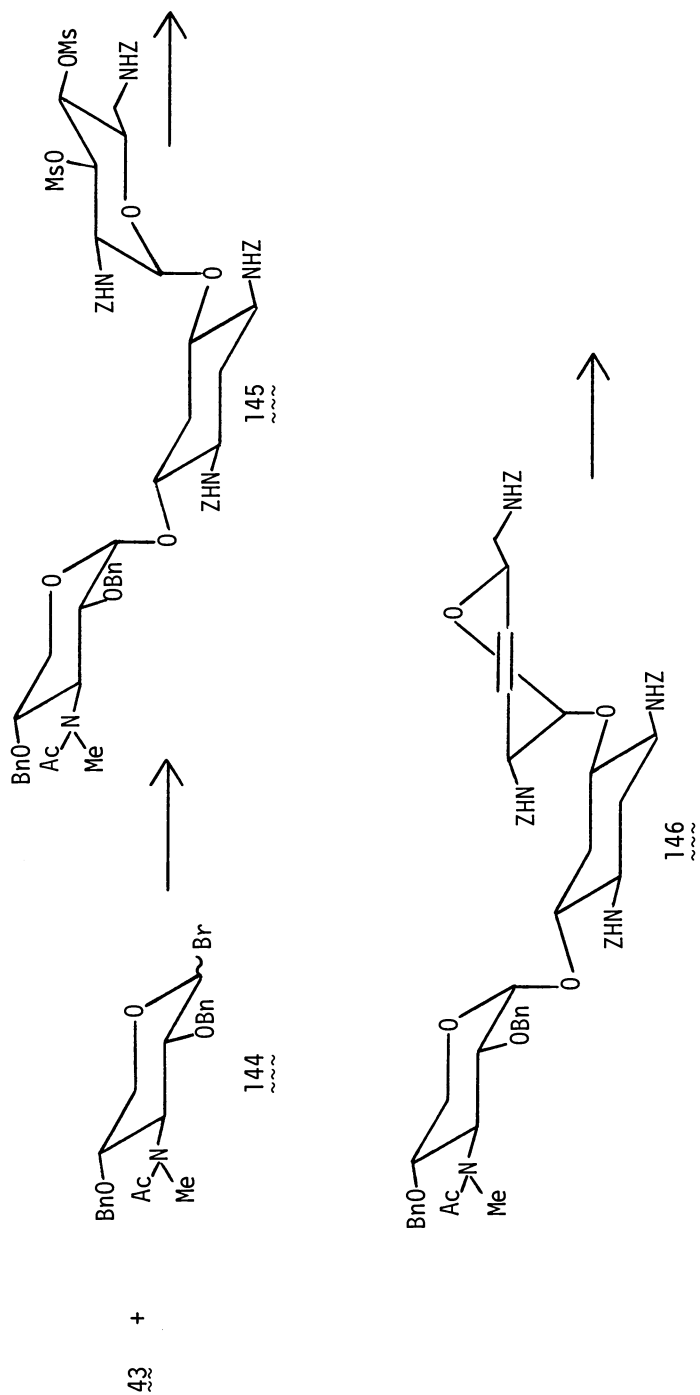
Comparison of the ¹³C-NMR spectra of the aminocyclitol antibiotics that were synthesized with that of dibekacin shows, once again, a 5 ppm upfield shift for C-1', indicating a substantial change in configuration between the streptamine ring and 4-O-glycoside ring (71,72,73). As proposed earlier, this change of configuration is probably due to the absence of hydrogen bonding between the amine function at C-2' and the hydroxyl at C-5 (see Figure 1). Similarly, C-4 and C-6 are shifted upfield by approximately 6 ppm due to the absence of a hydroxyl group at C-5, and C-1, C-2 and C-3 are shifted slightly for the same reason. C-2', C-3', C-4', C-1" and C-2" remain fairly constant in all five analogues. Upon N-methylation C-3" is shifted downfield,

Table II
¹³C-NMR Chemical Shifts (a)

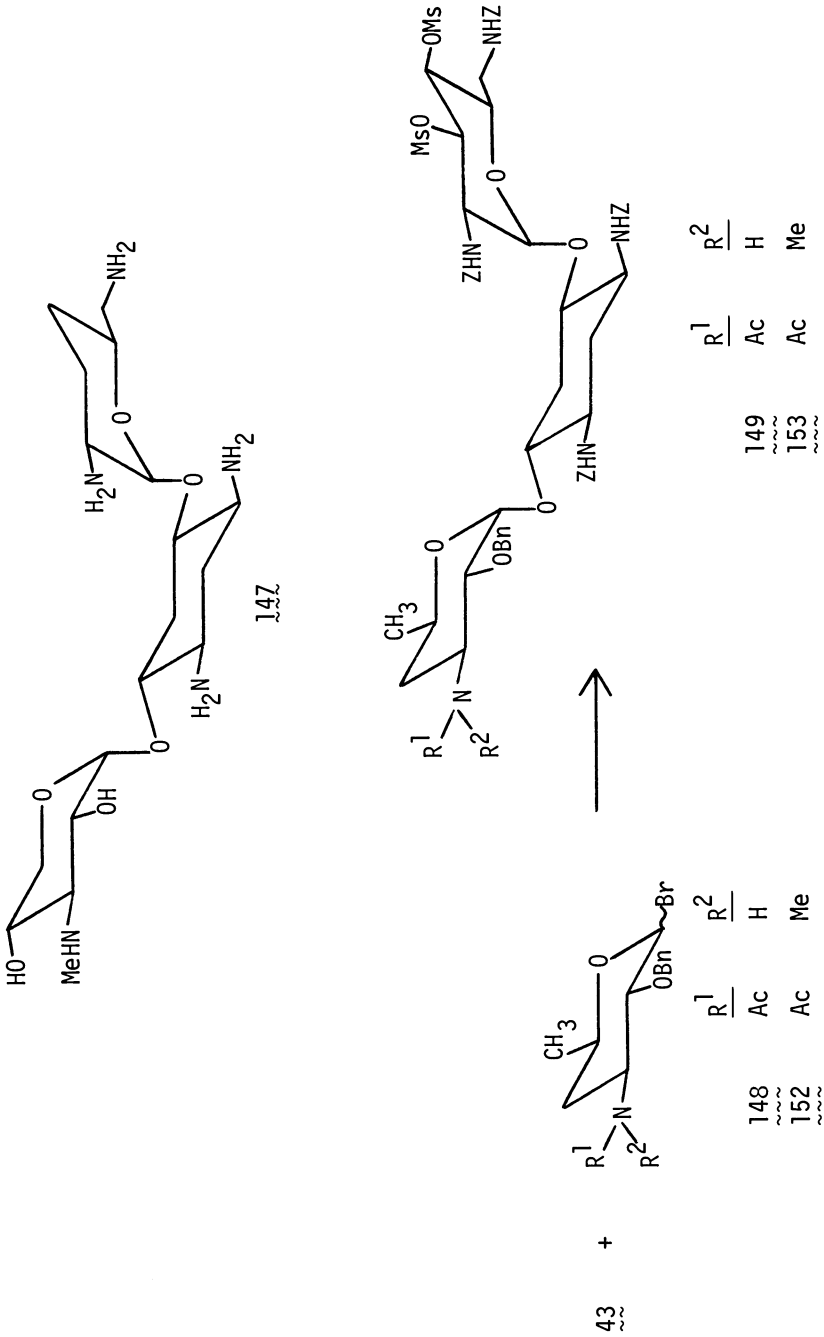
	N-Me	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6	1"	2"	3"	4"	5"	6"
56(b)		96.8	50.1	26.9	28.3	71.2	45.7	54.3	36.3	53.0	78.3	37.2	73.9						
Dibekacin		95.8	51.0	21.8	26.6	69.6	43.6	49.7	31.8	49.7	86.0	75.7	80.0	101.5	67.1	55.7	66.5	73.5	60.9
137		90.8	52.2	21.7	26.3	68.8	43.5	51.8	33.2	48.9	78.7	29.3	73.4	100.3	66.9	55.7	66.5	71.1	61.3
143		30.2	90.7	52.1	21.7	26.4	66.9	43.5	51.8	33.2	48.9	29.2	73.6	100.5	64.9	61.3	66.5	71.0	61.3
147		30.7	90.6	52.2	21.6	26.5	67.1	43.5	51.7	32.9	48.9	28.9	70.7	100.3	64.5	61.7	66.6	36.2	
151		90.5	52.3	21.7	26.4	66.6	43.5	51.7	32.7	48.9	78.6	29.0	70.5	100.9	65.9	49.3	36.1	70.1	20.3
155		30.4	90.8	52.3	21.7	26.3	69.3	43.4	51.8	33.5	48.9	29.1	70.9	100.9	66.0	56.3	32.9	70.9	20.5

(a) In parts per million downfield from tetramethylsilane, solvent D₂O.

(b) Solvent D₂O-NaOD, pH 12.



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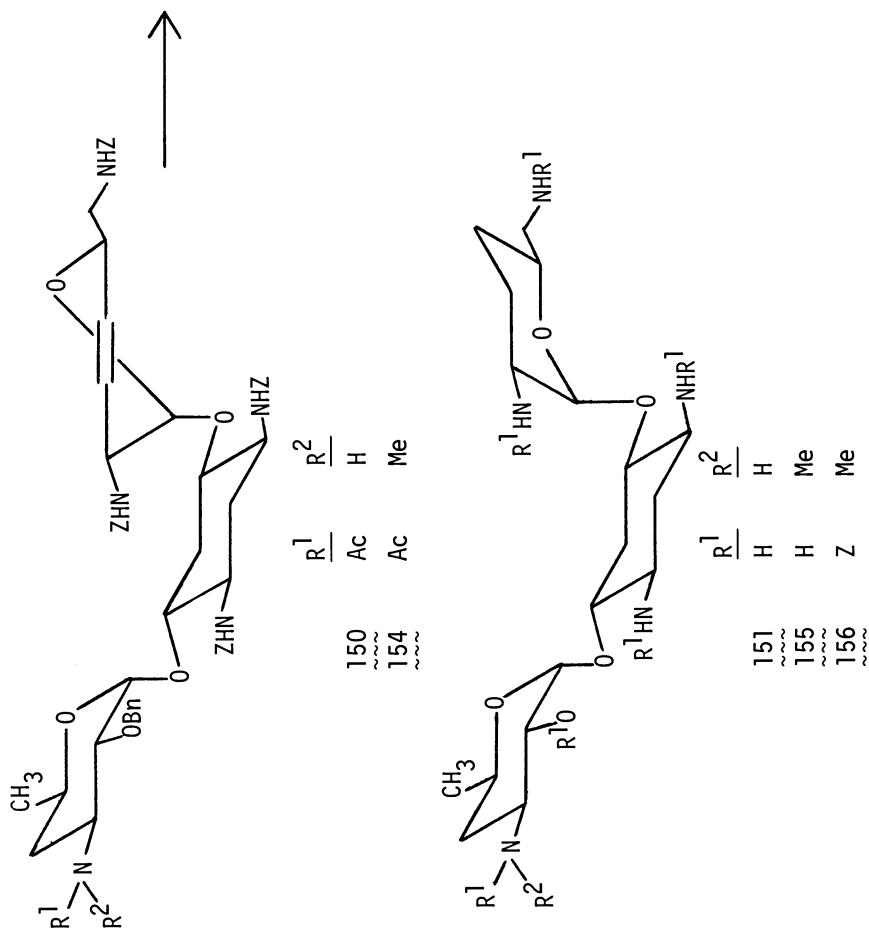


Table III
Antimicrobial Activity MIC ($\mu\text{g/mL}$) (a)

Test Organism	23	26	56	Dibekacin	137	143	147	151	155	Gentamicin
<i>S. aureus</i> Sa 482	125	25	25	0.8	6	0.8	8	0.8	25	0.4
Sa 481	---	---	50	1.6	12	1.6	8	0.8	25	3
Sa 435	---	---	---	---	---	0.025	4	0.025	1.6	0.8
<i>S. faecalis</i> Sf 545	---	---	---	---	---	>200	>200	200	25	25
<i>S. pyogenes</i> Sp 466	125	50	50	25	50	12	>200	25	---	6
<i>E. aerogenes</i> Er 555	---	---	---	---	---	25	32	12	50	3
<i>E. coli</i> Ec 13	250	50	200	6	25	25	64	12	25	6
<i>K. pneumoniae</i> Kp 447	---	---	---	---	12	12	16	6	12	1.6
Kp 461	64	25	50	1.6	---	---	---	---	---	---
<i>S. marcescens</i>	---	---	200	25	50	200	64	200	200	3
<i>P. aeruginosa</i> Pa 484	500	200	50	0.8	6	12	16	500	100	3
<i>P. mirabilis</i> Pr 428	500	100	---	---	---	25	16	25	25	3
<i>P. morgani</i> Pm 454	250	25	---	---	---	25	---	25	25	---
<i>P. vulgaris</i> Pv 467	250	50	100	12	50	25	---	25	25	---

(a) Minimal Inhibitory Concentrations (MIC) were determined using a broth microdilution technique. The growth medium used was trypticase soy broth (pH 7.0) and the inoculum was 5×10^5 CFU/mL. Results were read visually at 24 h after incubation at 35°C.

and upon deoxygenation C-4" and C-6" are shifted upfield as expected (see Table II).

The antimicrobial activities of 137, 143, 147, 151 and 155 are listed in Table III. In general, the MIC's of our analogues were above those of dibekacin, even those of 137, whose only structural difference consists of the deoxygenation of C-5. Methylation of the 3"-amine function of 137 appears to increase the activity against the Gram positive S. aureus but unfortunately does not improve the activity against the Gram negative organisms. Contrary to the above, removal of the 3"-methyl group of 155 also appears to increase the activity against S. aureus but once again does not substantially change the activity of 151 against the more important Gram negative organisms. In conclusion, it appears that deoxygenation at C-5 is detrimental to the antimicrobial activity of dibekacin and its related derivatives.

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Literature Cited

1. Nagaoka, K. and Demain, A. L., J. Antibiot. (1975), 28, 627.
2. Rinehart, Jr., K. L., Pure Appl. Chem. (1977), 49, 1361.
3. Shier, W. T., Rinehart, Jr., K. L. and Gottlieb, D., Proc. Natl. Acad. Sci. USA (1969), 63, 198.
4. Cleophax, J., Gero, S. D., Leboul, J., Akhtar, M., Barnett, J. E. G. and Pearce, C. J., J. Am. Chem. Soc. (1976), 98, 7110.
5. Kojima, M. and Satoki, A., J. Antibiot. (1973), 26, 551.
6. Suami, T., Ogawa, S., Uchino, H. and Funaki, Y., J. Org. Chem. (1975), 40, 456.
7. Testa, R. T., Wagman, G. H., Daniels, P. J. L. and Weinstein, M. J., J. Antibiot. (1974), 27, 917.
8. Dawn, S. J., Rosi, D. and Goss, W. A., J. Am. Chem. Soc. (1977), 99, 283.
9. Umezawa, H., Umezawa, S., Okazaki, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn. (1972), 45, 3624.
10. Miyake, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Carbohydr. Res. (1976), 49, 141.
11. Kawaguchi, H., Naito, T., Nakagawa, S. and Fujisawa, K., J. Antibiot. (1972), 25, 695.
12. Wright, J. J., J. Chem. Soc., Chem. Commun. (1976), 206.
13. For a review, see Umezawa, S., in "Advances in Carbohydrate Chemistry and Biology," Vol. 30, p. 111, R. S. Tipson and D. Horton, Eds., Academic Press, New York, 1974.

14. Umezawa, H., in "Advances in Carbohydrate Chemistry and Biology," Vol. 30, p. 183, R. S. Tipson and D. Horton, Eds., Academic Press, New York, 1974.
15. Umezawa, S., Yagisawa, M., Sawa, T., Takeuchi, T., Umezawa, H., Matsumoto, H. and Tazaki, T., J. Antibiot. (1975), 28, 845.
16. Le Goffic, F., Martel, A., Capmau, M. L., Baca, B., Goebel, P., Chardon, H., Soussy, C. J., Duval, J. and Bouanchaud, D. H., Antimicrob. Agents Chemother. (1976), 10, 258.
17. Santanam, P. and Kayson, F. H., J. Infect. Dis. (1976), 134, 33.
18. Rosi, D., Goss, W. A. and Daum, S. J., J. Antibiot. (1977), 30, 88.
19. Hayashi, T., Iwaoka, T., Takeda, N. and Ohki, E., Chem. Pharm. Bull. (1978), 26, 1786.
20. Cron, M. J., Smith, R. E., Hooper, I. R., Keil, J. G., Ragain, E. A., Schreiber, R. H., Schawb, G. and Godfrey, J. C., Antimicrob. Agents Chemother. (1969), 219.
21. Haskell, T. H., Rodebaugh, R., Plessas, N., Watson, D. and Westland, R. D., Carbohydr. Res. (1973), 28, 263.
22. Sanger, F., Biochem. J. (1945), 39, 507.
23. Loyd, P. F. and Stacey, M., Chem. Ind. (London) (1955), 917.
24. Umezawa, S. and Koto, S., J. Antibiot. (1966), 19A, 88; Bull. Chem. Soc. Jpn. (1966), 39, 2014.
25. Umezawa, S. and Koto, S., J. Antibiot. (1964), 17A, 186.
26. Bergman, M. and Zervas, L., Chem. Ber. (1931), 64, 975.
27. Umezawa, S. and Nishimura, Y., J. Antibiot. (1977), 30, 189.
28. Watanabe, I., Tsuchiya, T., Takase, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn. (1977), 50, 2369.
29. Umezawa, S., Tsuchiya, T., Jikihara, T. and Umezawa, H., J. Antibiot. (1971), 24, 711.
30. Umezawa, S., Koto, S., Tatsuta, K., Hineno, H., Nishimura, Y. and Tsumura, T., Bull. Chem. Soc. Jpn. (1969), 42, 537.
31. Pearlman, W. H., Tetrahedron Lett. (1967), 1663.
32. Suami, T., Nishiyama, S., Ishikawa, Y. and Katsura, S., Carbohydr. Res. (1978), 65, 57.
33. Jennings, H. J. and Jones, J. K. N., Can. J. Chem. (1962), 40, 1408.
34. Suami, T., Nishiyama, S., Ishikawa, Y. and Katsura, S., Carbohydr. Res. (1977), 53, 239.
35. Furst, A., Berlo, R. C. and Hooton, S., Chem. Rev. (1965), 65, 51.
36. Arita, H., Ueda, N. and Matsushima, Y., Bull. Chem. Soc. Jpn. (1972), 45, 567.
37. Grady, G. L. and Kuivila, H. G., J. Org. Chem. (1969), 34, 2014.
38. Suami, T., Ogawa, S., Uchino, H. and Funaki, F., J. Org. Chem. (1975), 40, 456.
39. Kavadias, G., Velkof, S. and Belleau, B., Can. J. Chem. (1978), 56, 404.

40. Sneider, H.-J. and Hoppen, V., Tetrahedron Lett. (1974), 579.
41. Jennings, H. J. and Jones, J. K. N., Can. J. Chem. (1965), 43, 2372.
42. Hasegawa, A., Nishimura, D. and Nakajima, M., Agric. Biol. Chem. (1972), 36, 1043.
43. Jikihara, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn. (1973), 46, 3507.
44. Umezawa, S., Okazaki, Y. and Tsuchiya, T., Bull. Chem. Soc. Jpn. (1972), 45, 3624.
45. Tipson, R. S. and Cohen, A., Carbohydr. Res. (1965), 1, 338.
46. Nishimura, T., Tsuchiya, T., Umezawa, S. and Umezawa, H., Bull. Chem. Soc. Jpn. (1977), 50, 1580.
47. Brimacombe, J. S., Bryan, J. G. H., Husain, A., Stacey, M. and Tolley, M. S., Carbohydr. Res. (1967), 3, 318.
48. Meyer zu Reckendorf, W., Chem. Ber. (1968), 101, 3802.
49. Barlow, C. B., Guthrie, R. D. and Murphy, D., J. Chem. Soc. (1965), 3870.
50. Gigg, R. and Warren, C. D., J. Chem. Soc. (1965), 2205.
51. Hasegawa, A. and Kiso, M., Carbohydr. Res. (1975), 44, 121.
52. Koto, S., Tsumura, T., Kato, Y. and Umezawa, S., Bull. Chem. Soc. Jpn. (1968), 41, 2765.
53. Baer, H. H., Chem. Ber. (1960), 93, 2865.
54. Jackman, L. M., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," p. 56, Pergamon, New York, 1959.
55. Jackman, L. M. and Sternhell, S., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Second Edition, p. 361, in International Series of Monographs in Organic Chemistry, Vol. 5, Pergamon, New York, 1969.
56. Ohri, H., Kuzuhara, H. and Emoto, S., Agric. Biol. Chem. (1970), 34, 375.
57. Tsuchiya, T., Suo, K. and Umezawa, S., Bull. Chem. Soc. Jpn. (1970), 43, 531.
58. Cheung, S, T. and Benoiton, L. N., Can. J. Chem. (1977), 55, 906.
59. Ghazan, J. B. and Gase, J. C., Tetrahedron Lett. (1976), 3145.
60. Richardson, A. C., Proc. Chem. Soc. (1963), 131.
61. Lemieux, R. U. and Shyluk, W. P., Can. J. Chem. (1953), 31, 528.
62. Honma, K., Nakajima, K., Uematsu, T. and Hamada, A., Chem. Pharm. Bull. (1976), 24, 394.
63. Ingle, T. R. and Bose, J. L., Carbohydr. Res. (1970), 12, 459.
64. Wallace, J. E. and Schroeder, L. R., J. Chem. Soc., Perkin Trans. II (1976), 1632.
65. Hasegawa, A., Kurihara, N., Nishimura, D. and Nakajima, M., Agric. Biol. Chem. (1968), 32, 1123.
66. Sitrin, R. D., Cooper, D. J. and Weisbach, J. A., J. Antibiot. (1977), 30, 836.

67. Schuerch, C. and Kronzer, F. J., Carbohydr. Res. (1973), 27, 379.
68. Lemieux, R. U., Hendricks, K. B., Stick, R. V. and James, K., J. Am. Chem. Soc. (1975), 97, 4056.
69. Barton, D. H. R. and Combie, S. W., J. Chem. Soc., Perkin Trans. I (1975), 1574.
70. Roussel-Uclaf, French Patent 2,351,660 (May 21, 1976), Ghazan, J. B. and Gase, J. C.
71. Lemieux, R. U. and Koto, S., Tetrahedron (1974), 30, 1933.
72. Nagabhushan, T. L. and Daniels, P. J. L., Tetrahedron Lett. (1975), 747.
73. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y., Tetrahedron Lett. (1977), 179.
74. Sano, H., Imura, S., Tsuchiya, T. and Umezawa, S., Bull. Chem. Soc. Jpn. (1978), 51, 3661.

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The Selective *N*-Acylation of Kanamycin A

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Amikacin, IIc, a 4-amino-2(S)-hydroxybutyryl derivative of Kanamycin A, first reported by Kawaguchi, et al.^(1,2) is a widely used antibacterial agent having greater activity than Kanamycin A against Gram negative and Gram positive bacteria.⁽³⁾ Its preparation in good yield by selective *N*-acylation of Kanamycin A has proved difficult. Acylation of Kanamycin A base, I, in aqueous tetrahydrofuran using 4-benzyloxycarbonylamino-2(S)-hydroxybutyric acid *N*-hydroxysuccinimide active ester (III) leads preferentially to substitution in the 6'-*N* position to give IIa, with only 5% formation of IIc.^(2,4,5) Acylation of 6'-*N*-benzyloxycarbonyl Kanamycin A in the form of its tri-Schiff's base leads to increased yields of IIc, but with significant formation of the other isomers.⁽⁶⁾ We now report a novel procedure for the selective *N*-acylation of Kanamycin A. In the work described below 4-benzyloxycarbonylamino-2(S)-hydroxybutyric acid *N*-hydroxysuccinimide active ester (III) was the preferred acylating agent. III can be easily obtained in a crystalline form and generally gives consistently higher yields than the corresponding norbornyl active ester IV.^(7,8)

When a suspension of Kanamycin A free base containing 1% Kanamycin A sulfate was refluxed in acetonitrile with hexamethyldisilazane (HMDS—7 moles/mole Kanamycin A) (Figure 1), complete dissolution of the Kanamycin base took place after 2½ hours. Separation of the polytrimethylsilylated Kanamycin A as an oil occurred shortly thereafter. The amount of oil increased over the 16-20 hour reflux period and corresponded to about 90% conversion of the starting material. After removal of the acetonitrile and excess HMDS *in vacuo* the polytrimethylsilylated Kanamycin obtained was recovered as an oil. The weight of product isolated indicated the presence of 8-10 trimethylsilyl groups per Kanamycin molecule. The material was readily soluble in most organic solvents. Acylation of this polytrimethylsilylated Kanamycin A in acetone with III gave primarily IIa (ca. 50%); smaller amounts of IIb (ca. 5%), IIc (ca. 5%), and polyacylated Kanamycin A (ca. 20%) were also obtained. IIc was not detected.

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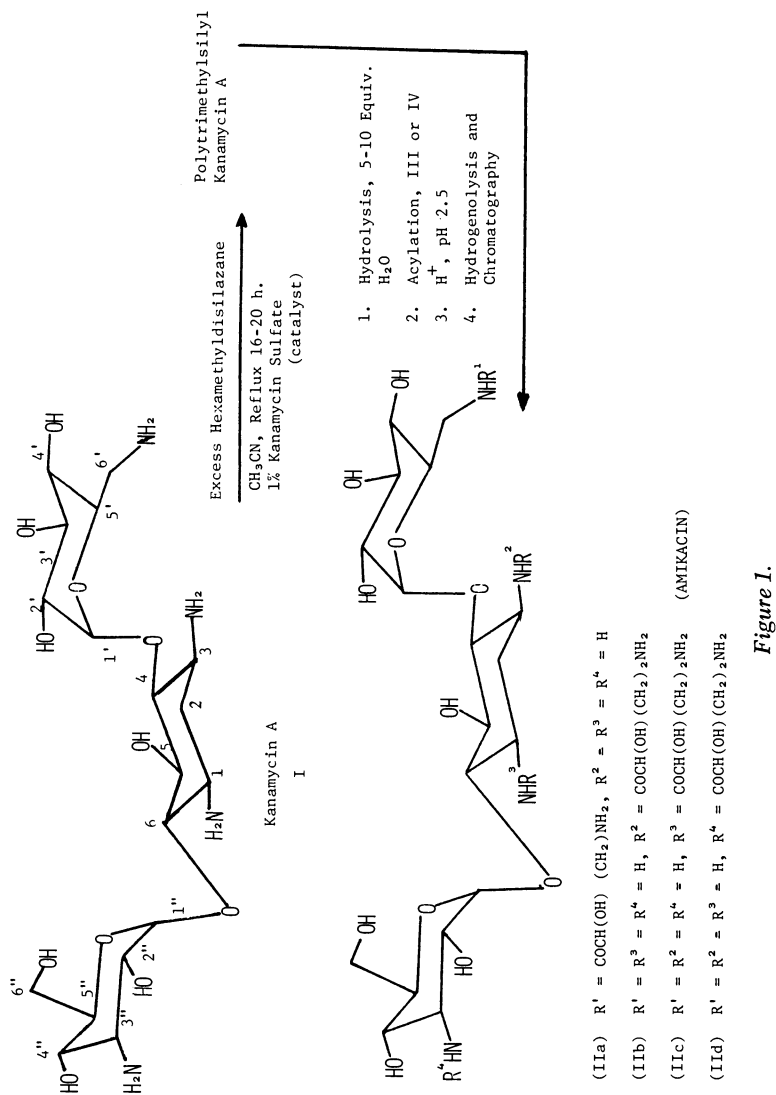


Figure 1.

About 20% of Kanamycin A was recovered.

During this work a number of acylation reactions were carried out on trimethylsilylated Kanamycin A containing less than 8-10 trimethylsilyl groups. In these cases a different pattern of products was produced. In particular, a notable increase in the yield of IIc was observed. A systematic study of the acylation of partially hydrolyzed polytrimethylsilylated Kanamycin A was undertaken leading to a procedure which favored the formation of IIc.

Polytrimethylsilylated Kanamycin A, containing 8-10 trimethylsilyl groups, was dissolved in acetone and stirred in vacuo or under nitrogen with 10 molar equivalents of water or methanol at 5°C for 30 minutes, and the resulting solution was acylated at 5°C for 1 hour. The mixture was hydrolyzed with water at pH 2.5, hydrogenolyzed and chromatographed on an Amberlite CG-50 (NH₄⁺) column. In this case the major product isolated was IIc (50%). Small amounts of IIa (6%), IIb (12%), polyacylated Kanamycin A (8%), and unchanged Kanamycin A (22%) were obtained. Again IIId was not detected.

In previously used procedures for the preparation of IIc,^(2, 4, 5, 6) the IIId isomer is formed in appreciable amounts and separation of the two by chromatography using Amberlite CG-50 is difficult. It is of interest that in the present procedure this undesirable isomer is not found.

A number of acylation reactions were carried out in which the ratio of acylating agent to trimethylsilylated Kanamycin A was varied from 0.5 to 1.2. In general, it was observed that with low values the ratio of IIc/IIId became larger, the degree of polyacylation decreased and the unreacted Kanamycin A levels were higher. With high values the IIc/IIb ratio became lower, the degree of polyacylation increased and the unreacted Kanamycin A levels were lower. Maximum productivity of IIc was obtained when the ratio of acylating agent to partially hydrolyzed polytrimethylsilyl Kanamycin A was in the 0.7-0.9 range.

We have shown that methanol and other hydroxylic reagents can replace water without affecting the reaction. On the other hand, changing the acylation solvent can cause dramatic effects on the selectivity of the reaction. Thus, when partially hydrolyzed polytrimethylsilyl Kanamycin A is acylated with III using heptane in place of acetone as solvent, acylation occurs primarily at the 6'-N position to yield IIa.

The acylation temperature is also important in determining the ratio of the products formed. In general, lower temperatures lead to better 1-N/3-N ratios.

Silylation of Kanamycin A sulfate using HMDS in acetonitrile solution yielded polytrimethylsilylated Kanamycin A sulfate. This material was also soluble in most organic solvents. Acylation with III or IV in acetone solution with or without prior hydrolysis with water led to the IIa isomer as the major product.

Only traces of the IIc and IIb were obtained. A similar result was obtained when 1 mole equivalent of N-hydroxynorbornene was incorporated during the trimethylsilylation of Kanamycin A base.

Trimethylsilylation of 6'-N-benzyloxycarbonyl Kanamycin A in acetonitrile solution using HMDS affords 6'-N-benzyloxycarbonyl polytrimethylsilyl Kanamycin A. Acylation and subsequent workup of this product by the procedure described yields IIc and IIb in an approximately 1:1 ratio along with smaller amounts of polyacylated derivatives. If the polytrimethylsilylated product is partially hydrolyzed with water before acylation, then an approximately 2:1 ratio of the IIc isomer to the IIb isomer is formed.

The results of this study are summarized in Table 1. The use of partially trimethylsilylated Kanamycin A, procedure 3, shows a significant increase in IIc (Amikacin) yield as compared to previously reported procedure 1 or 2.^(2,4,5,6) A higher degree of silylation, procedure 4, leads to a low yield of IIc. Use of the trimethylsilylated derivative of 6'-N-benzyloxycarbonyl Kanamycin A, procedures 5 and 6, again leads to reasonable IIc yields. In this case the degree of silylation is not as critical with respect to the IIc yield.

From examination of molecular models it would seem reasonable in the case of procedure 3 that the low yield of IIa and the absence of IIc result from the shielding effects of bulky O-trimethylsilyl groups in the 4', 2'' and 4'' positions. An explanation of the preferential acylation at the 6'-N group in the case of the more fully trimethylsilylated derivative, procedure 4, awaits a clearer understanding as to distribution of these groups in the molecule.

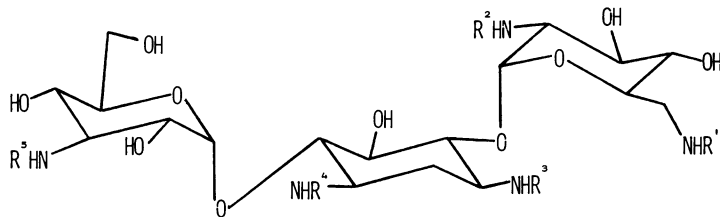
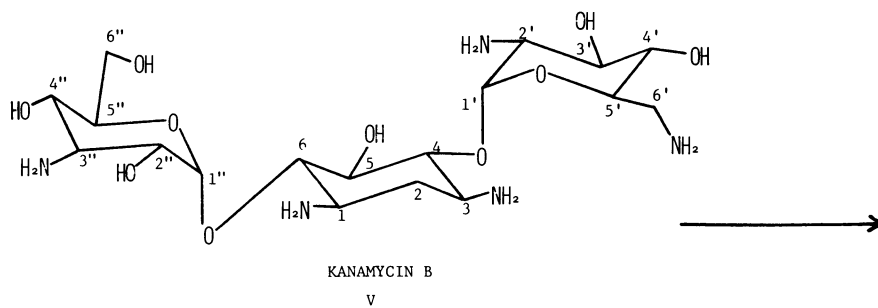
Studies parallel to those discussed for Kanamycin A, Figure 2, have been undertaken using Kanamycin B (V) with similar results. Acylation of partially trimethylsilylated Kanamycin B in acetone solution using either III or IV and using the conditions already described for Kanamycin A gave a mixture of products. The major component, isolated in 34% yield was the only one showing antibacterial activity. Acid hydrolysis of this major product gave 2,6-diaminoglucose and 3-aminoglucose but no deoxystreptamine, indicating the site of acylation must be on the deoxystreptamine moiety. Comparison of the Minimum Inhibitory Antibacterial spectra of the major product with that reported for an authentic sample of the 1-N isomer (VI_d)⁽⁴⁾ showed them to be identical. The 3-N isomer (VI_c) is biologically inactive. Thin layer chromatography of the product mixture gave a pattern very similar to that obtained from Kanamycin A. On this basis the residual Kanamycin B, the other product isomers, and the polyacylated materials were tentatively identified: VI_c, 10%; VI_a, 5%; VI_b and VI_e, not detectable (Figure 3).

TABLE 1

N-Acylation Yields from Kanamycin A Using 4-Benzylloxycarbonylamino-2(S)-Hydroxybutyric Acid

Procedure ^①	Results ^⑦					
	Kanamycin	6'-N	3-N	1-N	3"-N	Polyacetyls
1. Non-Blocked ^②	10-15	45-50	5-10	5	5	15-20
2. 6'-N-Benzylloxycarbonyl-1,3,3/-N-Tri Schiff Base ^③	-	-	-	19.2	-	-
3. Partially Trimethylsilylated ^{④⑤}	22	6	12	50	None	8
4. Polytrimethylsilylated ^④	20	50	5	5	None	20
5. 6'-N-Benzylloxycarbonyl Polytrimethylsilylated ^④	10-14	-	38-42	38-42	None	5
6. 6'-N-Benzylloxycarbonyl Partially Trimethylsilylated ^{④⑤}	20	-	20	45	None	5

- Key
- ① 0.9 mole equivalent of ester per mole of starting Kanamycin A at 5° for 1 hour.
 - ② Acylation in 50% aqueous tetrahydrofuran.
 - ③ Acylation in dimethylformamide.
 - ④ Acylation in acetone.
 - ⑤ Back hydrolyzed polytrimethylsilylated Kanamycin A, prior to acylation with water, 5-10 molar equivalents per mole of starting Kanamycin A, 5° for 30 minutes 'in vacuo'.
 - ⑥ Back hydrolyzed prior to acylation as in ⑤.
 - ⑦ Percent calculated from rotation at λ 578 of eluted Amberlite CG-50 (NH₄⁺) column cuts.



(VIa) $R^1 = \text{COCH(OH)(CH}_2)_2\text{NH}_2$, $R^2 = R^3 = R^4 = R^5 = \text{H}$

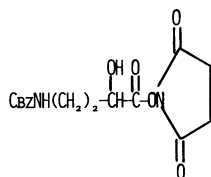
(VIb) $R^1 = R^3 = R^4 = R^5 = \text{H}$, $R^2 = \text{COCH(OH)(CH}_2)_2\text{NH}_2$

(VIc) $R^1 = R^2 = R^4 = R^5 = \text{H}$, $R^3 = \text{COCH(OH)(CH}_2)_2\text{NH}_2$

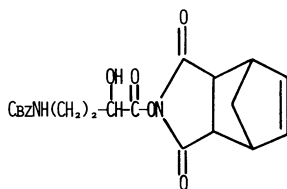
(VIId) $R^1 = R^2 = R^3 = R^5 = \text{H}$, $R^4 = \text{COCH(OH)(CH}_2)_2\text{NH}_2$

(VIe) $R^1 = R^2 = R^3 = R^4 = \text{H}$, $R^5 = \text{COCH(OH)(CH}_2)_2\text{NH}_2$

Figure 2.



III



IV

Figure 3.

ABSTRACT

1-N-(L-(-)-4-amino-2(S)-hydroxybutyryl) Kanamycin A (Amikacin) is a widely used antibacterial agent having greater activity than Kanamycin A against Gram negative and Gram positive bacteria. Treatment of Kanamycin A with hexamethyldisilazane in refluxing acetonitrile affords polytrimethylsilylated Kanamycin A. Partial hydrolysis of this material followed by acylation with 4-benzyloxycarbonylamino-2(S)-hydroxybutyric acid N-hydroxysuccinimide active ester has been found to proceed in a highly selective manner; acylation occurs primarily at the 1-N position rather than the usual 6'-N position.

LITERATURE CITED

- ¹Kawaguchi, H.; Naito, T.; Nakagawa, S. and Fujisawa, K.; J. Antibiotics, 1972, 25, 695.
- ²Naito, T.; Nakagawa, S.; Abe, Y.; Toda, S.; Fujisawa, K.; Miyaki, T.; Kashiya, H.; Ohkuma, H. and Kawaguchi, H. J. Antibiotics, 1973, 26, 297.
- ³Price, K. E.; Chisholm, D. R.; Misiek, M.; Leitner, F. and Tsai, Y. H.; J. Antibiotics, 1970, 25, 709.
- ⁴U.S. Pat. No. 3781268, 1973.
- ⁵U.S. Pat. No. 3792037, 1974.
- ⁶U.S. Pat. No. 3974137, 1976.
- ⁷Cron, M. J.; Keil, J. G.; Lin, J. S.; Ruggeri, M. V.; Walker, D.; Journal of the Chemical Society Chem. Comm., 1979, 266.
- ⁸Belg. Pat. No. 866586, 1978.

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Carbon-13 NMR Spectra of Aminoglycoside Antibiotics

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Carbon-13 nuclear magnetic resonance (cmr) spectroscopy has played an increasingly important role in the structure elucidation of organic molecules. Especially, Pulse-Fourier-Transform technique developed in late 1960's has enabled the determination of cmr spectra in natural abundance, which has provided a powerful tool for investigation of various classes of natural products and related compounds. To our knowledge, the first paper (1) dealing with cmr in the field of aminoglycoside antibiotics (AG) was published in 1970, which described the cmr spectra of hygromycin B and destomycin A, although the spectra were not fully assigned at that time. Four papers then appeared in 1973, which described the assignment of cmr spectra of kanamycins A and B (2), gentamicins C₁, C_{1a} and C₂ (3), ribostamycin (4) and butirosin A (5). Since then increasing number of papers (6-58) have been reported to date as shown in Table 1.

Table 1. Numbers of papers describing cmr data of aminoglycoside antibiotics (AG)

year	<u>1970</u>	<u>1971</u>	<u>1792</u>	<u>1973</u>	<u>1974</u>	<u>1975</u>	<u>1976</u>	<u>1977</u>	<u>1978</u>
No. of papers	1	0	0	4	4	7	7	13	22

There are presented lists of various types of AG's obtained from natural sources (Table 2), by semi-synthesis (Table 3) and either by mutational biosynthesis or by AG-modifying enzymes (Table 4), for which cmr spectra have been reported.

Some of the published papers gave only cmr spectra or chemical shifts of the compounds but most papers have described assignments of the C-13 signals of their compounds. The assignments of the cmr spectra of AG have been made by comparison with the cmr spectra of their building blocks, such as 2-deoxystreptamine (DOS), 2-amino-2-deoxyglucose (2-AG), 3-AG, 6-AG, 2,6-AG, garos-

Table 2. Naturally-occurring aminoglycoside antibiotics for which cmr data have been reported.

Antibiotic	Reference
4,6-Disubstituted DOS derivatives	
Kanamycin A	<u>2, 9, 13, 15, 35, 43, 44, 55</u>
Kanamycin B	<u>2, 8, 13, 46, 50, 51</u>
Kanamycin C	<u>32</u>
Tobramycin	<u>8, 19, 37, 46</u>
Nebramycins	<u>8, 46</u>
Gentamicin A's	<u>13, 15, 16</u>
Gentamicin B	<u>13, 25, 55</u>
Gentamicin C's	<u>3, 10, 11, 29</u>
Sagamycin (Gentamicin C _{2b})	<u>10, 11</u>
Gentamicin X ₂	<u>22</u>
Sisomicin	<u>3, 14, 18, 20, 33, 54</u>
66-40B, 40D	<u>14</u>
66-40C	<u>33</u>
66-40G	<u>54</u>
G-52	<u>18</u>
Seldomycin factor 1	<u>24, 25</u>
Seldomycin factor 5	<u>25, 29, 31, 36, 53</u>
4,5-Disubstituted DOS derivatives	
Neomycin B	<u>7, 40</u>
Neomycin C	<u>9</u>
Paromomycin	<u>41</u>
Ribostamycin	<u>4, 43, 56</u>
Xylostasin	<u>59</u>
Butirosin A	<u>5, 50, 56, 57, 59</u>
Bu-1975 C (4'-deoxybutirosin A)	<u>59</u>
Monosubstituted DOS derivatives	
Apramycin	<u>17, 38, 46</u>
Nebramycin factor 7 (3'-OH-apramycin)	<u>46</u>
Paromamine	<u>4, 5, 13, 15, 16, 22, 24, 42</u>

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Table 2. (cont'd)

<u>Antibiotic</u>	<u>Reference</u>
Monosubstituted DOS derivatives	
Nebramycin factor 9 (3'-deoxyparomamine)	<u>46</u>
Neamine	<u>4, 5, 8, 24, 41, 42, 46</u> <u>47, 51</u>
Nebramine (3'-deoxyneamine)	<u>8, 24, 46</u>
Seldomycin factor 2	<u>24, 25</u>
Gentamine C's	<u>3, 24, 27</u>
Seldomycin factor 2 (4'-deoxyneamine)	<u>24</u>
Hygromycins	<u>1</u>
Destomycins	<u>1, 12</u>
Miscellaneous	
Streptomycins	<u>6</u>
Fortimicins	<u>27, 31</u>
Sorbistins	<u>34, 49</u>
LL-BM 123 α	<u>28</u>
LL-BM 123 β, γ	<u>48</u>

Table 3. Semi-synthetic aminoglycoside antibiotics for which cmr data have been reported.

Antibiotic	Reference
4,6-Disubstituted DOS derivatives	
Kanamycin A, N-AHBA	<u>44</u> , <u>45</u> , <u>55</u>
N-acetyl	<u>44</u> , <u>59</u>
N-ethyl	<u>59</u>
6'-N-methyl	<u>59</u>
4'-deoxy	<u>35</u> , <u>59</u>
4'-deoxy-6'-N-Me	<u>59</u>
(N-CHO) ₃₋₄	<u>39</u>
Kanamycin B, 3'-deoxy	<u>35</u>
3',4'-dideoxy	<u>35</u> , <u>37</u>
5-deoxy	<u>50</u> , <u>51</u>
analogs	<u>47</u>
Kanamycin C, N-AHBA	<u>32</u>
Gentamicin A, 3',4'-dideoxy-6'-NH ₂	<u>30</u>
Gentamicin B, N-AHBA, AHPA	<u>55</u>
Gentamicin X ₂ , 2'-epi	<u>21</u>
analogs	<u>22</u>
Gentamicin, analogs	<u>23</u>
Sisomicin, dihydro	<u>20</u>
Seldomycin, 3'-epi	<u>36</u> , <u>53</u>
3'-deoxy	<u>29</u> , <u>53</u>
analogs	<u>52</u>
4,5-Disubstituted DOS derivatives	
Ribostamycin, 6'-OH	<u>40</u>
Xylostasin, (N-CHO) ₃₋₄	<u>39</u>
Butirosin A, 3'- & 6-deoxy	<u>50</u>
Monosubstituted DOS derivatives	
Paromamine, 3'-epi	<u>42</u>
Neamine, 3'-epi	<u>42</u>
deoxy derivatives	<u>51</u>

Table 4. Aminoglycoside antibiotics modified by inactivating enzymes or by mutational biosynthesis for which cmr data have been reported.

Antibiotic	Reference
AG modified by inactivating enzymes	
Tobramycin, 4'-O-adenylyl	<u>19, 37</u>
3',4'-Dideoxykanamycin B, 4''-O-adenylyl	<u>37</u>
Amikacin, 4'-O-adenylyl	<u>45</u>
3'-O-phosphoryl	<u>45</u>
Seldomycin factor 5, 3-N-acetyl	<u>31</u>
Apramycin, 3-N-acetyl	<u>38</u>
Fortimicin A, 1-N-acetyl	<u>31</u>
AG modified by mutational biosynthesis	
Gentamicin C ₁ , 2-OH	<u>26</u>
Butirosin A, 6'-N-Me	<u>57</u>
Butirosin A, 3',4'-dideoxy-6'-N-Me	<u>56</u>

amine, paromamine (PA), neamine (NA), gentamine (GA) etc., in consideration of (a) substitution and proximity effects by glycosidation, alkylation, acylation, deoxygenation etc., (b) β -carbon shifts that occur upfield on protonation of amino groups and (c) multiplicity by the single frequency off resonance decoupling method (3). Assignments of the cmr spectra of AG have been discussed in many papers. This report summarizes the published cmr data of AG together with our unpublished ones (59), hopefully to aid in making better use of the cmr spectra in the AG chemistry.

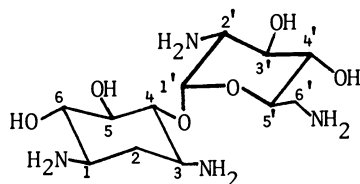
The cmr spectra of AG reported to date were mostly determined in D₂O using dioxane as an internal reference. The chemical shifts are indicated in ppm downfield from TMS using the following relation, $\delta(\text{TMS}) = \delta(\text{dioxane}) + 67.4$ ppm. Some of earlier papers (1, 5) reported chemical shifts in reference to external CS₂, which were, in this report, translated into the TMS scale by $\delta(\text{TMS}) = 193.7$ ppm - $\delta(\text{CS}_2)$. In the tables of this report, downfield shifts (deshielding) are expressed as positive values and upfield shifts (shielding) as negative values.

Morton et al. (3) observed in the cmr study on gentamicins C₁, C_{1a} and C₂ that the cmr data reported from various laboratories were generally in good agreement with each other and independent of techniques and instruments, by which they were determined. We also noted this fact on reviewing the published cmr reports of AG. Table 5 shows an example of neamine cmr data

reported from five institutions located in the United States, Canada and Japan. The last two columns of Table 5 show the mean value of the chemical shifts of neamine carbons obtained in the five laboratories and the ranges of deviation, indicating high reliance of cmr data.

Cmr data, however, are affected greatly by the sample used. Table 6 shows the cmr of kanamycin A in various forms of the sample determined in our laboratories (59). As shown in the last

Table 5. Cmr of neamine reported from various laboratories



Carbon	Chemical shift, δ (ppm)					Mean	Range of deviation (\pm)
	A	B	C*	D	E		
1	51.4	51.4	51.2	51.5	51.2	51.3	0.2
2	36.5	36.7	36.5	36.8	36.7	36.6	0.2
3	50.3	50.4	50.1	50.5	50.2	50.3	0.2
4	87.7	88.2	88.0	88.5	88.2	88.1	0.4
5	76.9	76.9	76.8	77.1	76.8	76.9	0.2
6	78.1	78.5	78.3	78.6	78.4	78.4	0.3
1'	101.5	101.8	101.6	101.9	101.7	101.7	0.2
2'	56.2	56.3	56.1	56.4	56.1	56.2	0.2
3'	74.4	74.6	74.4	74.7	74.5	74.5	0.2
4'	72.4	72.3	72.2	72.5	72.3	72.3	0.2
5'	73.4	74.0	73.9	74.3	74.0	73.9	0.5
6'	42.6	42.8	42.5	42.9	42.6	42.7	0.2

	Author	Institution	Year	Instrument used
A	Omoto et al. (4)	Meiji	1973	Varian XL-100-12
B	Koch et al. (8)	Lilly	1974	Varian DP-60
C	Koch et al. (46)	Lilly	1978	Jeol PFT-100
D	Hanessian et al. (41)	U. Montreal	1978	Brucker WH-90
E	Sitrin et al. (47)	SKF	1978	Varian CFT-20

* A 1.1 ppm correction has been added to the published values (8), because of difference in the reference chemical shift.

column of the table, some of the chemical shifts of kanamycin A vary considerably with the form of a sample dissolved in a solution. This pH-dependent character is largely due to the β -carbon shift caused by protonation of amino groups, which will be described later. Therefore, a great care should be taken for the preparation of cmr samples of AG, especially of the free base because AG readily absorbs carbon dioxide to form carbonate salt, resulting in C-13 signals which deviate significantly from those of the free base.

Fig. 1 shows chemical shift ranges for specific structural types of carbons usually existing in AG molecules. They are anomeric carbon, aglycon carbon, other oxygen-bearing carbons, nitrogen-bearing carbons and other. The carbon type, whether it is a methine, methylene or methyl, is shown by either closed, hatched or solid bar. Thus, the preliminary assignment of signals in a given spectrum would be made based on those carbon types, if necessary, in combination with the off resonance technique. For further assignments of the resonances, various substitution effects should be taken into consideration.

Table 6. ^{13}C -chemical shifts of kanamycin A in various forms (59) (solv., D_2O : internal ref., dioxane)

Carbon	Chemical shift (ppm, downfield from TMS)					Δ Max
	Base + NaOD (pD > 10)	Base	Base bubbled with CO_2	H_2SO_4 salt	Base + HCl (pD < 2)	
1	50.9	51.3	51.3	51.2	50.6	0.7
2	36.3	36.2	33.8	33.6	28.3	8.0
3	49.8	49.8	50.5	49.4	48.4	2.1
4	88.1	88.1	85.3	83.7	79.0	9.1
5	74.6	74.9	75.8	74.7	73.6	2.2
6	89.2	88.6	85.8	87.1	84.7	4.5
1'	100.1	100.3	101.0	98.4	96.3	4.7
2'	73.1	72.7	72.7	72.1	71.6	1.5
3'	74.6	73.7	73.2	73.2	73.0	1.6
4'	72.1	71.8	72.0	71.9	71.6	0.5
5'	74.1	73.7	72.4	71.0	69.5	4.6
6'	42.5	42.4	41.7	41.4	41.2	1.3
1''	101.6	100.8	101.4	100.9	101.2	0.8
2''	72.8	72.7	69.1	69.9	69.0	3.8
3''	55.4	55.0	55.6	55.5	55.8	0.8
4''	70.4	70.1	68.0	68.5	66.4	4.0
5''	73.3	72.9	73.2	73.2	73.0	0.4
6''	61.4	61.1	60.8	61.0	60.8	0.6

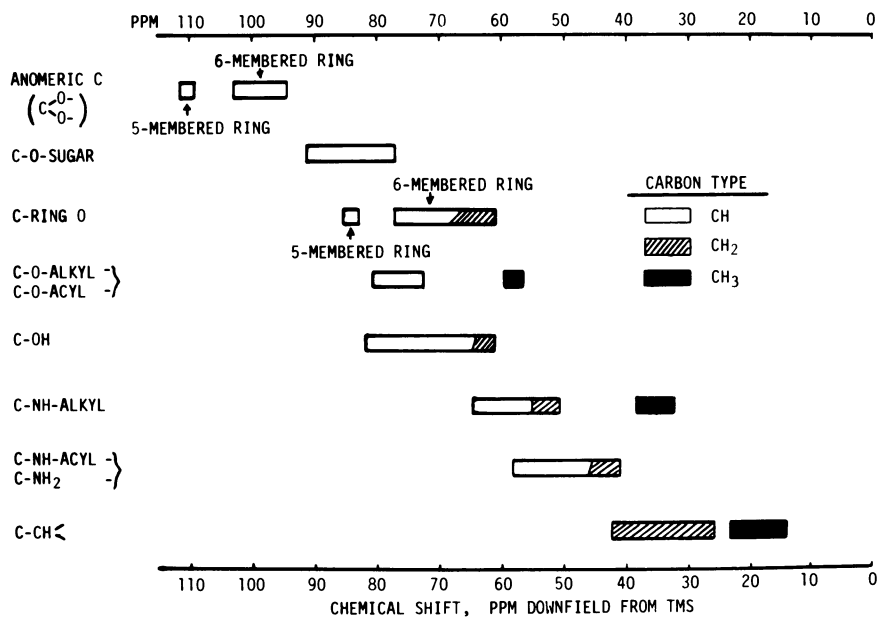


Figure 1. Typical ^{13}C chemical shifts of aminoglycoside antibiotics

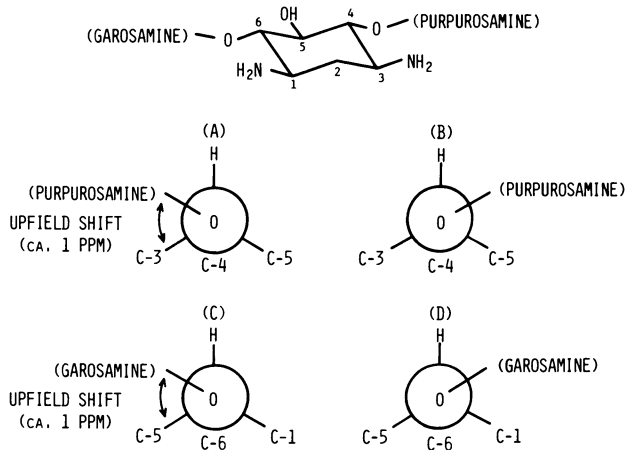


Figure 2. Interaction between carbons on the DOS moiety and aminosugars locating at the gauche position

Glycosidation shift of the DOS moiety

Most of AG are the 4,6-di-O-glycosylated DOS derivatives (kanamycin, gentamicin etc.) or the 4,5-di-O-glycosylated DOS (neomycin, ribostamycin etc.). The spectrum of DOS is easily assigned as shown in Table 7, because of its symmetric structure and the peak height. The effect of glycosidation on the cmr spectrum of DOS is expected to be similar to reported shifts for the methylation of hydroxyl groups in inositols (60): a 7 - 10 ppm downfield shift in the signal of α -carbon, a 4.5 ppm upfield shift on β -carbon bearing an axial hydroxyl group and a 0.5 ppm downfield shift on β -carbon bearing an equatorial hydroxyl group.

In cmr spectra of gentamines C₁, C_{1a} and C₂ (3), in which purpurosamines are linked glycosidically to the hydroxyl group at C-4 of DOS (Table 8), the C-4 and C-5 signals show expected downfield shifts by 9.6 - 10.3 ppm and 0.2 ppm, respectively, but the C-3 signals indicate an upfield shift of about 1 ppm.

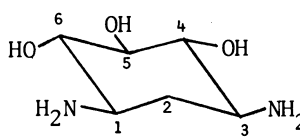
Garamine is another structural fragment of gentamicin in which an aminosugar named garosamine linked glycosidically at the 6-OH group of DOS. The cmr spectrum of garamine (20) showed expected downfield shifts of 9.4 ppm for the α -carbon, C-6 and 0.1 ppm for one of the β -carbons, C-1. However, the C-5 signal shows an upfield shift of 1.5 ppm as shown in Table 9.

Furthermore, gentamicins C₁, C_{1a} and C₂ (Table 10) undergo a combined shift due to both the 4- and 6-O-glycosidations, showing downfield shifts of 9 - 10 ppm for α -carbons, C-4 and C-6, and upfield shifts of around 1 ppm for two of the β -carbons, C-3 and C-5, and a small downfield shift for the remaining β -carbon, C-1. These data are consistent with those calculated from the glycosidation shifts of gentamine and garamine. The effect is essentially additive. Morton et al. (3) have described that the unexpected upfield shifts by about 1 ppm for C-3 and C-5 might be due to interaction with the aminosugars locating at the *gauche* position and, therefore, the preferred rotamer about the C-4-O bond should be formula A in Fig. 2 rather than formula B, and also the preferred rotamer about the C-6-O bond is like formula C. Both A and C satisfy the requirements of the exo-anomeric effect proposed by Lemieux and co-workers (61, 62).

Table 11 shows the glycosidation shifts of some 4-O-glycosyl DOS derivatives, such as paromamine, neamine, apramycin, aprosaminide and deoxygenated neamines, which show a downfield shift of 9 - 10 ppm for C-4, about 1 ppm upfield shift for C-3 and a small downfield shift for another β -carbon C-5. These shifts are in good agreement with those of gentamine C's.

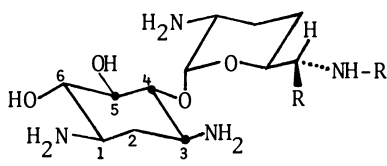
In several gentamicin derivatives listed in Table 12, were reported similar glycosidation shifts to those of gentamicin C's indicating a downfield shift of 9.0 - 10.1 ppm for α -carbons, C-4 and C-6, 1.3 - 1.6 ppm upfield shift for two of β -carbons, C-3 and C-5, 0.1 - 0.2 ppm downfield shift for the remaining β -carbon, C-1, as compared to chemical shifts of the corresponding carbon atoms of DOS.

Table 7. Cmr spectrum of 2-deoxystreptamine (3)



Carbon	Chemical shift
1,3	51.6 ppm
2	37.0
4,6	78.5
5	76.6

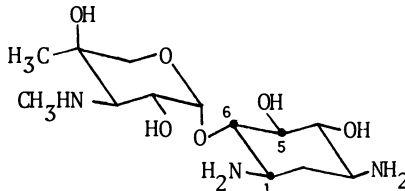
Table 8. Glycosidation shifts of gentamine C's (3)



	R	R'
C ₁	CH ₃	CH ₃
C _{1a}	H	H
C ₂	CH ₃	H

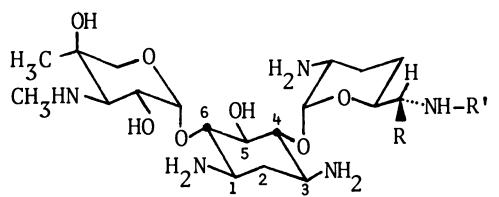
Carbon	Chemical shift, δ (ppm)				Difference, Δ δ (GA) - δ (DOS)
	DOS	GA-C ₁	GA-C _{1a}	GA-C ₂	
1	51.6	51.3	51.3	51.3	
2	37.0	36.8	36.8	36.8	
3	51.6	50.8	50.5	50.7	-0.8 ~ -1.1
4	78.5	88.8	88.1	88.7	+9.6 ~ +10.3
5	76.6	76.8	76.8	76.8	+0.2
6	78.5	78.4	78.3	78.3	

Table 9. Glycosidation shifts of garamine (20)



Carbon	Chemical shift, δ (ppm)		Difference Δ
	DOS	Garamine	
1	51.6	51.7	+0.1
2	37.0	36.6	
3	51.6	51.4	
4	78.5	78.8	
5	76.6	75.1	-1.5
6	78.5	87.9	+9.4

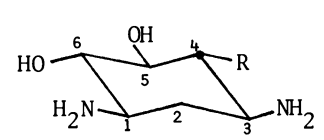
Table 10. Glycosidation shifts of gentamicin C's (3)



	R	R'
C ₁	CH ₃	CH ₃
C _{1a}	H	H
C ₂	CH ₃	H

Carbon	Chemical shift, δ (ppm)			Difference, Δ
	GM-C ₁	GM-C _{1a}	GM-C ₂	δ (GM) - δ (DOS)
1	51.8	51.7	51.8	+0.1 ~ +0.2
2	36.8	36.7	36.7	
3	50.9	50.6	50.8	-0.8 ~ -1.0
4	88.6	88.3	88.7	+9.8 ~ +10.2
5	75.4	75.4	75.3	-1.2 ~ -1.3
6	87.9	87.8	87.6	+9.1 ~ +9.4

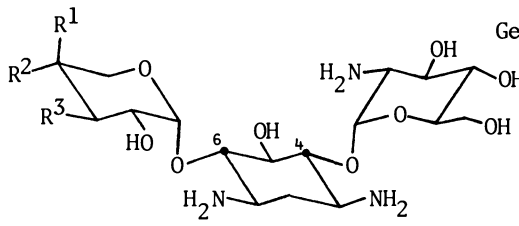
Table 11. Glycosidation shifts of 4-O-glycosyl DOS derivatives



Carbon	R							
	Paromamine		Neamine		Apramycin		Aprosamidine	
	δ	Δ	δ	Δ	δ	Δ	δ	Δ
1	51.2		51.4		51.3		51.3	
2	36.7		36.5		36.6		36.6	
3	50.4	-1.0	50.3	-1.1	50.4	-1.0	50.4	-1.0
4	88.7	+10.2	87.7	+9.2	87.8	+9.3	88.0	+9.5
5	76.7	+0.1	76.9	+0.3	76.8	+0.2	76.8	+0.2
6	78.2		78.1		78.5		78.3	
Ref.	(4)		(4)		(46)		(17)	
Carbon	3'-Deoxy NA		3'-Deoxy PA		4'-Deoxy NA		3',4'-Dideoxy NA	
	δ	Δ	δ	Δ	δ	Δ	δ	Δ
1	51.1		51.3		51.2		51.3	
2	36.6		36.6		36.6		36.7	
3	50.3	-1.1	50.5	-0.9	50.2	-1.2	50.5	-0.7
4	87.7	+9.2	88.1	+9.6	88.3	+9.8	88.3	+9.8
5	76.8	+0.2	76.9	+0.3	76.9	+0.3	76.9	+0.3
6	78.5		78.4		78.4		78.4	
Ref.	(46)		(46)		(24)		(3)	

δ = chemical shift, ppm Δ = δ (antibiotic) - δ (DOS)

Table 12. Glycosidation shifts of gentamicin analogs



	$\overline{R^1}$	$\overline{R^2}$	$\overline{R^3}$
Gentamicin A	H	OH	NHCH ₃
A ₁	OH	H	NHCH ₃
A ₂	H	OH	OH
A ₄	H	OH	N $\begin{matrix} \text{CH}_3 \\ \text{CHO} \end{matrix}$
X ₂	OH	CH ₃	NHCH ₃

Carbon	GM-A		GM-A ₁		GM-A ₂		GM-A ₄		GM-X ₂	
	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ
1	51.5	-0.1	51.5	-0.1	51.4	-0.2	51.5	-0.1	51.4	-0.2
2	36.5	-0.5	36.4	-0.6	36.2	-0.8	36.5	-0.5	36.6	-0.4
3	50.3	-1.3	50.3	-1.3	50.2	-1.4	50.2	-1.4	50.2	-1.4
4	88.6	+10.1	88.5	+10.0	88.1	+9.6	88.5	+10.0	88.6	+10.1
5	75.1	-1.5	75.2	-1.4	75.0	-1.6	75.1	-1.5	75.1	-1.5
6	87.9	+9.4	87.6	+9.1	87.9	+9.4	88.0	+9.5	87.5	+9.0
Ref.	(15)		(15)		(16)		(15)		(22)	

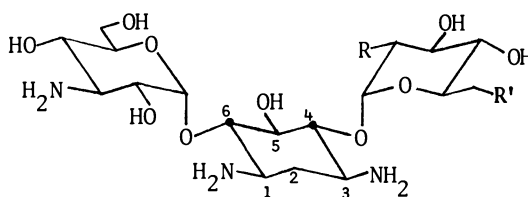
δ = chemical shift, ppm.

Δ = $\delta(\text{antibiotic}) - \delta(\text{DOS})$

The glycosidation shifts of other 4,6-di-O-glycosyl DOS derivatives, kanamycins A, B and C and deoxygenated kanamycins are shown in Table 13. The observed glycosidation shifts are similar to those of gentamicins, and this indicates that kanamycin is the same as gentamicin C in the stereochemistry about the two glycosidic oxygen atoms.

In the 4,5-di-O-glycosylated DOS derivatives (Table 14), the glycosidation shifts are quite different from those of the 4,6-di-O-glycosyl derivatives. As compared to DOS, ribostamycin and xylostasin show downfield shifts of about 4 ppm for C-4 and 8 ppm for C-5 with no significant shifts for other carbons of the DOS moiety. Similarly neomycin B and paromomycin undergo downfield shifts of about 5 ppm for both the C-4 and C-5 signals, without any significant shifts for the C-3 and C-6 signals as shown in Table 14.

Table 13. Glycosidation shifts of kanamycins



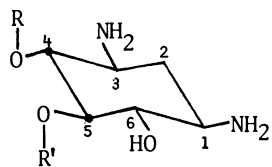
	R		R'	
A	OH		NH ₂	
B	NH ₂		NH ₂	
C	NH ₂		OH	

Carbon	Kanamycin A		Kanamycin B		Kanamycin C	
	δ	Δ	δ	Δ	δ	Δ
1	51.3		51.3		51.3	
2	36.2		36.5		36.2	
3	49.8	-1.6	50.3	-1.1	50.3	-1.1
4	88.1	+9.6	87.5	+9.0	87.9	+9.4
5	74.9	-1.7	75.2	-1.4	75.2	-1.4
6	88.6	+10.1	88.8	+10.3	88.5	+10.0

Ref.	(44)		(8)		(32)	
	4 ^L -Deoxy-KM-A		3 ^L -Deoxy-KM-B		DKB	
	δ	Δ	δ	Δ	δ	Δ
1	51.3		51.3		51.1	
2	36.3		36.3		36.3	
3	49.9	-1.5	50.1	-1.3	50.3	-1.1
4	88.3	+9.8	87.1	+8.6	86.9	+8.4
5	75.0	-1.6	75.5	-1.1	75.4	-1.2
6	88.6	+10.1	88.9	+10.4	89.0	+10.5

Ref.	(59)		(46)		(35)	
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Table 14. Glycosidation shifts of 4,5-di-O-glycosyl DOS



	R		R'	
Ribostamycin	Neosamine C		Ribose	
Xylostasin	Neosamine C		Xylose	
Neomycin B	Neosamine C		Neobiosamine B	
Paromomycin	2-Glucosamine		Neobiosamine B	

Carbon	Ribostamycin		Xylostasin		Neomycin B		Paromomycin	
	δ	Δ	δ	Δ	δ	Δ	δ	Δ
1	51.2		51.2		51.2		50.1	
2	36.7		36.5		36.5		36.5	
3	51.2		51.1		51.2		50.1	
4	83.0	+4.5	82.5	+4.0	83.2	+4.7	84.4	+5.9
5	85.0	+8.4	85.3	+8.7	82.4	+5.8	82.5	+5.9
6	78.4		78.6		78.4		78.3	

Ref.	(4)		(59)		(40)		(41)	
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Table 15. Deoxygenation shifts of glucose derivatives (66)

Carbon	Chemical shift, δ (ppm)		Difference $\delta(B) - \delta(A)$
	A	B	
1	99.9	98.9	
2	72.4	67.0	-5.4
3	70.5	32.6	-37.9
4	80.8	76.1	-4.7
5	62.0	63.4	
6	68.5	68.9	

Deoxygenated Derivatives

Bacterial resistance to AG (63) in clinical isolates is usually associated with the presence of plasmid-mediated enzymes, AG-phosphotransferases (APH), AG-nucleotidyltransferases (ANT) and AG-acetyltransferases (AAC), which O-phosphorylate, O-adenylate and N-acetylate antibiotics, respectively.

3',4'-Dideoxykanamycin B (DKB) synthesized by Umezawa et al. (64) was shown to be active against AG-resistant strains producing APH(3')-I and -II, which O-phosphorylate the 3'-OH group of AG. The naturally occurring 3'- and/or 4'-deoxygenated AG (65) such as tobramycin, gentamicin C's, sisomicin, lividomycin, Bu-1975 (4'-deoxybutirosin) etc., are also resistant to APH(3')-I and/or II. Therefore, deoxygenation of AG has become one of the major targets for the chemical modification and cmr spectra of many deoxygenated derivatives have been reported.

Conway and co-workers (66) reported deoxygenation effects in the cmr of glucose derivatives. By deoxygenation of the 3-OH group, the α -carbon, C-3, undergoes an upfield shift of about 38 ppm and β -carbons C-2 and C-4, a shift in the same direction by about 5 ppm as shown in Table 15.

Table 16 shows cmr spectral data of three 3'-deoxy derivatives of AG in terms of chemical shifts of the 2,6-AG moiety and chemical shift differences from those of the corresponding parent antibiotics. The deoxygenated C-3' (α -carbon) undergoes an upfield shift of 36.3 - 39.7 ppm and both C-2' and C-4' (β -carbons) by 5.3 - 6.9 ppm. The remaining carbons of the 3'-deoxy derivatives do not show any significant shifts as compared to those of parent antibiotics.

Deoxygenation effects of the 4'-deoxygenated AG (Table 17) are similar to those of the 3'-deoxy derivatives, showing an

Table 16. Deoxygenation shifts of 3'-deoxy derivatives of AG

	R^1	R^2	R^3	R^4
Neamine	H	H	H	NH ₂
Paromamine	H	H	H	OH
Kanamycin B	H	3-AG	H	NH ₂
Butirosin A	Xylose	H	AHBA	NH ₂

Carbon	3'-Deoxy-neamine		3'-Deoxy-paromamine		3'-Deoxy-kanamycin B		3'-Deoxy-butirosin A		Mean of $\Delta(3')$
	δ	$\Delta(3')$	δ	$\Delta(3')$	δ	$\Delta(3')$	δ	$\Delta(3')$	
1'	100.8	-0.8	101.0	-0.9	100.4	-0.3	100.8	-1.2	-0.8
2'	49.9	-6.2	50.0	-6.2	50.2	-5.9	51.9	-6.5	-6.2
3'	35.9	-36.3	35.8	-38.8	35.8	-36.5	36.5	-39.7	-37.8
4'	67.0	-6.9	65.5	-5.3	67.0	-5.9	68.9	-5.4	-5.9
5'	74.6	+0.2	74.4	+0.6	74.5	+0.3	76.5	+0.3	+0.4
6'	42.5	0	61.7	+0.2	42.6	+0.3	44.3	-0.2	+0.1

Ref. (46) (46) (46) (50)

δ = chemical shift, ppm $\Delta(3')$ = $\delta(3'\text{-deoxy-AG}) - \delta(\text{AG})$

Table 17. Deoxygenation shifts of 4'-deoxy derivatives of AG

	R^1	R^2	R^3	R^4	R^5
Kanamycin A	H	3-AG	H	OH	H
6'-N-Me-KM-A	H	3-AG	H	OH	CH ₃
Neamine	H	H	H	NH ₂	H
Butirosin A	Xylose	H	AHBA	NH ₂	H

Carbon	4'-Deoxy-kanamycin A		4'-Deoxy-6'-N-Me-KM-A		4'-Deoxy-neamine		4'-Deoxy-butirosin A		Mean of $\Delta(4')$
	δ	$\Delta(4')$	δ	$\Delta(4')$	δ	$\Delta(4')$	δ	$\Delta(4')$	
1'	101.3	+1.0	101.2	+1.1	102.6	+1.1	100.7	+0.8	+1.0
2'	74.6	+1.9	74.5	+1.9	57.6	+1.4	58.0	+1.6	+1.7
3'	68.1	-5.6	68.0	-5.6	69.1	-5.3	68.7	-5.3	-5.5
4'	36.3	-35.5	36.7	-35.0	36.9	-35.5	37.0	-35.3	-35.3
5'	71.7	-3.6	68.7	-3.7	71.3	-2.1	71.3	-2.7	-3.0
6'	45.5	+3.0	55.0	+2.7	45.7	+3.1	45.7	+3.1	+3.0

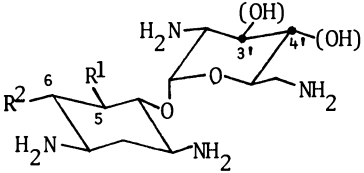
Ref. (59) (59) (24) (59)

$\Delta(4')$ = $\delta(4'\text{-deoxy-AG}) - \delta(\text{AG})$

α -carbon shift of 35 - 35.5 ppm and β -carbon shifts of 5.3 - 5.6 ppm for C-3 and 2.1 - 3.7 ppm for C-5. In contrast with the 3'-deoxy derivatives, the C-6' resonance of the 4'-deoxy derivatives causes an unexpected downfield shift of about 3 ppm. In the 3',4'-dideoxy compounds (Table 18), the deoxygenated methylene carbons appear at 26 - 29 ppm from TMS, showing a large deoxygenation upfield shift of 43.9 - 48.8 ppm for α -carbon, C-3' and C-4' and a smaller upfield shift for β -carbons, C-2' (5.5 - 6.1 ppm) and C-5' (2.1 - 4.2 ppm). The γ -downfield shift of about 3 ppm for the 6'-carbon is also observed in the 3',4'-dideoxy derivatives. The chemical shift differences observed for the carbons of deoxy sugar moiety are in good agreement with a calculated sum of the individual 3'- and 4'-deoxygenation effects, which is shown in the last column of Table 18.

Suami et al. (51) reported the cmr spectra of 5 and/or 6-deoxy neamines and 5-deoxykanamycin B (Table 19). 5-Deoxy-neamine and 5-deoxykanamycin B indicate the α -carbon shift of ca. 40 ppm, while the β -carbon shift for C-4 (9 - 10 ppm) is about twice as large as that for C-6 (4.5 - 4.9 ppm). The γ -carbons, C-1 and C-3, show downfield shifts of about 2 - 3 ppm. In addition, 5-deoxygenation causes a pronounced upfield shift of C-1' in both neamine (Δ 6.8 ppm) and kanamycin B (Δ 4.3 ppm), whereas no such shift of C-1'' was observed in the spectrum of 5-deoxykanamycin B. 6-Deoxyneamine and 6-deoxybutirosin A (50) show a similar α -carbon shift by about 40 ppm upfield, but the magnitude of β -carbon shifts for C-1 (-6.0 - -6.2 ppm) and C-5


Table 18. Deoxygenation shifts of 3',4'-dideoxy derivatives of AG

		R ¹		R ²			
		Kanamycin B		OH	-O-(3-AG)		
		Neamine		OH	OH		
		5,6-Dideoxyneamine		H	H		
Carbon	3',4'-Dideoxy-kanamycin B		3',4'-Dideoxy-neamine		3',4',5,6-Tetra-deoxyneamine		calcd. $\Delta(3'+4')$
	δ	$\Delta(3',4')$	δ	$\Delta(3',4')$	δ	$\Delta(3',4')$	
1'	101.1	+0.1	102.3	+0.8	96.6	+0.4	+0.2
2'	50.6	-5.8	50.7	-5.5	50.2	-6.1	-4.5
3'	26.2	-48.2	27.0	-47.5	27.2	-48.8	-43.3
4'	28.1	-44.2	28.5	-43.9	28.4	-45.0	-41.2
5'	70.2	-3.7	71.3	-2.1	71.0	-4.2	-2.6
6'	45.4	+2.7	46.0	+3.4	46.0	+3.0	+3.1
Ref.	(35)		(24)		(51)		

$$\Delta(3',4') = \delta(3',4'\text{-deoxy-AG}) - \delta(\text{AG})$$

$$\text{Calcd } \Delta(3',4') = \text{Mean of } \Delta(3') + \text{Mean of } \Delta(4')$$

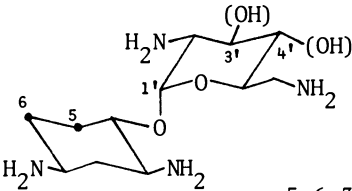
Table 19. Deoxygenation shifts of 5- and/or 6-deoxy derivatives of AG



Carbon	5-Deoxy-neamine		5-Deoxy-kanamycin B		6-Deoxy-neamine		6-Deoxy-butirosin	
	δ	$\Delta(5)$	δ	$\Delta(5)$	δ	$\Delta(6)$	δ	$\Delta(6)$
1	54.9	3.1	53.4	2.9	45.6	-6.2	45.7	-6.0
2	37.0	0.1	26.9	0.4	42.1	5.2	37.2	4.3
3	53.0	2.7	52.6	2.3	50.7	0.4	52.3	1.0
4	78.7	-10.2	78.1	-9.3	91.1	2.2	83.3	0
5	37.6	-39.8	34.9	-40.3	72.1	-5.3	80.7	-8.0
6	73.7	-4.9	84.1	-4.5	41.6	-37.0	37.2	-40.9
C-1'	96.4	-6.8	96.2	-4.3	102.3	0.1	98.4	1.1
C-1''			101.8	0.4			108.9	-5.6
Ref.	(51)		(51)		(51)		(50)	

$$\Delta(x) = \delta(x\text{-deoxy-AG}) - \delta(\text{AG})$$

Table 20. Deoxygenation shifts of 5,6-dideoxyneamines (51)



Carbon	5,6-Dideoxy-neamine		5,6,3',4'-Tetraoxo-neamine		Calcd. $\Delta(5,6)$
	δ	$\Delta(5,6)$	δ	$\Delta(5,6)$	
1	48.6	-3.2	48.7	-2.6	-3.1
2	41.9	5.0	41.9	5.2	5.3
3	53.1	2.8	53.3	2.8	3.1
4	80.4	-8.5	80.5	-7.8	-8.0
5	27.6	-49.8	28.0	-48.9	-45.1
6	33.6	-45.0	33.7	-44.7	-41.9
C-1'	96.2	-6.0	96.6	-5.7	-6.7

$$\text{Calcd. } \Delta(5,6) = \Delta(5) \text{ of 5-deoxyneamine} + \Delta(6) \text{ of 6-deoxyneamine}$$

(-5.3 - -8.0 ppm) is comparable, whereas the magnitude of γ -shifts for C-2 (4.3 - 5.2 ppm) and C-4 (0 - 2.2 ppm) is quite different from each other in 6-deoxyneamine and 6-deoxybutirosin. The latter compound shows an upfield shift of 5.6 ppm for C-1'', with a small downfield shift of C-1'.

Two derivatives of 5,6-dideoxyneamines were also reported by Suami et al. (51) (Table 20). The cmr spectra of the 5,6-dideoxy derivatives of neamine show that the signal shifts due to 5,6-dideoxygenation are in good agreement with the addition of two individual deoxygenation shifts observed for 5- and 6-deoxyneamines. This calculated sum is shown in the last column of Table 20.

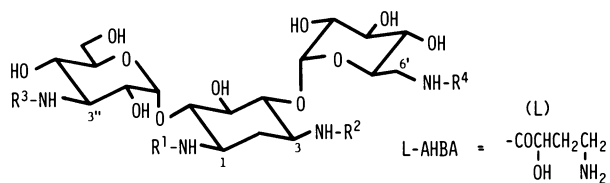
Amikacin and related derivatives

As described above, deoxygenation of AG afforded a number of interesting compounds such as DKB, which are resistant to AG-modifying enzymes due to the lack of target hydroxyl groups for enzymatic attack. Another direction for the chemical modification of AG was the attempt to inhibit the AG-modifying enzymes either at the binding site or at the active site on the AG molecule.

Amikacin (67), shown in Fig. 3, was thus synthesized in our laboratories by the selective 1-N-acylation of kanamycin with L(-)- γ -amino- α -hydroxybutyric acid (L-AHBA). Amikacin was found to be active against AG-resistant organisms that produce various types of AG-inactivating enzymes (68).

Since there are four acylable amino groups in the kanamycin molecule, three positional isomers of amikacin are possible as shown in Fig. 3. These isomers were also prepared in our laboratories from suitable N-protected intermediates (69). The antibacterial activities of the three isomers were very low, only about 1 % of amikacin, indicating the importance of acylation site for AG activity. Subsequently various AG were modified by acylation at the C-1 amino group with L-AHBA or its congeners. Facile determination of the actual site of acylation in AG molecule has been desired and the cmr provided a useful tool for such purpose.

Table 21 shows the cmr spectrum of amikacin compared with those of kanamycin and L-AHBA amide (44). Table 22 indicates acylation shift data of amikacin and its position isomers (44). 1-N-Acylation of kanamycin with L-AHBA induces a small upfield shift (0.8 ppm) of C-1, α to the acylated amino group. A marked upfield shift (7.3 ppm) occurs in C-6, β to the acylation site, while another β -carbon, C-2, undergoes a mild upfield shift (1.0 ppm). Isomer I, the 3-N-AHBA derivative, shows a similar type of shifts to that of amikacin, with an upfield shift of 1.1 ppm for α -carbon (C-3), a small shift (1.2 ppm) for one of β -carbons (C-2) and a large shift (6.5 ppm) for the other β -carbon (C-4). Isomer II (the 3''-N-AHBA isomer) is different from amikacin in the acylation shift which shows no significant shift for α -carbon (C-3'') and mild upfield shifts (1.8 - 1.9 ppm) for both of β -carbons



	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>
AMIKACIN (BB-K 8)	L-AHBA	H	H	H
ISOMER I (BB-K 29)	H	L-AHBA	H	H
ISOMER II (BB-K 11)	H	H	L-AHBA	H
ISOMER III (BB-K 6)	H	H	H	L-AHBA

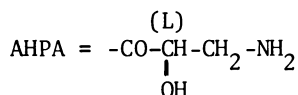
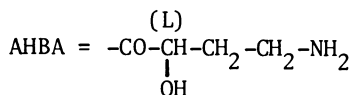
Figure 3. Amikacin and isomers

Table 21. C-13 chemical shifts of amikacin free base in D₂O (ppm downfield from TMS) (44)

Carbon	Kanamycin	Amikacin	Carbon	Kanamycin	Amikacin
1	51.2	50.4	1''	100.8	100.3
2	36.1	35.1	2''	72.6	72.5
3	49.7	49.4	3''	55.1	54.9
4	87.3	87.6	4''	70.0	70.1
5	74.9	75.4	5''	72.6	72.8
6	88.5	81.2	6''	61.1	61.2
1'	99.8	99.2			
2'	72.6	72.7			
3'	73.6	73.7			
4'	71.9	71.8			
5'	72.9	73.7			
6'	42.1	42.4			
				<u>AHBA amide</u>	
			C=O	180.7	177.2
			C-α	70.4	70.7
			C-β	36.8	36.5
			C-γ	37.9	38.1

Table 22. Acylation shifts of amikacin and related derivatives

Antibiotic	Acylation site	$\delta(\text{N-AHBA deriv.}) - \delta(\text{parent antib.})$		Ref.
		α -carbon	β -carbon	
Amikacin	1-N-AHBA	-0.8	-1.0(C-2) -7.3(C-6)	(44)
Isomer I	3-N-AHBA	-1.1	-1.2(C-2) -6.5(C-4)	(44)
Isomer II	3''-N-AHBA	-0.1	-1.8(C-2'') -1.9(C-4'')	(44)
Isomer III	6'-N-AHBA	-1.4	-1.7(C-5')	(44)
Gentamicin B	1-N-AHBA	-1.2	-1.2(C-2) -7.0(C-6)	(55)
Gentamicin B	1-N-AHPA	-1.2	-1.0(C-2) -7.1(C-6)	(55)
Kanamycin C	1-N-AHBA	-1.4	-2.4(C-2) -7.4(C-6)	(32)
Kanamycin C	3-N-AHBA	-1.3	-2.0(C-2) -6.7(C-4)	(32)



(C-2'' and C-4''). In the 6'-N-AHBA isomer, the α -carbon shift by N-acylation (1.4 ppm) is similar to the β -carbon shift of C-5' (1.7 ppm).

Analogous derivatives of gentamicin B (55) and kanamycin C (32) show nearly the same N-acylation effect as that of the corresponding kanamycin A derivatives as shown in Table 22.

N-Acetyl derivatives

Umezawa and co-workers (20) first elucidated in 1967 the biochemical mechanism of resistance of a kanamycin-resistant *E. coli* strain to be due to the enzymatic acetylation of the C-6' amino group of kanamycin. Since then other types of AG-acetylating enzymes have been isolated from AG-resistant bacteria and shown to acetylate the C-3 or C-2' amino group of AG (63). Identification of the site of acetylation was requisite to determine the resistance mechanism of resistant organisms.

All of the four mono-N-acetyl isomers of kanamycin A (44, 59) were prepared in our laboratories and the cmr determined as shown in Table 23, which shows that the resonance shifts of α - and β -carbons due to N-acetylation are in good agreement with those of the corresponding N-AHBA derivatives.

The 3-N-acetyl derivatives of ribostamycin (4), seldomycin factor 5 (31) and apramycin (38) show N-acetylation shifts nearly equal to those of 3-N-acetylkanamycin A (Table 23).

N-Alkyl derivatives

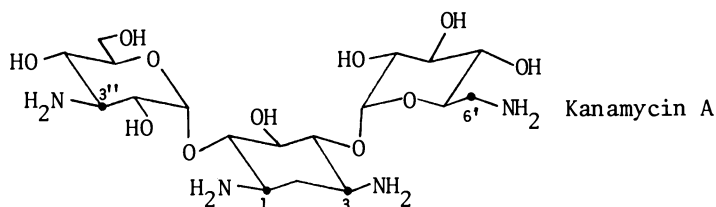
N-Alkylation is another important type of chemical modification of AG, because (a) 6'-N-methyl derivatives, obtained either chemically (71) or from natural sources (72, 73, 74, 75), are resistant to AAC (6')-producing strains, (b) 1-N-ethylsismicin (netilmicin) (76) has been reported to have excellent activity with reduced toxicity, and (c) a 2''-N-methyl group is present in gentamicins which are among the most active members of AG.

All of the four N-ethyl derivatives of kanamycin A were prepared in our laboratories (77). The cmr spectra of these compounds are shown in Table 24 for the α - and β -carbon shifts caused by N-ethylation. The table also includes data of the N-alkyl compounds which have been hitherto reported. In contrast to the N-acylation described above, N-alkylation causes pronounced downfield shifts of 6 - 10 ppm for α -carbons and mild upfield shifts of 0.6 - 3 ppm for β -carbons. 6'-N-Alkyl derivatives show somewhat larger α -carbon shifts (8.0 - 9.9 ppm) than other N-alkyl compounds (5.6 - 6.5 ppm) by 2 - 3 ppm.

O-Phosphoryl and O-adenylyl derivatives

The cmr spectra have been also found useful for the determination of the modification site in enzymatic inactivation

Table 23. Acylation shifts of N-acetyl derivatives of AG



Parent antibiotic	Acetylation site	$\delta(\text{N-acetyl deriv.}) - \delta(\text{parent antib.})$			Ref.
		α -carbon	β -carbon		
Kanamycin A	1-N	-1.1	-1.0(C-2)	-5.8(C-6)	(44)
Kanamycin A	3-N	-0.8	-1.1(C-2)	-6.3(C-4)	(44)
Kanamycin A	3''-N	0	-1.7(C-2'')	-1.5(C-4'')	(59)
Kanamycin A	6'-N	-1.1	-1.7(C-5')		(59)
Ribostamycin	3-N	-1.8	-1.4(C-2)	-5.9(C-4)	(4)
Seldomycin factor 5	3-N	-0.9	-1.4(C-2)	-6.7(C-4)	(31)
Apramycin	3-N	-1.0	-1.3(C-2)	-5.0(C-4)	(38)

Table 24. Alkylation shifts of N-alkyl derivatives of AG

Antibiotic	Alkylation site and alkyl group	$\delta(\text{N-alkyl deriv.}) - \delta(\text{parent antib.})$			Ref.
		α -carbon	β -carbon		
Kanamycin A	1-N-Et	6.5	-3.3(C-2)	-0.6(C-6)	(59)
Kanamycin A	3-N-Et	5.6	-3.0(C-2)	-2.5(C-4)	(59)
Kanamycin A	3''-N-Et	6.2	-1.3(C-2'')	-1.1(C-4'')	(59)
Kanamycin A	6'-N-Et	8.1	-1.1(C-5')		(59)
Kanamycin A	6'-N-Me	9.9	-1.3(C-5')		(59)
4'-Deoxy-KM-A	6'-N-Me	9.6	-2.3(C-5')		(59)
Gentamicin C ₂	6'-N-Me ^{*1}	7.8	-1.7(C-5')		(3)
Gentamicin C _{1a}	6'-N-Me ^{*2}	9.0	-3.0(C-5')		(10)
Gentamine C ₂	6'-N-Me ^{*3}	8.0	-1.7(C-5')		(3)
Ribostamycin	3-N-CH ₂ COOH	5.8	-4.5(C-2)	-3.7(C-4)	(4)
Sisomicin	6'-N-Me ^{*4}	9.1	-2.7(C-5')		(18)

*1 Gentamicin C₁, *2 Sagamicin, *3 Gentamine C₁,
*4 Antibiotic G-52

Table 25. O-Phosphorylation and O-adenylylation shifts of amikacin (45)

Carbon	Amikacin	3'-O-Phosphoryl-amikacin			4'-O-Adenylyl-amikacin		
	δ (ppm)	δ (ppm)	J _{c-p} (Hz)	Δ (ppm)	δ (ppm)	J _{c-p} (Hz)	Δ (ppm)
2'	71.7	71.2	2.4	-0.5			
3'	73.1	78.2	4.6	+5.1	72.2	2.8	-0.9
4'	71.7	71.1	4.8	-0.6	75.5	5.8	+3.8
5'	69.5				68.6	3.4	-0.9

Table 26. O-Adenylylation shifts of tobramycin and DKB (37)

Carbon	Tobramycin	4'-Adenylyltobramycin		
	δ (ppm)	δ (ppm)	J _{c-p} (Hz)	Δ (ppm)
3'	29.0	27.9	2.1	-1.1
4'	64.3	68.4	5.5	+4.1
5'	69.7	69.2	7.5	-0.5

Carbon	DKB	4''-Adenylyl-DKB		
	δ (ppm)	δ (ppm)	J _{c-p} (Hz)	Δ (ppm)
3''	54.7	54.3	ND	-0.4
4''	65.1	68.9	4.9	+3.8
5''	72.5	71.3	5.8	-1.2

ND: not determined

products (19, 31, 37, 38). Recently we reported (45) the structure determination of two amikacin derivatives modified by resistant strains of *S. aureus* isolated by J. Davies (78), and the products were identified as 3'-O-phosphorylamikacin and 4'-O-adenylylamikacin (Fig. 4). Both phosphoryl and adenylyl groups were easily recognized by ^{13}C - ^{31}P coupling. The α - and β -carbons to these groups gave doublet signals with $^2\text{J}(\text{c-p})$ of 4.6 - 5.8 Hz and $^3\text{J}(\text{c-p})$ of 2.4 - 4.8 Hz, respectively. As compared to amikacin, an introduction of phosphoryl or adenylyl group produces downfield shifts of α -carbons by 3.8 - 5.1 ppm and upfield shifts of β -carbons by 0.5 - 0.9 ppm (Table 25).

A resistant strain of *S. epidermidis* has been found to produce an enzyme which adenylylates the 4'-hydroxyl group of tobramycin and 4''-hydroxyl group of DKB (37). The resulting 4'-O-adenylyltobramycin and 4''-O-adenylyl-DKB give similar cmr data in both the ^{13}C - ^{31}P coupling and the substitution effect (Table 26).

β -Carbon shifts due to protonation of the amino groups

It has been known in H-1 nmr spectroscopy that protonation of amino groups in amino sugars causes a considerable downfield shift of the signal for the methine bearing the amino group (78). In contrast to this, the most pronounced upfield shift is seen on the β -carbons rather than the α -carbons, which was noted soon after the beginning of cmr studies in the AG field (2, 4, 5, 8).

Table 27 shows the pH effects on the C-13 chemical shifts of 2-deoxystreptamine (DOS) which is a constituent of representative aminoglycosides such as kanamycins, gentamicins and neomycins. For a pH change from 11.0 to 4.2, the signals of C-4 and C-6 located β to either 1- or 3-amino group are shifted upfield by 5.3 ppm and the signal of C-2, β to the two amino groups by 7.7 ppm, while the shifts of α -carbons to an amino group, C-1 and C-3, are only 0.3 ppm and that of the γ -carbon, C-5, is 1.0 ppm upfield.

Table 28 shows the β -carbon shifts on acidification observed in methyl glycosides of aminosugar components, 2-AG, 3-AG and 6-AG, in which carbon atoms β to the amino group are shielded by about 4 ppm.

Table 27. C-13 chemical shifts of 2-deoxystreptamine (4)

Carbon	pD							$\delta(\text{pD}4.2) - \delta(\text{pD}11)$
	11.0	10.0	9.0	8.0	6.8	6.0	4.2	
1,3	51.4	51.4	51.3	51.2	51.2	51.2	51.1	-0.3
2	36.9	36.0	34.2	32.0	29.5	29.2	29.2	-7.7
4,6	78.5	78.0	76.6	75.1	73.9	73.7	73.2	-5.3
5	76.6	76.4	76.2	76.0	75.7	75.7	75.6	-1.0

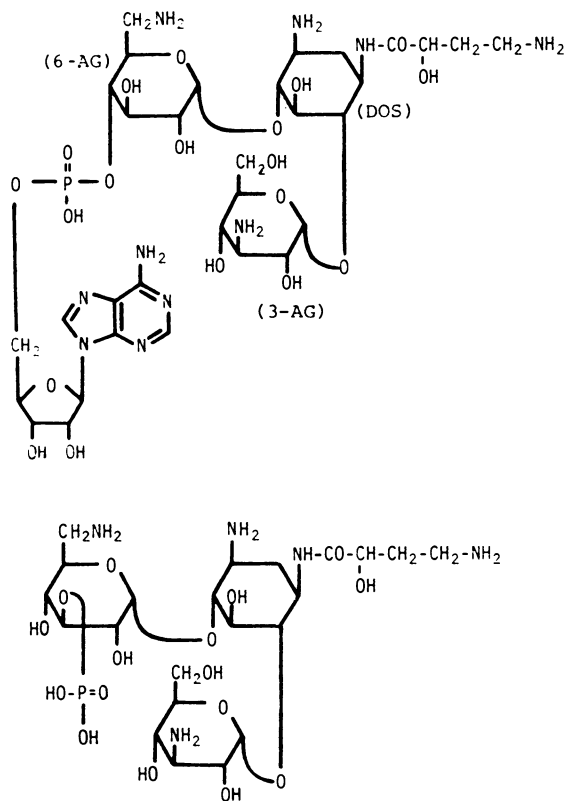
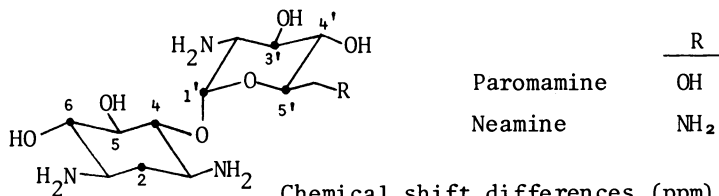


Figure 4. 4'-O-Adenylylamikacin (top) and 3'-O-phosphorylamikacin (bottom)

Table 28. C-13 chemical shifts (δ) and β -shifts on protonation ($\Delta\delta$) of aminodeoxyglucoses

Carbon	DOS		Me-2-AG		Me 3-AG		Me 6-AG	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
1	51.4		100.8	-4.2	99.8		100.1	
2	36.9	-7.7	51.0		72.8	-4.1	72.7	
3	51.4		75.2	-4.4	55.2		74.2	
4	78.5	-5.3	70.8		70.0	-4.0	72.3	
5	76.6		72.8		72.4		72.3	-3.9
6	78.5	-5.3	61.7		61.7		42.5	
OCH ₃			56.0		55.6		55.9	
Ref.		(4)		(8)		(8)		(8)

Table 29. Protonation shifts in pseudodisaccharides

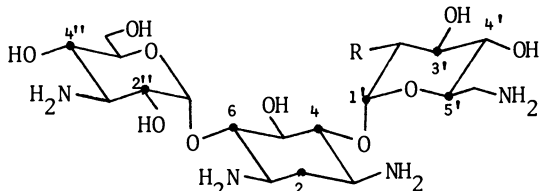
Chemical shift differences (ppm)
 $\delta(\text{acidic}) - \delta(\text{base})$

Compound	DOS moiety			Amino sugar moiety			Ref.
	C-2	C-4	C-6	C-1'	C-3'	C-5'	
Paromamine	-7.4	-7.4	-3.8	-4.1	-4.5	—	(15)
Neamine (NA)	-7.5	-10.3	-5.0	-5.5	-4.6	-4.8	(8)
3'-Deoxy-NA	-7.5	-9.7	-5.1	-5.9	-5.6	-3.8	(8)
4'-Deoxy-NA	-7.6	-11.1	-5.0	-6.0	-4.4	-5.5	(24)
5-Deoxy-NA	-7.7	-6.9	-5.6	-4.8	-6.4	-5.4	(51)
6-Deoxy-NA	-9.5	-10.7	-4.4	-5.7	-6.1	-5.1	(51)
5,6-Dideoxy-NA	-9.0	-6.6	-5.8	-4.7	-6.5	-5.6	(51)
3',4',5,6-Tetradeoxy-NA	-9.0	-7.4	-5.8	-6.1	-5.4	-4.7	(51)
Aprosaminide	-7.3	-8.9	-4.8	-5.4	-5.2	—	(17)

Table 29 shows the β -carbon shifts of paromamine, neamine and its deoxy derivatives, and aprosaminide, which indicate a large protonation shift of about 6.6 - 11 ppm for the C-4 as compared to the shift of DOS itself. Deoxygenation affects the magnitude of protonation shifts. The C-6 deoxygenation of neamines produces an upfield protonation shift of 9.0 - 9.5 ppm for the C-2, which is larger by about 2 ppm than that of other derivatives (7.5 - 7.7 ppm), while the 5-deoxyneamines show an upfield shift of 6.6 - 7.4 ppm for C-4, which is smaller by 3 - 4 ppm than others.

Kanamycin derivatives undergo the β -carbon shifts due to N-protonation similar to those in neamine derivatives as shown in Table 30. A relatively small upfield shift for the C-4 is observed again in 5-deoxykanamycin B as shown by square. It is notable that the C-1' of kanamycin A derivatives, bearing no amino group in the β position, undergoes an upfield shift of about 4 ppm. This will be discussed later.

Table 30. Protonation shifts in kanamycins



Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$							Ref.	
	C-2	C-4	C-6	C-1'	C-3'	C-5'	C-2''		C-4''
Kanamycin A									
unsubst.	-7.9	-9.1	-3.9	(-4.0)*		-4.2	-3.7	-3.7	(59)
4'-Deoxy	-7.9	-9.5	-4.0	(-3.7)		-4.6	-3.6	-3.8	(59)
6'-N-Me	-7.9	-9.0	-3.9	(-3.8)		-3.4	-3.6	-3.7	(59)
4'-Deoxy-6 ^L N-Me	-7.8	-9.3	-3.9	(-3.8)		-3.7	-3.7	-3.8	(59)
1-N-Et	-6.6	-8.5	-3.6	(-3.8)		-4.4	-3.8	-3.7	(59)
3-N-Et	-7.2	-7.2	-3.0	(-4.6)		-4.0	-3.7	-3.6	(59)
3''-N-Et	-7.9	-9.1	-3.7	(-4.0)		-4.1	-3.6	-3.9	(59)
6'-N-Et	-7.9	-9.4	-4.0	(-3.9)		-3.4	-3.7	-3.9	(59)
Kanamycin B									
unsubst.	-7.7	-9.6	-4.4	-4.8	-4.4	-4.9	-3.8	-3.8	(8)
3'-Deoxy	-7.8	-9.2	-4.5	-5.9	-5.5	-3.1	-3.7	-3.9	(8)
5-Deoxy	-7.8	\square -6.4	-5.5	-4.8	-5.8	-4.6	-4.0	-4.5	(51)

* no NH_2 group at the β -position

Table 31 shows data of the β -shift on protonation in gentamicin derivatives. In general, the C-4 of gentamicins indicates an upfield shift (6.6 - 8.7 ppm) which is smaller by 1 - 2 ppm than that of kanamycins. Gentamicin A₁, A₃ and B, which have an axial OH at the 4''-position, show upfield shifts of 1.3 - 2.1 ppm for the C-4'' and 3''-N-CH₃ which are smaller than those observed in gentamicin A possessing a 4''-equatorial OH group (3.7 - 4.4 ppm).

The protonation shifts reported for sisomicin and its relatives are summarized in Table 32. The effects of the stereochemistry at the 4''-position on the C-4'' and 3''-N-CH₃ in sisomicin-type antibiotics are similar to those indicated in Table 30. In the 4''-axial hydroxy derivatives (sisomicin and 66-40D) the protonation shifts are smaller than the usual values. The C-3' and C-4 signals show smaller upfield shifts by about 3 ppm than the usual shift probably due to the unsaturated sugar.

Table 33 shows the N-protonation shifts of the N-acylated derivatives of kanamycin A and gentamicin B. As expected there was no or decreased effect on the carbons β to the acylated amino groups. The magnitude of the β -shift of C-2, which is β to both the 1- and 3-amino group, decreases to about a half by 1-N- or 3-N-acylation. The large β -shift value of the C-4, which is β to the 3-N-amino group, does not disappear completely by 3-N-acylation.

The abnormal protonation shift of the C-1' which has no β -amino group, nearly disappears by 3-N-acylation as shown by square in Table 33. This implies that the C-1' shift may be caused by protonation of the 3-amino group which is located closely to the C-1'.

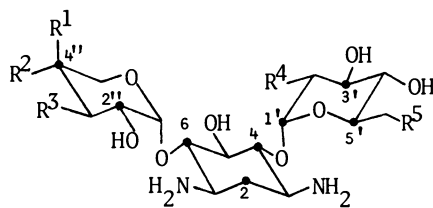
The 4,5-disubstituted DOS derivatives, xylostasin and its N-acyl derivatives, butirosins, also show protonation shifts similar to those of kanamycin and amikacin, respectively (Table 34). But the magnitude of the upfield shift of C-4 is smaller by 1.5 - 2 ppm than that of kanamycins and amikacin.

Protonation shifts of anomeric and aglycon carbons

The major group of AG consists of 4,6-disubstituted DOS derivatives which contain two glycopyranosyl units in the ⁴C₁ conformation linked axially at the 4- and 6-OH groups of DOS. Recently Nagabhushan and Daniels (13) reported an empirical rule correlating protonation shifts for anomeric and aglycon carbons with stereochemistry of the two sugar moieties in the 4,6-disubstituted DOS which possess 4-R-1'-R-axial and 6-S-1''-R-axial type configurations as shown by a partial structure in Table 35. According to the empirical rule, protonation of the amino groups causes shielding of C-1', C-4, and C-6 resonances by 3.8 - 4.2, 7.4 - 8.8 and 3.4 - 4.1 ppm, respectively, and 0 to a small downfield shift (deshielding) for C-1'', as shown in Table 35.

McAlpine and co-workers (25) reported the cmr spectra of seldomycins. Factor 1 and factor 5 of seldomycin showed proto-

Table 31. Protonation shifts in gentamicin A and B derivatives

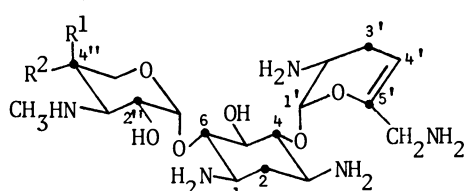


Gentamicin	R ¹	R ²	R ³	R ⁴	R ⁵
A	H	OH	NHCH ₃	NH ₂	OH
A ₁	OH	H	NHCH ₃	NH ₂	OH
A ₂	H	OH	OH	NH ₂	OH
A ₃	OH	H	NHCH ₃	OH	NH ₂
A ₄	H	OH	N $\begin{matrix} \text{CH}_3 \\ \text{CHO} \end{matrix}$	NH ₂	OH
B	OH	CH ₃	NHCH ₃	OH	NH ₂

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$								Ref.	
	C-2	C-4	C-6	C-1'	C-3'	C-5'	C-2''	C-4''		N-Me*
Gentamicin A	-8.0	-7.7	-3.7	-4.0	-4.9	—	-3.8	-4.4	-3.7	(15)
A ₁	-7.0	-6.6	-3.2	-3.8	-4.4	—	-3.8	-1.4	-1.7	(15)
A ₂	-6.7	-7.2	-4.1	-4.2	-4.7	—	—	—	—	(15)
A ₃	-7.8	-8.6	-3.4	-3.8*	—	-3.7	-3.6	-1.3	-1.6	(15)
A ₄	-7.9	-7.4	-3.8	-3.3	-4.8	—	—	—	—	(15)
B	-7.9	-8.7	-3.1	-3.5*	—	-3.3	-2.5	-1.8	-2.1	(55)

* no NH₂ group at the β -position

Table 32. Protonation shifts in sisomicin and 4',5'-unsaturated aminoglycosides



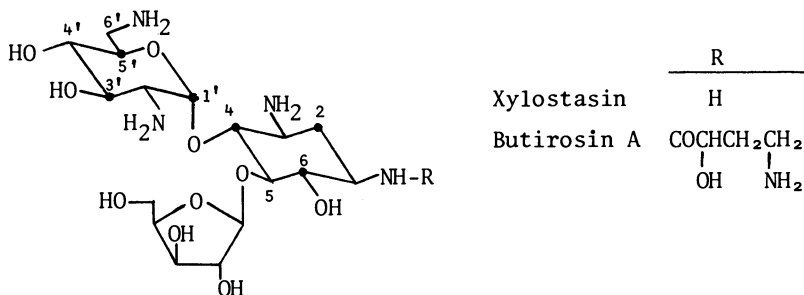
	R ¹	R ²
Sisomicin	OH	CH ₃
66-40B	H	OH
66-40D	OH	H

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$								Ref.	
	C-2	C-4	C-6	C-1'	C-3'	C-5'	C-2''	C-4''		N-Me
Sisomicin	-8.2	-5.5	-4.0	-2.8	-1.7	-6.1	-2.9	-2.2	-2.4	(33)
Antib.66-40B	-5.5	-4.2	-2.9	-3.0	-1.9	-6.5	-3.1	-4.1	-3.7	(14)
Antib.66-40D	-8.0	-5.6	-4.0	-3.0	-1.5	-6.3	-2.3	-1.0	-1.9	(14)

Table 33. Protonation shifts in N-acylated kanamycins and gentamicin B's

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$							Ref.	
	C-2	C-4	C-6	C-1'	C-5'	C-2''	C-4''		
Kanamycin A									
unsubst.	-7.8	-8.4	-3.9	-3.5	-3.4	-3.7	-3.6	(44)	
1-N-AHBA	-4.2	-7.8	0	-3.0	-4.2	-3.7	-3.7	(44)	
1-N-Acetyl	-4.2	-7.6	-0.4	-3.3	-4.0	-3.7	-3.7	(44)	
3-N-AHBA	-4.2	-2.7	-3.2	-0.9	-4.0	-3.5	-3.8	(44)	
3-N-Acetyl	-4.3	-1.8	-3.6	-0.5	-2.9	-2.7	-3.8	(59)	
3''-N-AHBA	-8.1	-9.4	-3.7	-4.1	-4.3	-0.3	-0.3	(44)	
3''-N-Acetyl	-7.7	-9.3	-3.9	-4.1	-4.2	-0.2	-0.4	(59)	
6'-N-AHBA	-7.8	-8.2	-4.8	-1.9	-0.8	-3.7	-3.8	(44)	
6'-N-Acetyl	-7.9	-8.1	-5.3	-1.8	-0.7	-3.7	-4.0	(44)	
6'-N-Cbz	-7.8	-8.0	-5.4	-2.3	-1.3	-3.7	-3.8	(59)	
Gentamicin B									
unsubst.	-7.9	-8.7	-3.1	-3.5	-3.3	-2.5	-1.8	-2.1	(55)
1-N-AHPA	-4.4	-7.7	0	-3.1	-3.5	-2.2	-2.0	-1.7	(55)
1-N-AHBA	-4.1	-6.9	0	-2.7	-3.1	-2.2	-1.3	-1.3	(55)

Table 34. Protonation shifts in Xylostasin and butirosins



Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$						Ref.
	C-2	C-4	C-6	C-1'	C-3'	C-5'	
Xylostasin	-7.7	-7.0	-5.4	-4.2	-4.1	-5.2	(59)
Butirosin A	-4.0	-6.6	-0.9	-4.1	-5.2	-3.6	(59)
4'-Deoxy-butirosin A	-4.0	-6.7	-0.8	-4.3	-5.3	-4.5	(59)
6'-N-Me-Butirosin A	-4.5	-5.9	-0.7	-3.7	-5.1	-2.1	(57)
3',4'-Dideoxy-6'-N-methyl-butirosin B	-4.3	-5.4	-0.7	-3.5	-3.6	-1.3	(56)

nation shifts of C-1' and C-4', which were in good agreement with the Nagabhushan and Daniels rule, but the acid shifts of C-1'' did not fit the rule as shown by square in Table 36. However, chemical shift differences of seldomycin factor 5 calculated from data at pH 9 and pH 6.4 come within the range expected from the rule. They interpreted this phenomenon as being that, in the lower pH range, the 2,3-diaminopentose linked at C-6 has inverted from the 4C_1 chair form to the 1C_4 form. In the latter form the two adjacent ammonium groups are in the trans-diaxial conformation, as shown in Table 36.

Table 37 shows that the empirical rule is applicable to the N-alkyl and deoxy derivatives of kanamycin A with a slight extension of the range for each of the carbons. The ranges of protonation shifts shown in the bottom of Table 37 are deduced from data described by Nagabhushan and Daniels (13) and those of the kanamycin derivatives listed in the table.

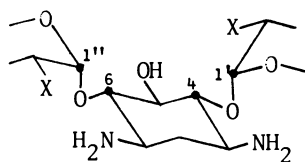
However, deviations are observed when the rule is applied to the N-acyl derivatives of AG as indicated by a square frame in Table 38. In the 1-N-acyl kanamycin derivatives the C-1' and C-4 resonances show the regular protonation shifts, but the C-1'' signal shows an unexpected upfield shift by 1.1 - 1.6 ppm and the C-6 signal unchanged. A similar tendency is observed in gentamicin B analogs. In contrast with this, the 3-N-acyl derivatives fit the rule for the protonation shifts of the C-1'' and C-6, whereas the C-1' and C-4 resonances show much smaller shifts than predicted values (0.5 - 0.9 ppm for C-1' and 1.8 - 2.7 ppm for C-4). In the 6'-N-acyl derivatives the protonation shift for C-1' is about one-half (1.8 - 2.3 ppm) of the ordinary value, while other anomeric and aglycon carbons show normal acid shifts. The 3''-N-acyl derivatives of kanamycin A have protonation shifts for all of the anomeric and aglycon carbons which are in good agreement with the empirical rule, as in the case reported for 3''-N-formyl-gentamicin A.

Among the five carbons which do not fit the rule, the C-6 of 1-N-acyl derivatives and the C-4 of 3-N-acyl derivatives are located at the β -position to the acylamino groups and, therefore, are not affected any more by the protonation.

The abnormal shifts observed for the C-1'' of the 1-N-acyl AG and the C-1' of 3-N- and 6'-N-acyl derivatives have not been fully interpreted at the present time, but it is interesting that all of the anomeric carbons showing abnormal shifts possess common structural feature. As shown in Fig. 5, they are located at the δ -position relative to the respective acylamino groups with a similar sequence of intervening atoms.

Fig. 6 gives a summary of this paper and shows the magnitude of α - and β -carbon shifts caused by substitutions and also that of protonation shifts for β -carbons to amino groups and anomeric and aglycon carbons. The α -carbon shifts are indicated by striped bars and the β -carbon shifts and the protonation shifts by open bars. As discussed in this paper, substitution and protonation

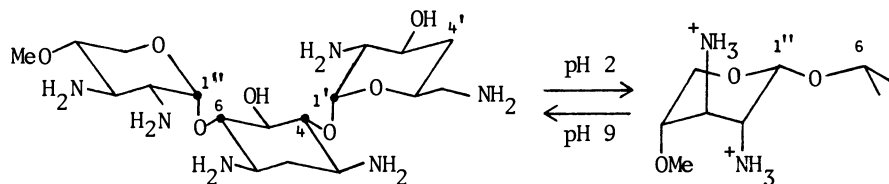
Table 35. Protonation shifts of anomeric and aglycon carbons
 — Nagabhushan-Daniels empirical rule (13)



6-S-1''-R-axial 4-R-1'-R-axial

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$			
	C-1'	C-4	C-1''	C-6
Gentamicin A	-4.0	-7.7	+0.6	-3.7
Gentamicin A ₃	-3.8	-8.6	+0.9	-3.4
Gentamicin A ₄	-3.8	-7.4	+1.2	-3.8
Gentamicin B	-3.9	-8.5	+0.9	-4.2
Paromamine	-4.1	-7.4	—	-3.8
Kanamycin A	-3.8	-8.8	+0.3	-4.1
Kanamycin B	-4.2	-8.1	+0.7	-4.0
Range	-3.8 ~ -4.2	-7.4 ~ -8.8	0 ~ small positive value	-3.4 ~ -4.2

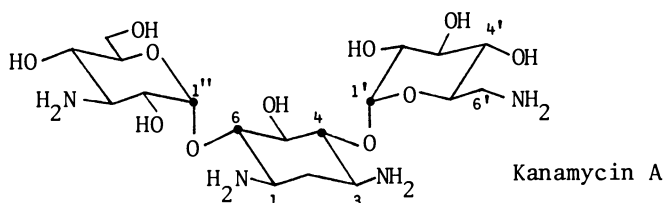
Table 36. Protonation shifts of anomeric and aglycon carbons of seldomycin factors (25)



Seldomycin factor 5

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$			
	C-1'	C-4	C-1''	C-6
Seldomycin factor 1	-3.2	-6.4	-4.7	-4.3
Seldomycin factor 2	-4.5	-8.4	—	-3.6
Seldomycin factor 5				
pH 9 — pH 6.4	-4.1	-7.0	+0.2	-2.9
pH 9 — pH 2	-4.1	-7.9	-5.2	-4.8
Gentamicin B	-3.9	-8.5	+0.9	-4.2

Table 37. Protonation shifts of anomeric and aglycon carbons of deoxy and N-alkyl derivatives of kanamycin A (59)



Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$			
	C-1'	C-4	C-1''	C-6
Kanamycin A				
unsubst.	-4.0	-9.0	+0.1	-3.9
4'-Deoxy	-3.7	-9.5	+0.2	-4.0
6'-N-methyl	-3.8	-9.0	+0.4	-3.9
4'-Deoxy-6'-N-Me	-3.8	-9.3	+0.4	-3.9
1-N-Et	-3.8	-8.5	-0.1	-3.6
3-N-Et	-4.6	-7.2	+0.2	-3.0
3''-N-Et	-4.0	-9.1	+0.4	-3.7
6'-N-Et	-3.9	-9.4	+0.4	-4.0
Range	-3.7 ~ -4.6	-7.2 ~ -9.5	-0.1 ~ +0.4	-3.0 ~ -4.0

Table 38. Protonation shifts of anomeric and aglycon carbons of N-acyl derivatives of AG

Antibiotic	$\delta(\text{acidic}) - \delta(\text{base}), \text{ppm}$				Ref.
	C-1'	C-4	C-1''	C-6	
Kanamycin	-3.5	-8.4	+0.4	-3.9	(44)
1-N-Acyl deriv.					
1-N-AHBA-KM A	-3.0	-7.8	-1.6	0	(44)
1-N-Ac-KM A	-3.3	-7.6	-1.2	-0.4	(44)
1-N-AHBA-GM B	-3.1	-7.7	-1.1	0	(55)
1-N-AHPA-GM B	-2.7	-6.7	-1.1	0	(55)
3-N-Acyl deriv.					
3-N-AHBA-KM A	-0.9	-2.7	+0.3	-3.4	(44)
3-N-Ac-KM A	-0.5	-1.8	+0.3	-3.6	(44)
6'-N-Acyl deriv.					
6'-N-AHBA-KM A	-1.9	-8.2	0	-4.8	(44)
6'-N-Ac-KM A	-1.8	-8.1	-0.3	-5.3	(59)
6'-N-Cbz-KM A	-2.3	-8.0	-0.5	-5.4	(59)
3''-N-Acyl deriv.					
3''-N-AHBA-KM A	-4.1	-9.4	+0.9	-3.7	(44)
3''-N-Ac-KM A	-4.1	-9.2	+1.0	-3.9	(59)
3''-N-Formyl-GM A	-3.8	-7.4	+1.2	-3.8	(13)

shifts usually fall into certain ranges of chemical shifts. However, deviations from the typical shift ranges are sometimes observed in carbons of the specific positions or in specific groups of compounds. A broad bar shows the typical shift range caused by substitution or protonation shift and narrow bar shows the shift range of a specific carbon or in a specific group, which is stated beside the bar.

Literature Cited

- 1 Neuss N., Koch K. F., Molloy B. B., Day W., Huckstep L. L., Dorman D. E., and Roberts J. D., Helv. Chim. Acta, (1970) 53, 2374.
- 2 Kotowycz G. and Lemieux R. U., Chem. Rev., (1973) 73, 669
- 3 Morton J. B., Long R. C., Daniels P. J. L., Tkach R. W., and Goldstein J. H., J. Am. Chem. Soc., (1973) 95, 7464.
- 4 Omoto S., Inouye S., Kojima M., and Niida T., J. Antibiotics, (1973) 26, 717.
- 5 Woo P. W. K., and Westland R. D., Carbohyd. Res., (1973) 31, 27.
- 6 Bock K., Pedersen C., and Heding H., J. Antibiotics, (1974) 27, 139.
- 7 Rinehart, Jr. K. L., Malik J. M., Nystrom R. S., Stroshane R. M., Truitt S. T., Taniguchi M., Rolls J. P., Haak W. J., and Ruff B. A., J. Am. Chem. Soc., (1974) 96, 2263.
- 8 Koch K. F., Rhoades J. A., Hagaman E. W., and Wenkert E., J. Am. Chem. Soc., (1974) 96, 3300.
- 9 Yamaoka N., Usui T., Sugiyama H., and Seto S., Chem. Pharm. Bull., (1974) 96, 2196.
- 10 Egan R. S., DeVault R. L., Mueller S. L., Levenberg M. I., Sinclair A. C., and Stanaszek R. S., J. Antibiotics, (1975) 28, 29.
- 11 Daniels P. J. L., Luce C., Nagabhushan T. L., Jaret R. S., Schumacher D., Reimann H., and Ilavsky J., J. Antibiotics, (1975) 28, 35.
- 12 Shimura M., Sekizawa Y., Iinuma K., Naganawa H. and Kondo S., J. Antibiotics, (1975) 28, 83.
- 13 Nagabhushan T. L., and Daniels P. J. L., Tetrahedron Lett., (1975) 747.
- 14 Davies D. H., Greeves D., Mallams A. K., Morton J. B., and Tkach R. W., J. Chem. Soc. Perkin I, (1974), 814.
- 15 Nagabhushan T. L., Turner W. N., Daniels P. J. L., and Morton J. B., J. Org. Chem., (1975) 40, 2830.
- 16 Nagabhushan T. L., Daniels P. J. L., Jaret R. S., and Morton J. B., J. Org. Chem., (1975) 40, 2835.
- 17 Wenkert E., and Hagaman E. W., J. Org. Chem., (1976) 41, 701.
- 18 Daniels P. J. L., Jaret R. S., Nagabhushan T. L., and Turner W. N., J. Antibiotics, (1976) 29, 488.
- 19 Le Goffic F., Martel A., Capmau M. L., Baca B., Goebel P., Chardon H., Soussy C. J., Duval J., and Bouanchaud D. H.,

- Antimicrob. Agents & Chemoth., (1976) 10, 258.
- 20 Kugelman M., Mallams A. K., Vernay H. F., Crowe D. F., and Tanabe M., J. Chem. Soc. Perkin I, (1976), 1088.
- 21 Kugelman M., Mallams A. K., Vernay H. F., Crowe D. F., Detre G., Tanabe M., and Yasuda D. M., J. Chem. Soc. Perkin I, (1976) 1097.
- 22 Kugelman M., Mallams A. K., and Vernay H. F., J. Chem. Soc. Perkin I, (1976) 1113.
- 23 Mallams A. K., Saluja S. S., Crowe D. F., Detre G., Tanabe M., and Yasuda D. M., J. Chem. Soc. Perkin I, (1976) 1135.
- 24 Egan R. S., Sinclair A. C., De Vault R. L., McAlpine J. B., Mueller S. L., Goodley P. C., Stanaszek R. S., Cirovic M., Mauritz R. J., Mitscher L. A., Shirahata K., Sato S., and Iida T., J. Antibiotics, (1977) 30, 31.
- 25 McAlpine J. B., Sinclair A. C., Egan R. S., De Vault R. L., Stanaszek R. S., Cirovic M., Mueller S. L., Goodley P. C., Mauritz R. J., Wideburg N. E., Mitscher L. A., Shirahata K., Matsushima H., Sato S., and Iida T., J. Antibiotics, (1977) 30, 39.
- 26 Daum S. J., Rosi D., and Goss W. A., J. Antibiotics, (1977) 30, 98.
- 27 Egan R. S., Stanaszek R. S., Cirovic M., Mueller S. L., Tadanier J., Martin J. R., Collum P., Goldstein A. W., De Vault R. L., Sinclair A. C., Fager E. E., and Mitscher L. A., J. Antibiotics, (1977) 30, 552.
- 28 Ellestad G. A., Martin J. H., Morton G. O., Sassiver M. L., and Lancaster J. E., J. Antibiotics, (1977) 30, 678.
- 29 Matsushima H., Mori Y., and Kitaura K., J. Antibiotics, (1977) 30, 890.
- 30 Sitrin R. D., Cooper D. J., and Weisback J. A., J. Antibiotics, (1977) 30, 836
- 31 Sato S., Iida T., Okachi R., Shirahata K., and Nara T., J. Antibiotics, (1977) 30, 1025.
- 32 Kondo S., Miyasaka T., Yoshida K., Iinuma K., and Umezawa H., J. Antibiotics, (1977) 30, 1150.
- 33 Davies D. H., Mallams A. K., McGlotten J., Morton J. B., and Tkach R. W., J. Chem. Soc. Perkin I, (1977) 1407.
- 34 Nara K., Katamoto K., Suzuki S., Akiyama S., and Mizuta E., Chem. Letters, (1977) 229.
- 35 Miyake T., Tsuchiya T., Umezawa S., and Umezawa H., Bull. Chem. Soc. Japan, (1977) 50, 2362.
- 36 Matsushima H., Kitaura K., and Mori Y., Bull. Chem. Soc. Japan, (1977) 50, 3039.
- 37 Schwotzer U., Kayser F. H., and Schwotzer W., FEMS Microb. Letters, (1978) 3, 29.
- 38 Davies J., and O'Connor S., Antimicrob. Agents & Chemoth., (1978) 14, 69,
- 39 Horii S., Fukase H., Kameda Y., and Mizokami N., Carbohydr. Res., (1978) 60, 275.
- 40 Hanessian S., Takamoto T., Massé R., and Patil G., Can. J.

- Chem.*, (1978) 56, 1482.
- 41 Hanessian S., Massé R., and (in part) Ekborg G., *Can. J. Chem.*, (1978) 56, 1492.
- 42 Hanessian S., Massé R., and Nakagawa T., *Can. J. Chem.*, (1978) 56, 1509.
- 43 Hanessian S., and Patil G., *Tetrahedron Lett.*, (1978) 1031.
- 44 Toda S., Nakagawa S., Naito T., and Kawaguchi H., *Tetrahedron Lett.*, (1978) 3913.
- 45 Toda S., Nakagawa S., Naito T., and Kawaguchi H., *Tetrahedron Lett.*, (1978) 3917.
- 46 Koch K. F., Merkel K. E., O'Connor S. C., Ocolowitz J. L., Paschal J. W., and Dorman D. E., *J. Org. Chem.*, (1978) 43, 1430.
- 47 Sitrin R. D., Cooper D. J., and Weisbach J. A., *J. Org. Chem.*, (1978) 43, 3048.
- 48 Ellestad G. A., Cosulich D. B., Broschard R. W., Martin J. H., Kunstmann M. P., Morton G. O., Lancaster J. E., Fulmor W., and Lovell F. M., *J. Am. Chem. Soc.*, (1978) 100, 2515.
- 49 Nara K., Katamoto K., Suzuki S., and Mizuta E., *Chem. Pharm. Bull.*, (1978) 26, 1091.
- 50 Hayashi T., Iwaoka T., Takeda N., and Ohki E., *Chem. Pharm. Bull.*, (1978) 26, 1786.
- 51 Suami T., Nishiyama S., Ishikawa Y., and Umemura E., *Bull. Chem. Soc. Japan*, (1978) 51, 2354.
- 52 Matsushima H., Mori Y., and Kitaura K., *Bull. Chem. Soc. Japan*, (1978) 51, 3553.
- 53 Carney R. E., McAlpine J. B., Jackson M., Stanaszek R. S., Washburn W. H., Cirovic M., and Mueller S. L., *J. Antibiotics*, (1978) 31, 441.
- 54 Kugelman M., Jaret R. S., Mittelman S., and Gau W., *J. Antibiotics*, (1978) 31, 643.
- 55 Nagabhushan T. L., Cooper A. B., Tsai H., Daniels P. J. L., and Miller G. H., *J. Antibiotics*, (1978) 31, 681.
- 56 Takeda K., Kinumaki A., Hayasaka H., Yamaguchi T., and Ito Y., *J. Antibiotics*, (1978) 31, 1031.
- 57 Takeda K., Kinumaki A., Okuno S., Matsushita T., and Ito Y., *J. Antibiotics*, (1978) 31, 1039.
- 58 Pearce C. J., Akhtar M., Barnett J. E. G., Mercier D., Sepulchre A. M., and Gero S. D., *J. Antibiotics*, (1978) 31, 74.
- 59 BBRI data, unpublished.
- 60 Dorman D. E., Angyal S. J. and Roberts J. D., *J. Am. Chem. Soc.*, (1970) 92, 1351.
- 61 Lemieux R. U., Ravia A. A., and Watanabe K. A., *Can. J. Chem.*, (1979) 47, 4427.
- 62 Lemieux R. U., Nagabhushan T. L., Clemetson K. J., and Tucker L. C. N., *Can. J. Chem.*, (1973) 51, 53.
- 63 Mitsuhashi S., *R. Factor. Drug Resistance Plasmid.*, Ed., University Tokyo Press, (1977) pp.195-251.
- 64 Umezawa H., Tsuchiya T., and Okazaki Y., *J. Antibiotics*,

- (1971) 24, 485.
- 65 Kawaguchi H., Japanese J. Antibiotics, (1977) 30, S-190.
- 66 Conway E., Guthrie R. D., Gero S. D., Lukacs G., Sepulchre A.-M., Hagaman E. W., and Wenkert E., Tetrahedron Lett., (1972) 4847.
- 67 Kawaguchi H., Naito T., Nakagawa S., and Fujisawa K., J. Antibiotics, (1972) 25, 695.
- 68 Price K. E., DeFuria M. D., and Pursiano T. A., J. Infec. Dis., (1976) 134, S249.
- 69 Naito T., Nakagawa S., Abe Y., Toda S., Fujisawa K. Miyaki T., Koshiyama H., Ohkuma H., and Kawaguchi H., J. Antibiotics, (1973) 26, 297.
- 70 Umezawa H., Okanishi M., Utahara R., Maeda K., and Kondo S., J. Antibiotics, (1976) 20, 136.
- 71 Umezawa H., Nishimura Y., Tsuchiya T., and Umezawa S., J. Antibiotics, (1972) 25, 743.
- 72 Weinstein M. J., Wagman G. H., Oden E. M., and Marquez J. A., J. Bact., (1967) 94, 789.
- 73 Cooper D. J., Daniels P. J. L., Yudis M. D., Marigliano H. M., (in part) Guthrie R. D., and Bukhari S. T. K., J. Chem. Soc. (C), (1971) 3126.
- 74 Okachi R., Kawamoto I., Takasawa S., Yamamoto M., Sato S., Sato T., and Nara T., J. Antibiotics, (1974) 27, 793.
- 75 Marquez J. A., Wagman G. H., Testa R. T., Waitz J. A., and Weinstein M. J., J. Antibiotics, (1976) 29, 483.
- 76 Wright J., J. Chem. Soc. Chem. Comm., (1976) 206.
- 77 Nakagawa S., Toda S., Abe Y., Yamashita H., Fujisawa K., Naito T., and Kawaguchi H., J. Antibiotics, (1978) 31, 675.
- 78 J. Davies, Personal communication.
- 79 Inouye S., Chem. Pharm. Bull. (1966) 14, 1210.

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The Structures of Minor Components of the Fortimicin Complex

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The fortimicins are a new group of potent aminoglycoside antibiotics discovered under a joint research agreement between the Kyowa Hakko Kogyo Company and Abbott Laboratories. They are produced by fermentation of a novel micromonospora species, *M. olivoasterospora* (1,2). The structures of a number of fortimicins have already been elucidated by Egan, et. al. (3) and Shirahata, et. al. (4) and that of fortimicin B confirmed by an x-ray determination (5) on a crystal of the free base. They consist of ψ -disaccharides of purpurosamine C or 6-epi-purpurosamine B and a novel 1,4-diaminocyclitol of chiro-stereochemistry. One oxygen and adjacent nitrogen of the cyclitol each carry methyl groups. Fortimicins A and D each bear a glycyI group attached in an amide linkage with the secondary amine, and fortimicin C has a hydantoic acid residue in this position.

From the fermentation beers of *Micromonospora olivoasterospora* a large number of minor fortimicins have been isolated both at Abbott Laboratories and the Kyowa Hakko Kogyo Company. These have been isolated using techniques of ion exchange, silica gel and gel filtration chromatography involving a wide variety of solvent systems and resins. The structures of a number of components have been determined mainly by spectroscopic means and no attempt will be made in the following discussion to outline the isolation procedures involved in obtaining these metabolites. It has been shown (6) that the glycyI group of fortimicin A is very labile to basic conditions. As such conditions are employed in many of the chromatographies the possibility exists that some of these compounds may not be true metabolites but artefacts of the isolation procedure.

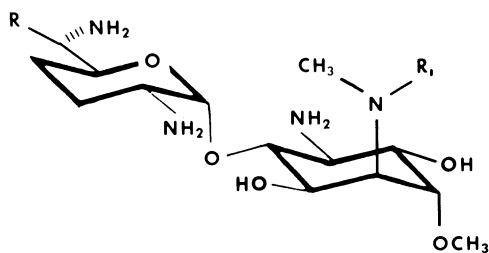
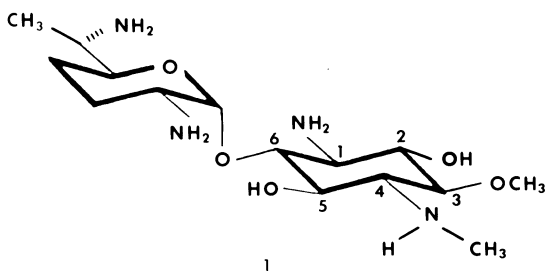
Fortimicin E had an $M+1^+$ peak in its mass spectrum at 349 mmu and the spectrum was essentially identical to that of fortimicin B. Moreover the carbon magnetic resonance spectrum of this component indicates that it contains fifteen carbons and that the difference between the structures of fortimicins B and E lies in the cyclitol portion of the molecule. All

oxygenated carbons of the cyclitol exhibit β shifts in the CMR on acidification of the sample indicating that we are again dealing with a 1,4-diaminocyclitol. The proton magnetic resonance spectrum, even when measured at 350 MHz, was not amenable to complete first order analysis. Overlap and ambiguity with respect to assignment of signals prevented a unique definition of parameters; however, it was possible to determine that none of the vicinal proton couplings of the cyclitol was less than 8.5 Hz. This allows the formulation of fortimicin E as the 3,4-diepimer of fortimicin B having the all trans or scyllo stereochemistry. The tetra-N-acetate of fortimicin E was mercaptanolyzed with ethanethiol to give the same di-N-acetyl-6'-epi-purpurosamine B diethylthioacetal as had been obtained from fortimicin B (3), thus confirming the stereochemistry of the sugar.

Fortimicin AP had an M+1 peak in the mass spectrum at 335 mmu, 14 units less than those of fortimicin B and E, and all the peaks associated with the sugar moiety in the mass spectrum of fortimicin B were also present in the spectrum of this component. The proton magnetic resonance spectrum of fortimicin AP differs strikingly from those of any of the fortimicins discussed so far in that it lacks the signal due to the protons of the methoxy group. At 270 MHz complete first order analysis of this spectrum is possible and shows that all of the vicinal proton couplings of the cyclitol protons are large, the smallest being 9 Hz indicating that the cyclitol of fortimicin AP like that of fortimicin E has scyllo stereochemistry. The carbon magnetic resonance spectrum of fortimicin AP confirms the presence of 6'-epi-purpurosamine B as the sugar portion and the presence of four oxygenated carbons showing β -shifts on protonation indicates that we are again dealing with a 1,4-diamino cyclitol. Single frequency heteronuclear decoupling experiments allowed the specific cyclitol carbon assignments shown in Table 1. Fortimicin AP is thus formulated as O-demethylfortimicin E. It cochromatographed closely and was separated only with difficulty from fortimicin AM.

Fortimicin AM had an M+1 peak in the mass spectrum at 335.2288 which indicates a molecular formula of $C_{14}H_{30}N_4O_5$. Moreover the degradation pattern is essentially that of fortimicin AP. The proton magnetic resonance spectrum of fortimicin AM in deuterium oxide at 100 MHz confirmed the close relationship to fortimicin AP. No O-methyl signal was present and the signals for the protons of the cyclitol were well resolved and amenable to extensive spin decoupling experiments. These, in conjunction with a 220 MHz spectrum led to the analysis given in Table 2. This clearly indicates that we are dealing with a novel cyclitol in which the proton at C-3 is equatorial and accordingly the cyclitol is a derivative of myo-inositol. The CMR spectrum of fortimicin AM confirms that it incorporates the same sugar moiety as fortimicin AP and the

FORTIMICIN E



	R	R ₁
FORTIMICIN	A	CH ₃ COCH ₂ NH ₂
	B	CH ₃ H
	C	CH ₃ COCH ₂ NH-CO-NH ₂
	D	H COCH ₂ NH ₂
	KE	H H

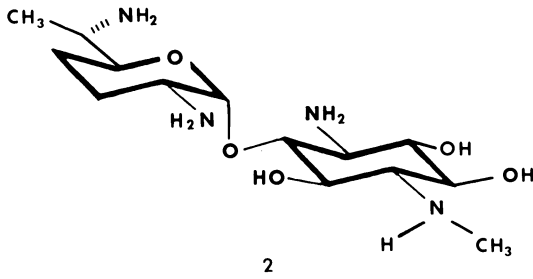
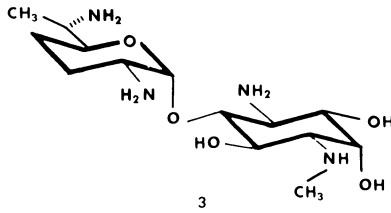
FORTIMICIN AP**FORTIMICIN AM**

Table 1
CMR SPECTRA

Fortimicin	B	E	AH	AI	AK	AL	AM
C-1'	102.4	102.3	100.8	101.1	102.2	101.2	102.1
C-2'	50.6	51.0	56.2	56.2	50.0	47.8	50.7
C-3'	27.1	27.0	18.9	18.9	26.8	25.7	26.8
C-4'	27.4	27.5	22.4	22.4	26.8	152.5	27.3
C-5'	75.1	74.9	68.5	68.5	70.8	48.9	74.7
C-6'	50.4	50.6	182.6	182.6	65.0	20.6	50.5
C-7'	18.7	18.4	22.5	22.5	----	----	18.2
C-1	53.8	55.1	53.6	54.6	53.5	54.8	52.9
C-2	71.1	75.3	71.0	75.2	73.2	75.6	73.1
C-3	79.9	83.7	79.8	83.1	79.5	73.4	68.5
C-4	60.9	62.4	61.0	62.0	62.1	63.3	62.0
C-5	71.1	73.7	70.7	72.7	74.0	73.1	73.2
C-6	84.1	85.0	80.6	82.2	86.3	83.2	85.9
OCH ₃	59.3	60.3	59.2	60.1	62.7	----	----
NCH ₃	36.0	33.9	35.3	33.3	33.8	33.2	33.3

Fortimicin	AN	AO	AP	AQ	AS	iso	∞	†
C-1'	99.3	102.0	102.1	101.0	100.1	98.9	101.6	102.3
C-2'	50.2	56.1	50.7	50.6	50.9	50.8	56.3	50.7
C-3'	26.6	74.6	26.8	27.0	26.5	23.7	74.8	27.0
C-4'	26.8	70.8	27.3	27.3	27.3	26.9	70.8	28.5
C-5'	74.4	73.7	74.5	75.0	73.9	74.4	73.8	71.3
C-6'	50.5	61.6	50.4	50.3	50.2	49.9	61.7	46.0
C-7'	18.2	----	18.5	18.5	17.7	18.2		
C-1	53.0	54.9	54.9	55.0	54.8	54.3		
C-2	69.7	75.6	75.6	71.7	71.9	71.1		
C-3	72.0	73.4	73.7	77.8	76.8	79.8		
C-4	64.8	63.2	63.2	62.4	61.1	61.2		
C-5	69.1	73.7	73.4	71.5	71.4	71.0		
C-6	76.3	85.8	85.9	81.0	80.3	82.3		
OCH ₃	----	----	----	57.9	59.9	59.2		
NCH ₃	35.3	33.6	33.3	44.0	40.5	35.4		

[∞]Seldomycin factor 1 (7)

[†]Gentamine Cla (6)

Spectra were determined in D₂O solution at pD 10 with dioxane as internal reference taken as 67.4 ppm downfield from TMS. They were measured on a Varian XL-100-15/TT-100 spectrometer system. Additional peaks occurred as indicated in the spectra of the following compounds: 45.1, 176.5 (fortimicin AN) 57.4, 67.0 (fortimicin AS), 44.8, 175 (isofortimicin).

cyclitol resonances show the expected upfield shifts, greatest at C-3, associated with the epimerization of a substituent from an equatorial to an axial orientation. Thus fortimicin AM is formulated as 3-epi-fortimicin AP.

Fortimicin AK has a molecular ion at 355.2071 in the mass spectrum indicative of a molecular formula of $C_{14}H_{29}N_3O_6$. The mass spectral degradation pattern includes the same strong tetrad of peaks associated with the cyclitol as seen in the spectra of fortimicins B and E. A strong peak at m/e 130 peak matched for $C_6H_{12}NO_2$ and has to be ascribed to the sugar.

The proton magnetic resonance spectrum measured at both 350 and 100 MHz allowed for the analysis shown in Table 3 and indicates that the cyclitol of fortimicin AK like that of fortimicin AM has the myo-configuration. Moreover the sugar is established as a 2-amino 2,3,4-trideoxyhexose as suggested by the mass spectrum. Carbon magnetic resonance spectra data of fortimicin AK are shown in Table 1. Although no similar figures for a model glycoside of 2-amino-2,3,4-trideoxyglucose were found, the purpurosamine C ring of gentamicin C_{1a} provided a surprisingly good model (7) for the carbon resonances of the sugar. Those of the cyclitol were very similar to the carbon resonances of the cyclitol of fortimicin AM with the exception of a downfield shift at C-3 arising from methylation of that hydroxyl group. The multiplicities of these carbons were confirmed by an ORSFD experiment in which the carbon assigned to C-6' gives rise to a triplet identifying it as an oxygenated methylene. Although the sugar moiety of fortimicin AK has not been isolated nor its stereochemistry defined the available evidence suggests that it is 2-amino-2,3,4-trideoxyglucose and accordingly fortimicin AK is formulated as shown.

Fortimicin AL has a molecular ion at 332.2056 mass matched for the molecular formula $C_{14}H_{28}N_4O_5$ (calculated 332.2060). The typical tetrad of peaks associated with the cyclitol occurs at 221 (44%), 203 (90%), 193 (44%) and 175 (70%) indicative of one of the 0-demethyl cyclitols. The stoichiometry of the sugar moiety indicates it to possess two hydrogens less than 6-epi-purpurosamine B and this is confirmed by strong peaks in the spectrum at m/e 158 (40%), 141 (60%) and 140 (100%).

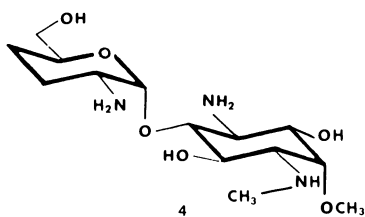
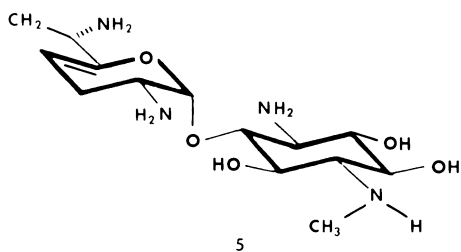
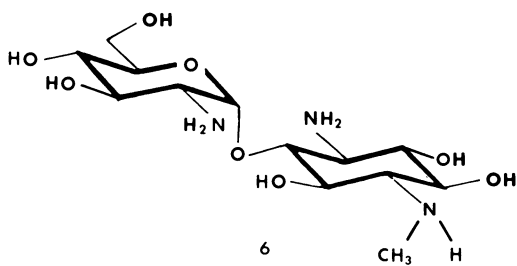
The carbon magnetic resonance spectrum of fortimicin AL (Table 3) indicates that the cyclitol portion of this component is the same as that present in fortimicin AP; namely, the 0-demethylfortamine of scyllo-stereochemistry. The carbon resonances of the sugar together with their multiplicities in an ORSFD experiment establish the sugar as a 4,5 unsaturated sugar. The proton magnetic resonance spectrum of fortimicin AL in deuterium oxide shows the anomeric proton signal with a coupling of 2.5 Hz at δ 5.77 and the signal assigned to H-4' is a rough triplet at δ 5.35. Fortimicin AL is tentatively formulated as shown; however, no evidence has been obtained to support the configuration at C-6'.

Table 2
Proton Magnetic Resonance Spectral Parameters
Fortimicin AM

	Chemical Shift (δ)		Coupling (Hz)
H-1	3.52	J _{1,2}	10.0
H-2	3.88	J _{2,3}	2.8
H-3	4.59	J _{3,4}	2.7
H-4	2.88	J _{4,5}	8.5
H-5	4.16	J _{5,6}	10.5
H-6	3.77	J _{6,1}	10.0
NCH ₃	33.3		
H-1'	5.59		
H-2'	3.26		
CH ₂ -3'			
CH ₂ -4'	1.7-2.4		
H-5'	4.04		
H-6'	3.30		
CH ₃ -7'	1.48		

Table 3
Proton Magnetic Resonance Spectral Parameters
Fortimicin AK

	Chemical Shift (δ)		Coupling (Hz)
H-1	3.48	J _{1,2}	10.0
H-2	3.97	J _{2,3}	3.0
H-3	4.32	J _{3,4}	2.5
H-4	2.94	J _{4,5}	11.0
H-5	4.12	J _{5,6}	9.5
H-6	3.76	J _{6,1}	10.0
NCH ₃	2.90		
OCH ₃	4.05		
H-1'	5.57	J _{1',2'}	3.4
H-2'	3.32		
CH ₂ -3'			
CH ₂ -4'	1.8-2.4		
H-5'	4.42		
CH ₂ -6'	4.05		

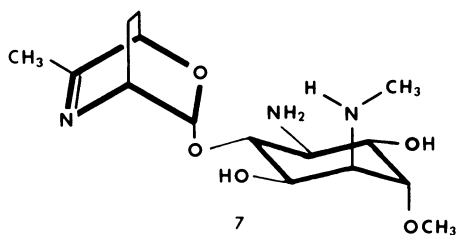
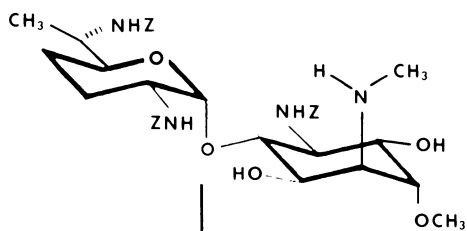
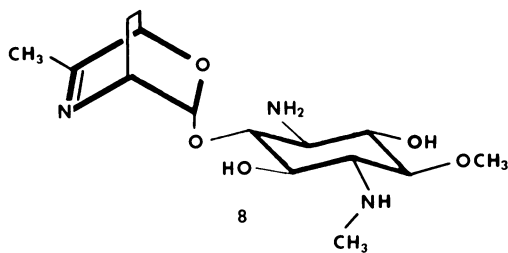
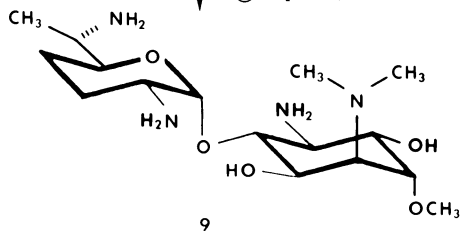
FORTIMICIN AK**FORTIMICIN AL****FORTIMICIN AO**

Fortimicin A0 has a protonated molecular ion m/e 354 in its mass spectrum which was mass matched at 354.1868 indicative of a molecular formula of $C_{13}H_{27}N_3O_8$. The same strong tetrad of peaks associated with the cyclitol as seen in the mass spectra of fortimicins AL and AP were also present in the spectrum of fortimicin A0. The CMR spectrum of fortimicin A0 shows a striking comparison between the resonances assigned to the cyclitol with those similarly assigned in the spectrum of fortimicin AP. Those assigned to the sugar portion of fortimicin A0 can be correlated with those of the hexose (2-aminoglucose) of seldomycin factor 1 (8). This leads to the suggested formulation of fortimicin A0 as shown.

Fortimicin AH and AI behaved similarly on chromatography and their spectral properties indicated a close structural analogy. The mass spectra of these two compounds were virtually identical and showed a molecular ion at m/e 329 mmu which was peak matched for $C_{15}H_{27}N_3O_5$. Peaks associated with a cyclitol of fortamine stoichiometry were present in the spectra of both samples and the predominant ion arising from the sugar fragment appeared to have the surprising formula, $C_7H_{10}NO$, for both compounds. Despite the seven carbon nature of this sugar the proton magnetic resonance spectra of both compounds were lacking the typical $6'-CH_3$ doublets as seen in the spectra of fortimicins containing 6'-*epi*-purpurosamine B. Both spectra contained a three proton singlet at $\delta 2.65$ which was exchangeable on standing in D_2O . Analysis of the spectra indicated that fortimicin AH possessed the fortamine cyclitol with *chiro* stereochemistry whereas fortimicin AI had the analogous cyclitol of *scyllo* stereochemistry. The sugar moieties of both compounds appear to be identical and give rise to anomeric proton signals at $\delta 5.7$ ppm with less than 1 Hz coupling to H-2' signal at $\delta 4.8$. These observations are accommodated by formulating fortimicins AH and AI as shown. The same sugar has recently been formulated (9) as present in a minor component from the gentamicin complex although no physical or spectroscopic data were presented.

This is further confirmed by analysis of the carbon spectra of these compounds. The signals assigned to C-6' and C-7' of fortimicins AH and AI were not evident in the spectra of these compounds in D_2O , but were present when the solvent was water. Partly on this evidence and on the multiplicity of the 18.9 ppm signal; a triplet in an ORSFD experiment the assignments are as shown rather than the assignments of the 18.9 signal to the 7'-carbon as may have been expected from initial visual inspection. The assignments to C-3' and C-4' may be interchanged.

The proton magnetic resonance spectrum of fortimicin AQ was very similar to that of fortimicin B with the exception that the *N*-methyl singlet was of 6 proton intensity. The carbon magnetic resonance spectra also supported a close relationship between these two compounds. A single peak at $\delta 44.0$ in the *N*-methyl region of greater intensity than others in the spectrum led

FORTIMICIN AH**FORTIMICIN AI****TRI-Z-FORTIMICIN B**1: $\text{CH}_2\text{O} / \text{H}_2 / \text{Pt}_2\text{O}$ ② $\text{H}_2 / \text{Pd} / \text{C}$ **FORTIMICIN AQ**

to the suggestion that fortimicin AQ was 4-N-methylfortimicin B. This was shown to be the case by conversion of fortimicin B into fortimicin AQ. Reductive methylation of 1,2',6-tri-N-carbo-benzyloxyfortimicin B followed by deprotection gave fortimicin AQ.

Fortimicin AS was also rapidly identified. It was shown spectroscopically to be identical to a sample of 4-N- β -hydroxyethylfortimicin B prepared by Kyowa Hakko scientists (10) by diborane reduction of 4-N-glycolylfortimicin B.

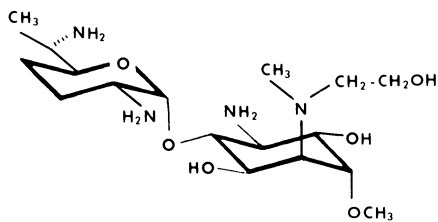
Fortimicin AN has a molecular ion at m/e 391, the cyclitol associated peaks in its mass spectrum were 14 mass units less than the corresponding peaks in the spectrum of fortimicin A. Hydrolysis of fortimicin AN by aqueous alkali gave glycine and a semisynthetic derivative of fortimicin B, 0-demethylfortimicin B (11). The proton magnetic resonance spectrum of fortimicin AN indicated the absence of a methoxy group in the structure. A two proton singlet at δ 3.81 was ascribed to the methylene protons of a glycol group. The 4-N-methyl singlet was at δ 2.82 indicating that this was not the point of attachment of the glycol group. Spin decoupling experiments located H-2' and H-6' at δ 3.21, the point of irradiation which caused the collapse of both the anomeric doublet at δ 5.77 and the C-methyl doublet at δ 1.46. Thus the glycol group was not located at the 4-amino group the 2'-amino nor the 6'-amino group and by elimination is on the 1-amino group. Thus fortimicin AN is formulated as 2'-N-glycolylfortimicin B.

One minor component of the fortimicin mixture has been given the name isofortimicin. Its molecular formula detected by mass spectrometry was identical to that of fortimicin A. Alkaline hydrolysis of isofortimicin gave rise to fortimicin B and glycine. The proton magnetic resonance spectrum of isofortimicin shows the N-methyl singlet as an amine rather than an amide nitrogen at δ 2.81. Spin decoupling experiments place H-2', the point of irradiation at which the anomeric doublet at δ 5.56 collapses, at δ 4.32 and identifies the 2' amine as the point of attachment of the glycine. Thus isofortimicin is formulated as 2'-N-glycolylfortimicin B.

The cyclitols that we have encountered, to date, in the fortimicins are shown together with the fortimicins in which they occur. Three of four possible diastereomers arising from various configurations at C-3 and C-4 have been found with or without the 0-methyl group at the 3 position. The fourth possible diastereomer has not yet been discovered in the fortimicin fermentation.

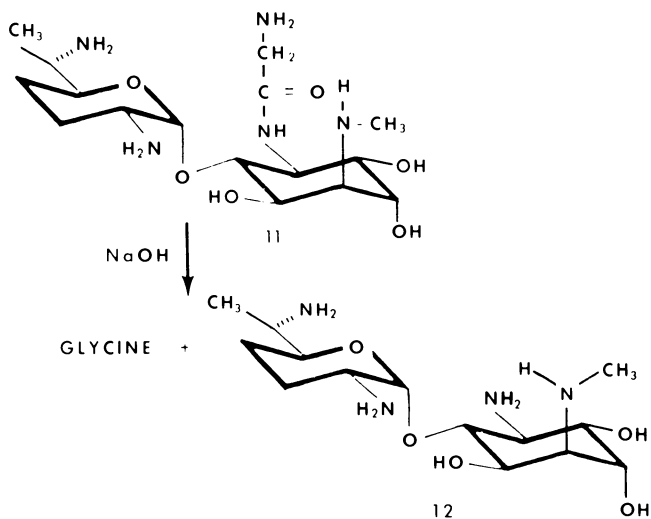
The sugars encountered in the fortimicins are shown and 6'-epi-purpurosamine B is by far the most commonly encountered. The 2-amino-2,3,4-trideoxyglucose of fortimicin AK is of some biogenetic interest. In the biosynthesis of the gentamicins as proposed by Testa and Tilley (12) removal of the hydroxyl groups at C-3 and C-4 occurs subsequent to branching and amination at C-6. The position of fortimicin AK in the biosynthetic pattern

FORTIMICIN AS

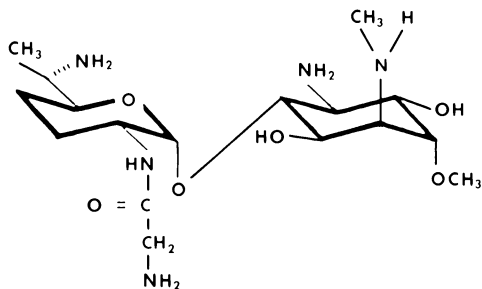


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FORTIMICIN AN

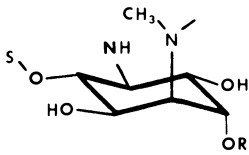


ISOFORTIMICIN



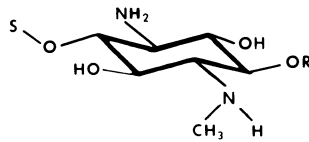
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FORTIMICIN CYCLITOLS



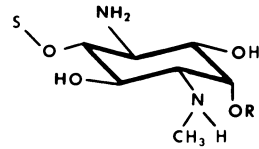
CHIRO

R = H AN
 R = CH₃ A, B, C, D, KE,
 AH, AQ, AS,
 ISO



SCYLLO

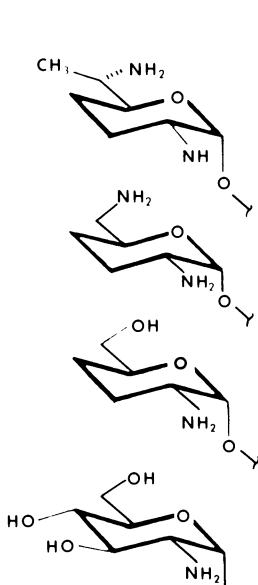
AL, AP, AO
 E, AI



MYO

AM
 AK

FORTIMICIN SUGARS

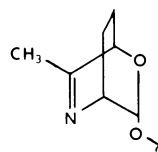


A, AP
 B, AN
 C, AQ
 E, AS
 ISO

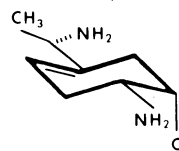
D, KE

AK

AO



AH, AI



AL

of the fortimicins is unknown but its existence suggests the possibility of a biosynthetic pathway to 6'-epi-purpurosamine B in the fortimicins different to that leading to purpurosamine B in the gentamicins.

Literature Cited

1. Nara T., Yamamoto, M., Kawamoto, I., Takayama, K., Okachi, R., Takasawa, S., Sato, T. and Sato, S., J. Antibiotics (1977) 30, 533~40.
2. Okachi, R., Takasawa, S., Sato, T., Sato, S., Yamamoto, M., Kawamoto, I. and Nara, T., J. Antibiotics (1977) 30, 541~551.
3. Egan, R. S., Stanaszek, R. S., Cirovic, M., Mueller, S. L., Tadanier, J., Martin, J. R., Collum, P., Goldstein, A. W., DeVault, R. L., Sinclair, A. C., Fager, E. E. and Mitscher, L. A., J. Antibiotics (1977) 30, 552~63.
4. Iida, T., Sato, M., Matsubara, I., Mori, Y. and Shirahata, K. The Structures of Fortimicins C, D and KE, J. Antibiotics (in press).
5. Hirayama, N., Shirahata, K., Ohashi, Y., Sasada, Y. and Martin, J. R., Acta Cryst. (1978).
6. Tadanier, J., Martin, J. R., Collum, P., Goldstein, A. W., and Hallas, R., Carbohydrate Research (in press).
7. Morton, J. B., Long, R. C., Daniels, P.J.L., Tach, R. W. and Goldstein, J. H., J. Am. Chem. Soc. (1973) 95, 7464~7469.
8. Egan, R. S., Sinclair, A. C., DeVault, R. L., McAlpine, J. B. Mueller, S. L., Goodley, P. C., Stanaszek, R. S., Cirovic, M. Mauritz, R. J., Mitscher, L. A., Shirahata, K., Sato, S. and Iida, T., J. Antibiotics (1977) 30, 31~38.
9. Berdy, J., Pauncz, K., Vajna, Z. M., Horvarth, G., Gyimesi, J. and Koczka, I., J. Antibiotics (1977) 30, 945~954.
10. Sato, M. and Mori, Y., J. Antibiotics (in press).
11. Tadanier, J., Martin, J. R., Kurath, P., Goldstein, A. W. and Collum, P., Carbohydrate Research (in press).
12. Testa, R. T. and Tilley, B. C., J. Antibiotics (1976) 29, 140~146.

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The Structures of New Fortimicins Having Double Bonds in Their Purpurosamine Moieties

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In 1972, Dr. Nara and his co-workers of Kyowa Hakko discovered a new type of aminoglycosides, named fortimicins (1,2), in the fermentation broths of *Micromonospora* species. Fortimicin is a pseudo disaccharide composed of a 6-epi-purpurosamine B and a novel 1,4-diaminocyclitol. Fortimicin A (FM-A) is the major component and its structure has been determined by the Abbott group together with that of a minor component, fortimicin B (FM-B) (3). The structure of FM-B has also been determined by single X-ray crystal analysis in our laboratories (4). A couple of years later we isolated three minor components, fortimicins C, D, and KE, from the fermentation broth of the same organism (5, 6) (Figure 1). Recently Dr. Deushi and his co-workers of Kowa reported isolation of sporaricins A and B which are 1-epi-2-deoxyfortimicins A and B, respectively (7). We have continued to search for fortimicins having substantial antibiotic activity and have isolated a number of minor components. Several of them have double bonds in their purpurosamine moieties. This paper will describe the chemical structures of five new fortimicins having double bonds.

Fortimicin KF and KG

The molecular formulas of the fortimicins were determined by high resolution mass measurements and elemental analysis. The PMR spectrum of fortimicin KG (FM-KG) free base showed a secondary methyl, an N-methyl, and an O-methyl signals. An anomeric proton was observed at 5.28 ppm and a triplet due to an olefinic proton at 4.86 ppm. The PMR spectrum of fortimicin KF (FM-KF) closely resembles that of FM-KG lacking only a secondary methyl group. The prominent peaks of the FM-KG's mass spectrum (Figure 2) can be attributed to the fragmentation showed in Scheme 1. The m/e 247 peak is characteristic of the fortimicins having double bonds and is considered to be generated by a retro Diels-Alder type cleavage showing that the double bond must be located between the 4' and 5'-carbons. In the case of FM-KF, each fragment containing the

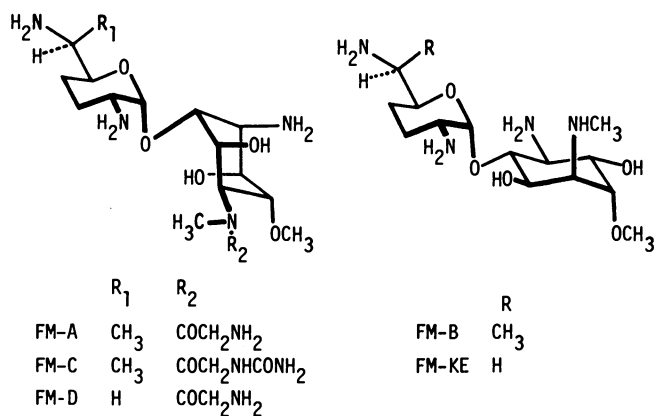
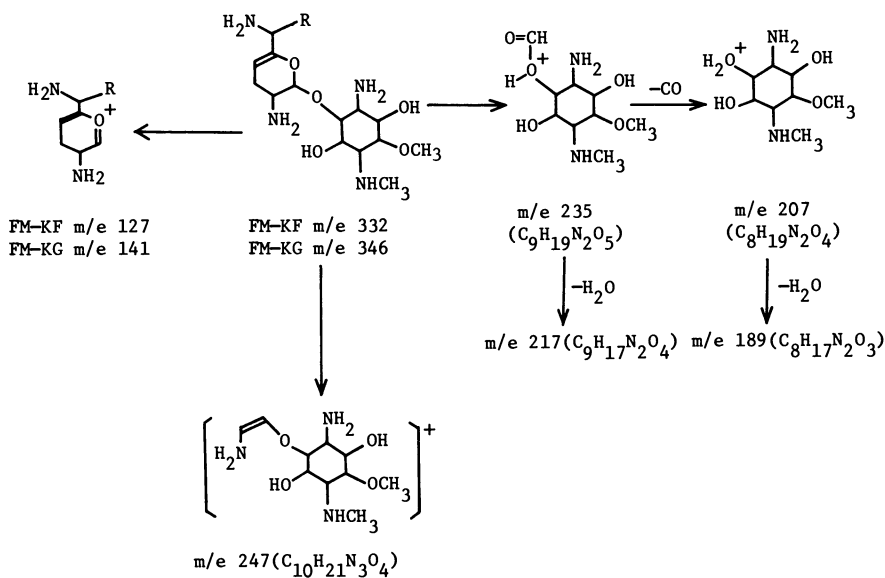


Figure 1. Structures of Fortimicins A, B, C, D, and KE



Scheme 1. Mass fragmentations of FM-KF and KG

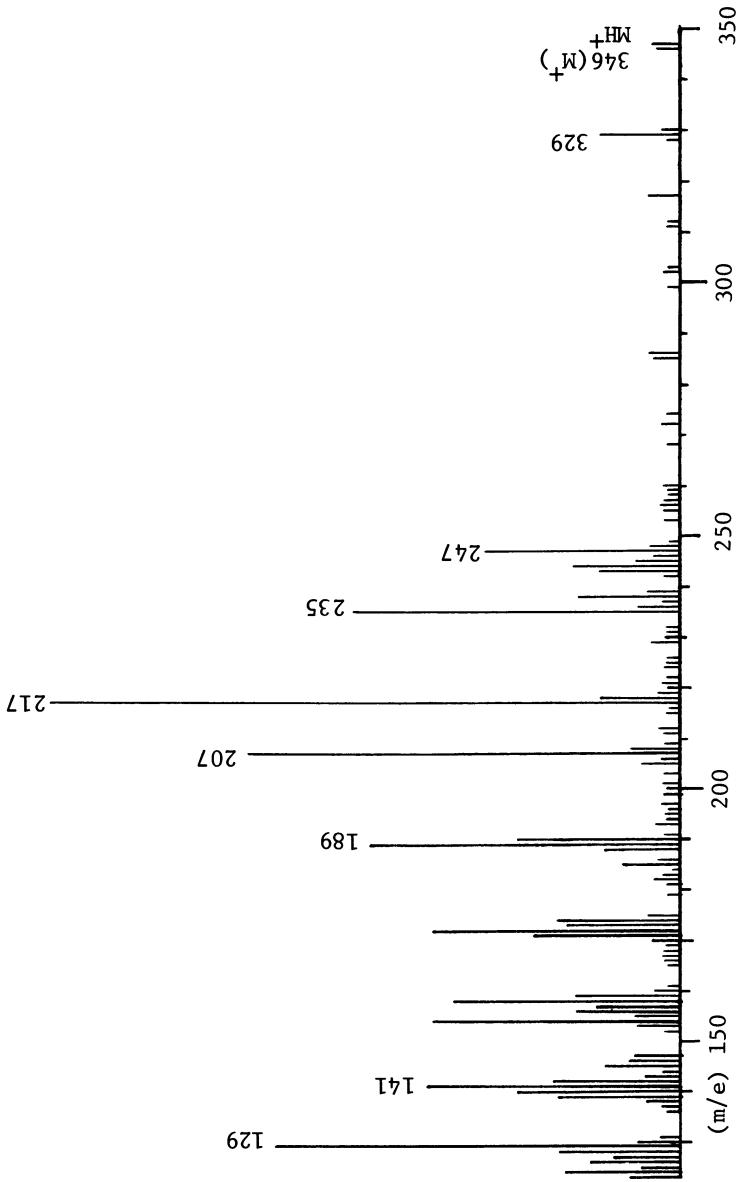


Figure 2. Part of MS spectrum of FM-KG

purpurosamine moiety is 14 atomic mass units less than those of the corresponding peaks of FM-KG. The purpurosamine moiety of FM-KF is thus 4,5-dehydropurpurosamine-C, which is known to be a component of sisomicin (8, 9).

The ^{13}C NMR (CMR) of FM-KG free base exhibited all the fifteen carbons' signals. When the CMR were taken at various pDs, the resonance of each carbon, depending on its environment, gave titration curves (Figure 3). Eight carbons showed protonation shifts (deuteration shifts) and it could be concluded the fortamine of this antibiotic is a 1,2 or 1,4-diaminocyclitol. The signals due to the dehydropurpurosamine moieties of FM-KF and KG were assigned by consideration of the titration curves and by comparison with chemical shifts of sisomicin (Table 1). As the

Table 1. CMR Chemical Shifts (ppm from TMS)

carbon	FM-KG (pD=11.0)	FM-KF (pD=10.7)	sisomicin(10) (free base)	fortamine KF (pD=10.3)
1'	101.3	100.8	100.8	
2'	47.5	47.3	47.6	
3'	25.8	25.5	25.6	
4'	94.9	97.1	96.5	
5'	153.7	149.8	150.4	
6'	48.9	43.2	43.5	
6'-Me	20.8	-	-	
1	53.3	53.2		54.3
2	74.0*	73.9*		73.0*
3	79.5	79.5		79.6
4	62.2	62.2		42.2
5	73.2*	73.3*		74.0*
6	84.2	83.2		76.5
3-OMe	62.8	62.8		62.8
4-NMe	33.8	33.8		33.7

The chemical shifts were measured in D_2O from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. The assignments of the resonances marked with * may be interchanged.

remaining eight carbons of both compounds resonate within 1.0 ppm of the same frequencies, it could also be concluded that FM-KF and KG have the same fortamine moiety, namely fortamine KF, with identical stereochemistry.

Acid hydrolysis of tetra-N-acetylfortimicin KG gave fortamine KF, the PMR of which showed an N-methyl at 2.42 ppm and an O-methyl at 3.57 ppm. Although the six protons on the cyclitol

appeared as multiplets, they were fairly simple, and first order analysis was possible (Figure 4). One proton, at the lowest frequency (3.58 ppm), appeared as a triplet with small coupling constants of 2.7 Hz. All others were coupled to each other with 8.6 Hz or larger coupling constants. This means that only the proton which appears at 3.58 ppm is in the equatorial orientation.

In addition, it was clearly demonstrated that the equatorial proton is attached to C-3, which carries the methoxy group, by means of the proton-selective decoupling CMR. The assignments of H₁ and H₄, attaching to the carbons which carry nitrogen atoms, were also confirmed by proton-selective decoupling CMR and there was found to be no interaction between H₁ (2.43 ppm) and H₄ (2.87 ppm); fortamine KF is a 1,4-diaminocyclitol.

The CMR of fortamine KF were assigned by means of single frequency irradiation at its protons (the CMR and PMR of the same compounds were measured on the same samples in the same sample tube with using a proton/carbon dual probe, JEOL model NM 3835, through this study). By comparison with the chemical shifts of the fortamine KF, the fortamine carbons of FM-KG were assigned (Table 1). Both are consistent except one pair. The 76.5 ppm signal of fortamine KF appeared at 84.2 ppm in the spectrum of FM-KG. This 7.7 ppm down field shift corresponds to an alkylation shift of a carbinol. Thus, fortamine KF should be attached to the purpurosamine moiety through the oxygen atom at the position-6 in FM-KG and also in FM-KF. The absolute configuration of the new fortamine was determined by measuring difference of the rotations of its di-N-acetyl derivative between in a solution of cuprous chloride in a concentrated aqueous ammonia and in water ($\Delta[M]_{\text{CuAm}} = +1160^\circ$) to be shown in Figure 5.

Because the 5'-carbon has no proton, the PMR can not give enough information for the stereochemistry at the 6'-position. But, if the double bond can be cleaved oxidatively, alanine and aspartic acid will be formed from the upper part and the lower part of the purpurosamine of FM-KG, respectively. Actually, the tetra-N-Z derivative of FM-KG was treated with ozone, followed by potassium iodide, acid hydrolysis, then oxidation with silver nitrate of the resulting aldehyde groups, and finally hydrogenolysis to remove the amine-protecting group to afford an amino acid fraction where alanine and aspartic acid were detected by an amino acid analyzer (Scheme 2). A part of the amino acid fraction was freeze-dried and converted into its N-trifluoroacetyl-L-menthyl ester. Gas chromatographic analyses (Figure 6) showed that the alanine derived from FM-KG is in L-form by comparison with the retention times of the same derivatives of authentic D and L-alanine(11). This finding indicates that the absolute configuration at the 6'-position must be S in FM-KG. As the derivative of the aspartic acid obtained under the same conditions could not be separated on a gas chromatograph, an enzyme reaction was applied to the aspartic acid.

It is known that aspartate aminotransferase catalyzes the

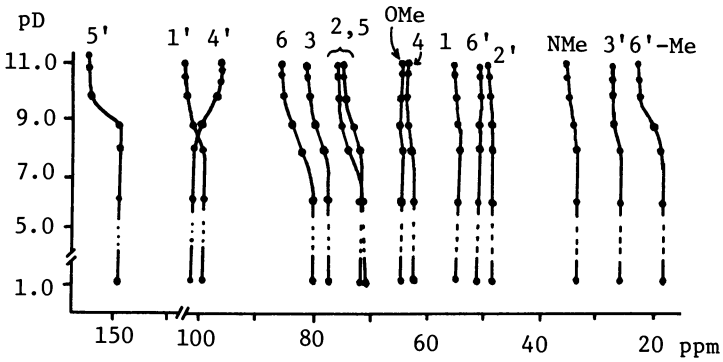


Figure 3. Titration curves for the ^{13}C resonances of FM-KG

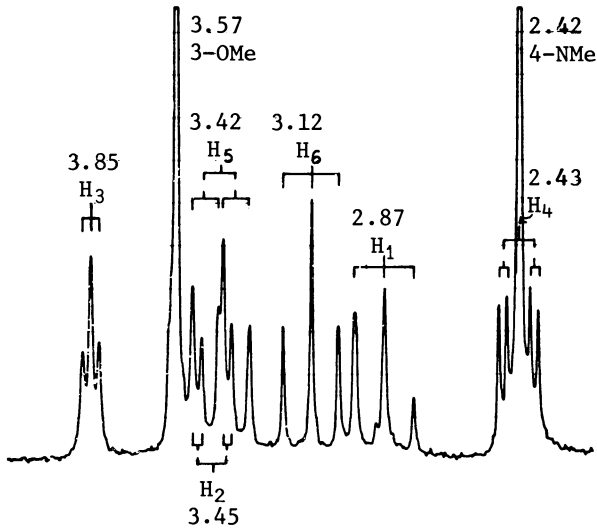


Figure 4. The 100 MHz PMR spectrum of fortamine KF free base in D_2O measured in WEFT (water-eliminated Fourier transform) mode

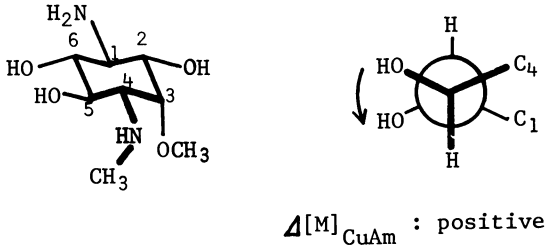
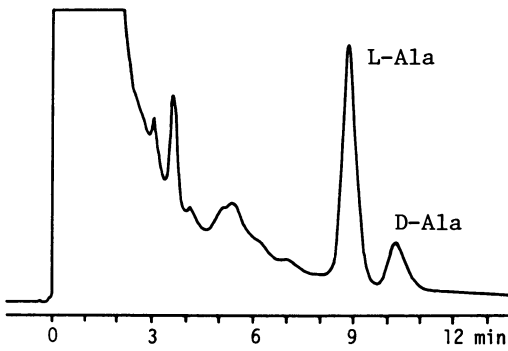
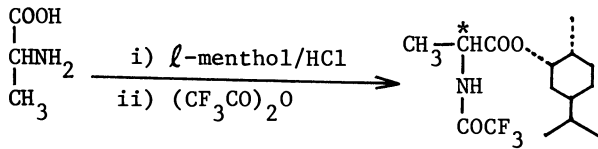
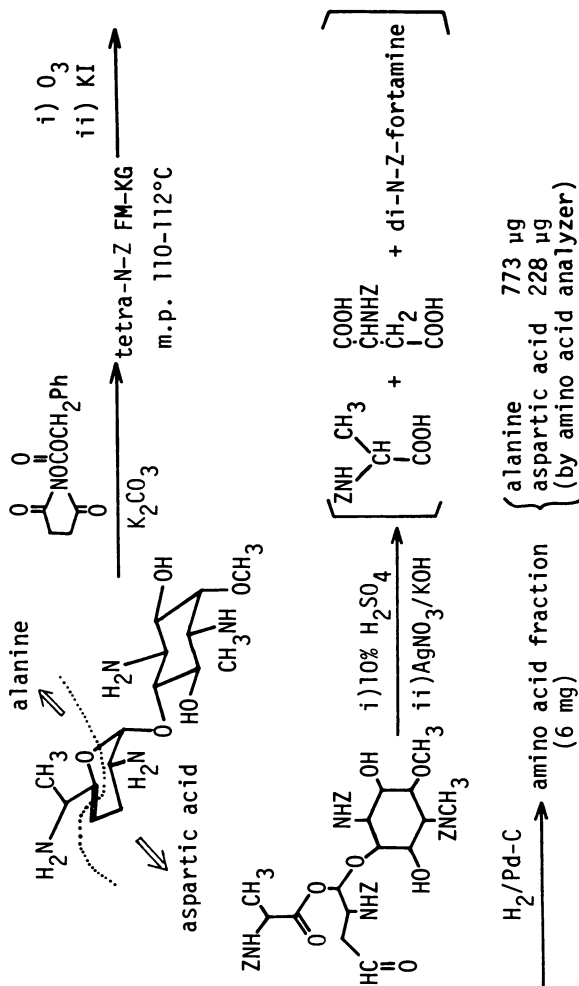


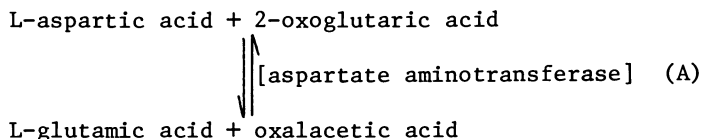
Figure 5. Absolute configuration of fortamine KF



Column 20% PEG 20M
 Temperature 170°C
 Carrier He

Figure 6. Gas chromatogram of N-TFA-l-menthyl ester of the alanine





reaction(A) (12). Authentic L-aspartic acid was converted into glutamic acid by the enzyme even in the presence of the amino acid fraction obtained from FM-KG. On the other hand, no glutamic acid was detected in the reaction mixture of the amino acid fraction after treatment with the enzyme. The aspartic acid derived from FM-KG, therefore, must be in the D-form and consequently the absolute configuration of the 2'-position of the antibiotic is R. In the case of FM-KF, the 2'(R) configuration was also revealed by a similar procedure.

Fortimicin KG₁, KG₂, and KG₃

Fortimicin KG₃(FM-KG₃) is unstable, especially in alkaline medium. Several intense peaks were observed in its mass spectrum and could be attributed to such fragmentation as shown in Figure 7. Treatment of KG₃ with barium hydroxide yielded fortimicin KG₂(FM-KG₂), which was also isolated from the broth of the same fermentation. The mass spectrum of FM-KG₂ (C₁₅H₃₀N₄O₅) could be explained by fragmentations similar to those of FM-KG showing that FM-KG₂ is a stereoisomer of FM-KG. Seven resonances of FM-KG₂ in its CMR were consistent with those of the dehydropurpurosamine moiety of FM-KG and the remaining eight carbons were observed at almost the same chemical shifts as those of FM-B. Similarly, the resonances of the fortamine carbons of FM-KG₃, including the glycyll group, were closely consistent with those of FM-A (Table 2). On the basis of these facts, it was concluded that FM-KG₂ is 4',5'-dehydrofortimicin B and FM-KG₃ is 4-N-glycyllfortimicin KG₂ (Figure 8).

PMR and mass spectrum of fortimicin KG₁(FM-KG₁) suggested that this antibiotic is a stereoisomer of FM-KG (and FM-KG₂). On the other hand, another minor component, fortimicin KH has been isolated and its structure has been established (which will be published elsewhere in the near future). The CMR spectra demonstrated that FM-KG₁ and KH have the same fortamine. Because only a small amount of FM-KG₁ was isolated, the fortamine (fortamine KH) used in this study was obtained by degradation of FM-KH. There were observed six proton multiplets as well as an O-methyl and an N-methyl signals in the PMR of fortamine KH. Decoupling experiments demonstrated that the two protons on the carbons carrying amino groups had no interaction with each other. Fortamine KH, therefore, is also a 1,4-diaminocyclitol. Moreover, the six protons on the ring appeared with coupling constants of larger than 8 Hz. It is evident that all the substituents on the cyclitol are in equatorial orientation.

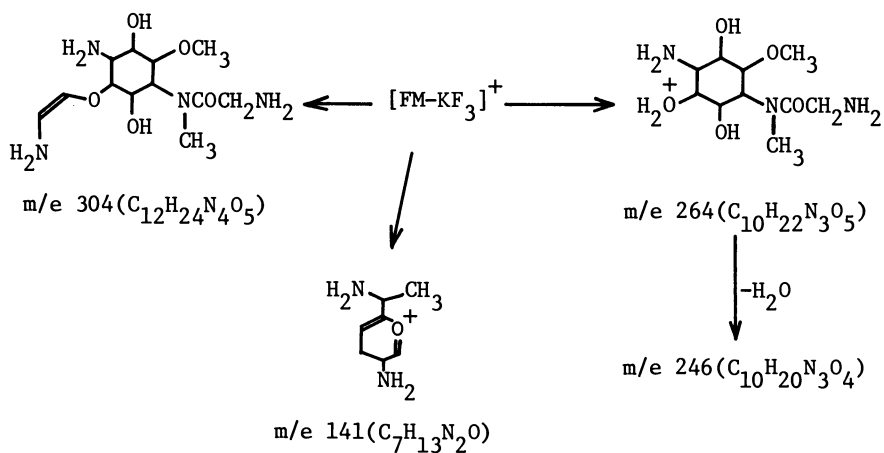


Figure 7. Fragment ions of FM-KG₃.

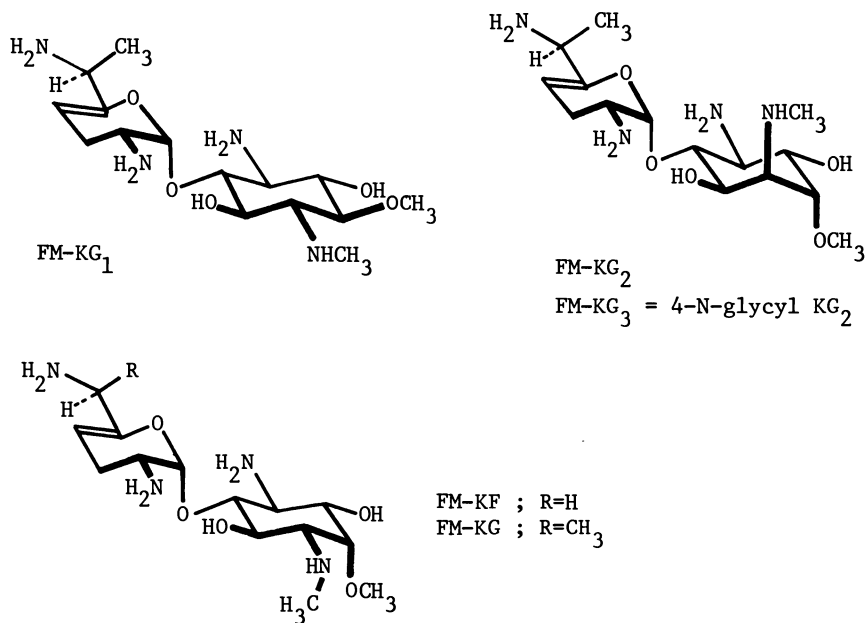


Figure 8. Structures of fortimicins KF, KG, KG₁, KG₂, and KG₃. The fortamine conformation in FM-KG₃ is similar to that of FM-A (3).

Table 2. CMR Chemical Shifts (ppm from TMS)

pD carbon	FM-KG ₂	FM-B	FM-KG ₃	FM-A	FM-KG ₁	fortamine KH
	10.6	11.3	10.7	11.5	10.7	10.0
1'	101.3		100.2		101.2	
2'	47.3		47.1		47.4	
3'	25.6		25.5		25.7	
4'	95.3		96.1		95.2	
5'	153.1		152.2		153.1	
6'	48.9		49.0		48.9	
6'-Me	20.5		20.3		20.5	
1	53.8	53.8	55.1	55.4	54.8	55.9
2	71.1	71.1	71.1	71.1	75.1	75.2*
3	78.0	79.9	74.1	73.7	83.2*	83.5
4	61.1	60.9	52.7	52.5	62.2	62.2
5	71.3	71.3	72.9	72.9	73.4	73.2
6	82.2	84.0	79.7	78.4	83.4*	75.5*
3-OMe	59.3	59.3	56.4	56.4	60.1	60.1
4-NMe	35.4	35.4	32.4	32.2	33.6	33.6

The assignments of the resonances marked with * may be interchanged. CMR of FM-A and B are discussed in the reference(6).

The assignments of the carbon resonances of fortamine KH were accomplished by pD-titration experiments in addition to the selective-proton decoupling method. The purpurosamine moiety of KG₁ is evident to be same to that of FM-KG. Resonances due to carbons of the purpurosamine and fortamine moieties of FM-KG₁ were easily assigned by comparison with those of FM-KG and fortamine KH, respectively (Table 2). Although the assignment of 3-carbon could not be strictly distinguished from 6-carbon in this manner, it was concluded that fortamine KH links to the purpurosamine through the oxygen atom at the position-6 in FM-KG₁ (Figure 8), as in FM-KH.

Among the new fortimicins, FM-KG₃ is the most active and is comparable to FM-A in its antibacterial activity (Table 3).

Table 3. Antimicrobial Activities of Fortimicins (MIC; mcg/ml)

organisms	FM-KF	FM-KG	FM-KG ₁	FM-KG ₂	FM-KG ₃	FM-A
E. coli NIH JC-2	4.2	2.8	78	124	0.17	0.33
KY8302 (APH-3')		2.8	78	124		
KY8327 (ANT-2'')		2.8	78	63	0.17	0.33
KY4348 (AAC-3-I)		1.4	78	200	5.2	10.4
KY8530 (AAC-6'-II)		1.4	78	63	0.08	0.17
S. aureus 209P	2.1	0.36	39	32	0.04	0.08
KY8970 (AAD-4')		0.18	10	7.1	0.01	0.02
P. vulgaris ATCC 6897	4.2	2.8	78	200	0.33	0.66
Providencia 164		83	200	200	1.3	1.3
P. aeruginosa #1		21	200	200	2.6	10.4

Literature Cited

1. Nara, T., Yamamoto, M., Kawamoto, I., Takayama, K., Okachi, R., Takasawa, S., Sato, T., and Sato, S., J. Antibiot., 1977, **30**, 533.
2. Okachi, R., Takasawa, S., Sato, T., Sato, S., Yamamoto, M., Kawamoto, I., and Nara, T., J. Antibiot., 1977, **30**, 541.
3. Egan, R. S., Stanaszek, R. S., Sandra, M. C., Mueller, L., Tadanier, J., Martin, J. R., Collum, P., Goldstein, A. W., De Vault, R. L., Sinclair, A. C., Fager, E. E., and Mitscher, L. A., J. Antibiot., 1977, **30**, 552.
4. Hirayama, N., Shirahata, K., Ohashi, Y., Sasada, T., and Martin, J. R., Acta Cryst., 1978, **B34**, 2648.
5. Sugimoto, M., Ishii, S., Okachi, R., and Nara T., J. Antibiot. 1979, **32**. September issue (in press).
6. Iida, T., Sato, M., Matsubara, I., Mori, Y., and Shirahata, K. J. Antibiot. 1979, in press.
7. Deushi, T., Nakayama, M., Watanabe, I., Mori, T., Naganawa, H. and Umezawa, H., J. Antibiot., 1979, **32**, 187.
8. Reimann, H., Cooper, D. J., Mallams, A. K., Jaret, R. S., Yehaskel, A., Kugelman, M., Vernay, H. F., and Schumacher, D., J. Org. Chem. 1974, **39**, 1451.
9. Daniels, P. J. L., Mallams, A. K., Weinstein, J., Wright, J. J., and Milne, G. W. A., J. Chem. Soc. Perkin I, 1976, 1078.
10. Morton, J. B., Long, R. C., Daniels, P. J. L., Tkach, R. W., and Goldstein, J. H., J. Am. Chem. Soc., 1973, **95**, 7464.
11. Hasegawa, M., and Matsubara, I., Anal. Biochem., 1975, **63**, 308
12. Barman, T. E., Ed. "Enzyme Handbook, Vol. I"; Springer-Verlag: Germany, 1969; p. 345.

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Enzymes Modifying Aminocyclitol Antibiotics and Their Roles in Resistance Determination and Biosynthesis

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The aminocyclitol antibiotics constitute a group of highly active antibacterial agents that are used extensively in the treatment of severe Gram-negative infections (1). At present a number of these compounds are in use (Table 1); they can be conveniently divided into several distinct chemical classes. The 4,5-disubstituted and 4,6-disubstituted 2-deoxystreptamines represent the two largest subclasses; with the exception of the novel compound apramycin, the remaining aminocyclitol antibiotics do not contain 2-deoxystreptamine but other cyclitols.

The recent development of new aminocyclitols, either isolated from nature (e.g., sorbistin, fortimicin) or by the chemical modification of existing compounds (e.g., amikacin, netilmicin) indicates that interest in this important group of agents is maintained, and one can anticipate that aminocyclitols will continue to be of use in the treatment of infectious disease. The continuing development of new members of this group is necessitated by the continued appearance of new forms of antibiotic resistance in clinical isolates (2).

Whereas, resistance to antibiotics such as the β -lactams is due to the presence of one type of enzymatic modification (β -lactamase), resistance to the aminocyclitols has been shown to involve any of several different enzymatic modifications that include O-phosphorylation, O-adenylylation, or N-acetylation. To date, some 12 different enzymatic modifications have been characterized in clinical isolates of Gram-negative and Gram-positive bacteria (Table 2). As we will discuss later, similar types of activities are found in antibiotic-producing organisms.

The structure modifications occur at several of the hydroxy- and amino-groups as exemplified in the case of kanamycin B (Figure 1). Not all of these modifications are known to occur simultaneously, although there are a number of examples in which bacterial strains encode as many as four of these different modifications at the same time (3). The enzymatic modifications are usually plasmid-coded and often transferable; aminoglycoside resistance is a common characteristic of resistance plasmids isolated from bacteria in clinical situations. Within each

Table I. Aminocyclitol Antibiotics in Clinical Use (Human and Veterinary)

STREPTOMYCIN	NEOMYCIN	KANAMYCIN A,B	AMIKACIN
DIHYDROSTREPTOMYCIN	PAROMOMYCIN	TOBRAMYCIN	NETILMICIN
	LIVIDOMYCIN	GENTAMICIN	
SPECTINOMYCIN	RIBOSTAMYCIN	SISOMICIN	APRAMYCIN

Table II. Enzymes Modifying Aminocyclitol Antibiotics Found in Resistant Gram-Negative and Gram-Positive Isolates

Modification	Enzyme	Typical Substrates*
Acetylation	AAC (2')	Gentamicin, tobramycin
	AAC (6')	Tobramycin, kanamycin, amikacin, neomycin (gentamicin [†])
Adenylylation	AAC (3)	Gentamicin, tobramycin, kanamycin
	AAD (4')	Amikacin, tobramycin, kanamycin
	AAD (2'')	Gentamicin, tobramycin, kanamycin
	AAD (3'')	Streptomycin, spectinomycin
Phosphorylation	AAD (6)	Streptomycin
	APH (3')	Kanamycin, neomycin
	APH (3'')	Streptomycin
	APH (2'')	Gentamicin
	APH (5'')	Ribostamycin

*Not all substrates are listed; each enzyme exists in a variety of forms with different substrate ranges.

[†]Gentamicin C_{1a} is a substrate for AAC (6'), another component of the gentamicin complex, gentamicin C₁, is not.

group of enzymes (modifying a given site) there exists different isozymic variants of the enzymes that differ in their aminoglycoside substrate range. The different forms appear to be elicited in response to different antibiotic selection pressures; for example, the original isolates of gentamicin resistant organisms (that were sensitive to tobramycin) possessed a 3-N-acetyltransferase that favored gentamicin as substrate (4). (Apparently) with increasing use of other aminoglycosides, new 3-N-acetyltransferases with broader substrate ranges, associated with broader resistance phenotypes, have been characterized (5) (Table 3). Newer resistance phenotypes in bacteria may imply new resistance mechanisms, but they can also be the result of combinations of pre-existing types. The enzyme content of a resistant strain cannot be predicted on the basis of resistance phenotype alone.

The function of the aminoglycoside-modifying enzymes is obviously related to the resistance mechanism. The enzymatic modification can be shown to be directly related to the determination of resistance by the isolation of point mutants that reduce or eliminate the enzyme activity (6), and also by the existence of transposable resistance elements that have coding capacity sufficient only for the aminoglycoside modifying enzyme (7). In spite of these studies the exact biochemical mechanism of R-plasmid coded aminoglycoside resistance is not known.

The aminoglycosides exert their inhibitory action on bacteria by binding to ribosomes and interfering with protein synthesis (8). Drugs such as amikacin and gentamicin bind to both ribosome subunits (9) (Fig. 2) in contrast to streptomycin, that binds only to the 30 S subunit (10). The mechanism of resistance could be due to detoxification of the drug or interference with drug transport as a result of enzymatic modification. Since binding to ribosomes is believed to be an essential component of the entry of aminoglycosides into the cell, it is difficult to distinguish between these two possibilities. Studies with radioactively-labelled gentamicin have shown that drug uptake is drastically reduced in resistant strains, and that there is no detoxification of antibiotic in the culture medium (7). Bryan and his collaborators have proposed a reasonable model for aminoglycoside transport and resistance, but convincing proof is lacking (11). A role for a specific polyamine-transport system in the uptake of aminoglycosides has been indicated (12). Notwithstanding this controversy, the role of the aminoglycoside-modifying enzymes in resistant clinical isolates is clear. Antibiotic modification and resistance are correlated.

However, the roles of these enzymes in other bacterial strains is less evident. In studies of the possible origins of aminoglycoside-modifying enzymes, it has been shown that most aminoglycoside-producing organisms (*Streptomyces*) possess enzyme

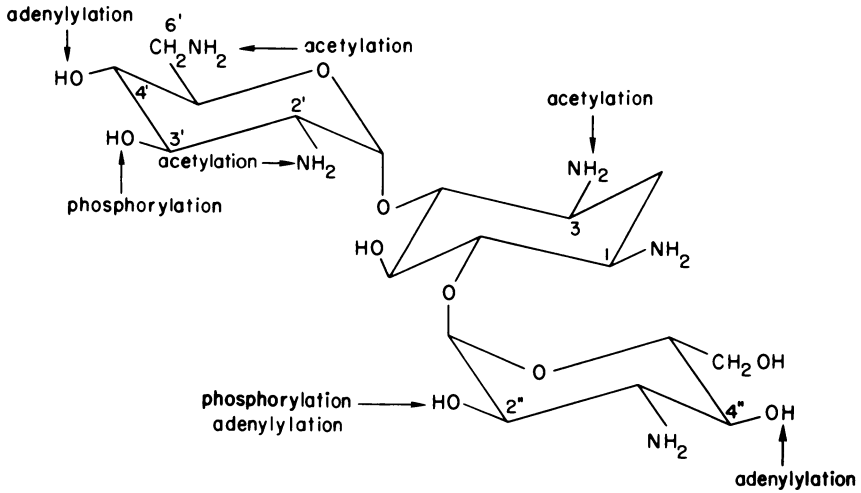


Figure 1. Enzymatic modification of kanamycin B by resistant strains

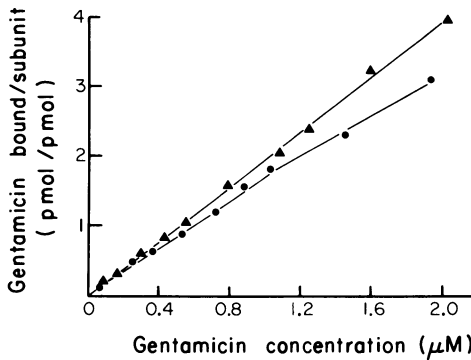


Figure 2. Binding of ^3H -gentamicin to 30S (\circ) and 50S (Δ) ribosome subunits of resistant *E. coli*. Experiments performed by equilibrium dialysis (S. Perzynski).

activities similar to those found in clinical isolates (13) (Table 4). The activities are similar in that they catalyze the same type of reaction (e.g., 3'-O-phosphorylation) and share many of the same substrates. However, examination of possible sequence homologies, at the nucleic acid or protein level, between aminoglycoside-modifying enzymes in resistant clinical isolates and those of aminoglycoside-producing strains, have proved negative (14). Thus, there is no direct evidence that producing organisms are the origins of the resistance determinants. That they can serve as resistant determinants is evident from gene transfer experiments; the 3'-O-phosphotransferase of Bacillus circulans (producing butirosin) acts as typical phosphotransferase resistance determinant in E. coli (15). Even if the aminoglycoside-modifying enzymes of resistant isolates did originate in the corresponding antibiotic producing organisms, the evolution of the plasmid encoded enzymes may have been so divergent as to eliminate any direct sequence homologies in the genes. It is of interest to note that plasmid encoded mechanisms of resistance to several different antibiotics in clinical isolates are identical to those found in producing organisms or closely related Streptomyces (Table 5).

What is the function of aminoglycoside-modifying enzymes in producing strains? The two obvious roles are: a) to protect the producing organism from the antibiotic(s) that it makes, or b) to catalyze the formation of a specifically protected or activated intermediate. Of course, a dual function might also be involved. One might also imagine that the aminoglycoside-modifying enzymes play no role in the biosynthesis or resistance to an antibiotic, and might be involved with other functions. In this case, the aminoglycoside would be assumed to be a gratuitous member of the enzyme's substrate range.

Studies of the effects of so-called "curing" agents, have thrown some light on this matter. These chemicals promote the segregation of plasmid-free progeny during bacterial cell division; this is their primary mode of action. Since the presence of plasmids has now been implicated in the biosynthesis of several different antibiotics (Table 6), we can examine the effects of curing agents on biosynthesis and resistance in aminoglycoside-producing organisms. The most extensive studies, so far, have been done by Yagisawa *et al.* (23), who examined neomycin resistance and 3'-O-phosphotransferase production in a number of variants of Streptomyces fradiae obtained by treatment with the curing agent, acridine orange. In addition, the ability of the "cured" strains to synthesize neomycin when grown in the presence of the precursor 2-deoxystreptamine, was tested.

The results of these experiments can be summarized as follows:

1. Treatment of neomycin-producing Streptomyces fradiae with acridine orange, produces at least two distinct classes of neomycin nonproducing variants.

Table III. Aminocyclitol-3-N-Acetyltransferases of Different Substrate Ranges

- I GENTAMICIN, SISOMICIN
- II GENTAMICIN, SISOMICIN, TOBRAMYCIN
- III GENTAMICIN, SISOMICIN, TOBRAMYCIN, NEOMYCIN
- IV GENTAMICIN, SISOMICIN, TOBRAMYCIN, NEOMYCIN, APRAMYCIN

Table IV. Aminocyclitol-Modifying Enzymes in Aminocyclitol-Producing Strains

<u>Strain</u>	<u>Antibiotic Produced</u>	<u>Modifying Enzyme</u>
<u>S. fradiae</u>	neomycin	AAC(3), APH(3')
<u>B. circulans</u>	butirosin	AAC(3), APH(3')
<u>M. chalcea</u>	neomycin	AAC(3), APH(3')
<u>S. tenebrarius</u>	tobramycin	AAC(6'), AAC(2')
<u>S. kanamycelicus</u>	kanamycin	AAC(6')
<u>S. griseus</u>	Streptomycin	APH(3''), APH(6)
<u>S. bikiniensis</u>		

Table V. Antibiotic Resistance Mechanisms in Streptomycetes

<u>Antibiotic</u>	<u>Organism</u>	<u>Mechanism of Resistance</u>	<u>Reference</u>
Aminoglycosides	Numerous <u>Streptomyces</u> and related species	Enzymatic modification of amino or hydroxy groups	see Table IV
Chloramphenicol	Numerous <u>Streptomyces</u>	Enzymatic acetylation of hydroxy-group (chlaramphenicol acetyl- transferase)	16
β -Lactams	Numerous <u>Streptomyces</u> and related species	Enzymatic hydrolysis of β -lactam ring (β -lacta- mase)	17
Erythromycin and other macrolides	<u>Streptomyces</u> <u>erythreus</u>	Enzymatic modification of 23S ribosomal RNA	18
Thiostrepton	<u>Streptomyces</u> <u>azureus</u>	Enzymatic modification of 23S ribosomal RNA	19
Lincomycin	Numerous <u>Streptomyces</u>	Enzymatic modification of hydroxy-group	20

Table VI. Antibiotic Biosynthesis in Which Plasmid Involvement Has Been Suggested

<u>Antibiotic</u>	<u>Reference</u>
Kasugamycin, aureothricin	21
Chloramphenicol	22
Neomycin	23
Kanamycin	24
Methylenomycin	25
Actinomycin	26
Streptomycin	27
Macrolides	28
Tetracycline	29
Leupeptin	30

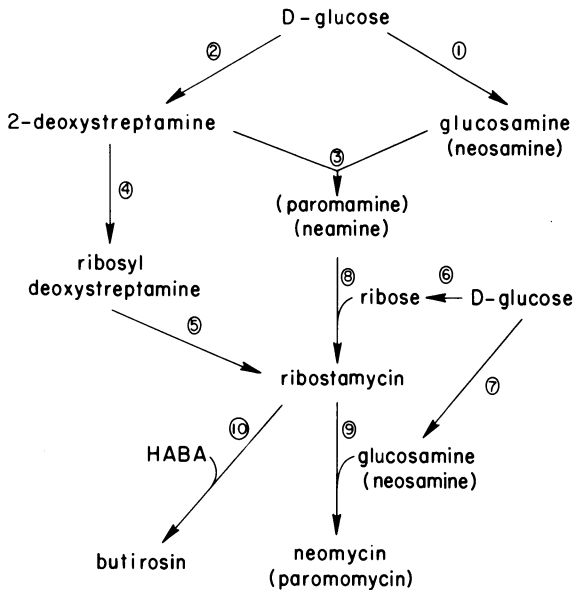


Figure 3. Outline of the biosynthetic pathway to neomycin

2. One class of nonproducers (I) was neomycin-resistant, synthesized normal amounts of 3'-O-phosphotransferase and would produce antibiotic when grown in the presence of 2-deoxystreptamine.
3. The second class of nonproducers (II) was neomycin-sensitive, did not contain phosphotransferase, and would not produce antibiotic on 2-deoxystreptamine feeding.

Although there are obviously several interpretations of these data, it has been suggested that, in *S. fradiae*, both 3'-O-phosphotransferase and at least part of the neomycin biosynthetic pathway (that concerned with 2-deoxystreptamine synthesis) are plasmid encoded. The 3'-O-phosphotransferase is required for neomycin resistance in *S. fradiae* and possibly also for a step in biosynthesis, although there is no evidence for the latter. The class I nonproducers have presumably lost the capacity to synthesize deoxystreptamine but retain the rest of the neomycin biosynthetic pathway. These strains will produce neomycin when supplemented with 2-deoxystreptamine, since they are neomycin resistant (they have the 3'-O-phosphotransferase). Similar results have been obtained with the paromomycin-producer *S. rimosus* forma paromomycinus and the neomycin-producer, Micromonospora chalcea (31).

The finding of plasmids in antibiotic-producing *Streptomyces* and their implication in antibiotic biosynthesis, has a number of interesting consequences for studies of antibiotic production. In the first place, curing agents can be used to produce a new class of idiotrophs (32). In the past *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) has been favored for the production of antibiotic nonproducing derivatives that can be used for "feeding" of precursors to produce novel antibiotics (33). Since NTG is a powerful mutagen that is known to cause multiple mutations (34, 35), the use of curing agents might increase the chances of obtaining plasmid derivatives specifically involved with antibiotic (or other secondary metabolite) synthesis. In addition, NTG may cause a number of lethal mutations and mutations in primary metabolism that affect both cell growth and the ability to produce antibiotics, that could not be supplemented exogenously. One can anticipate that curing agents, that would not affect primary metabolism, may give different classes of idiotrophs in which normal cell metabolism would not be affected.

The evidence for plasmid involvement in antibiotic synthesis in *Streptomyces* is still largely coincidental. But, if one considers the biosynthesis of an antibiotic such as neomycin (Fig. 3) it can be reasoned that several of the steps involve primary metabolism more critically than others. For example, the biosynthesis of ribose is a primary metabolic function, being required for cell-wall biosynthesis among other things. On the other hand, 2-deoxystreptamine is almost certainly a secondary metabolite, concerned only with antibiotic biosynthesis. The

biosynthesis of 2-deoxystreptamine is likely to be plasmid-determined while ribose is chromosomally encoded. Consistent with this notion is the fact that a high proportion of the idiotrophs of *S. fradiae* producing by curing agents, will produce antibiotic on supplementation with 2-deoxystreptamine (23). As we know more about the biosynthetic pathways, other intermediates may be used for feeding; for example, we were able to obtain one idiotroph of *S. rimosus* forma paromomycinus that would produce antibiotic in the presence of paromamine but not 2-deoxystreptamine (31). This constituted a new class of idiotroph for this organism.

It is probable that, in many instances, mechanisms of antibiotic resistance in *Streptomyces* (being concerned with secondary metabolites) may also be plasmid-encoded. This suggests the possibility of moving these antibiotic resistances into other members of the species (or even different genera) where an augmentation of antibiotic resistance might lead to higher capacity for the production of an antibiotic.

In conclusion, studies of the nature, distribution, and function of aminoglycoside-modifying enzymes have provided us with directions to new aminoglycosides, and their isolation in new producing strains can be used to predict probable resistance mechanisms to appear in clinical isolates. The presence of plasmids in *Streptomyces* that determine both antibiotic biosynthesis and resistance (although still not proven) indicates that much of the "genetic engineering" with respect to antibiotic biosynthesis has already been done by Nature. What is required now is a greater knowledge of the biosynthetic pathways involved, and the roles of plasmid-coded functions (directly and indirectly) in these pathways. With this knowledge it should be possible to use recent developments in the genetics to *Streptomyces*, such as transformation (36) and fusion (37), to make intelligent approaches to improving the yields of antibiotics (not only the aminocyclitols) and in producing modified compounds by microbiological methods.

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References

1. Daniels, P. J. L. "Aminoglycosides"; Kirk-Othmer, Encyclo-
pedia of Chemical Technology", John Wiley and Sons, Inc.
New York, 1978; 2, p. 819.
2. Davies, J.; Smith, D. I. Ann. Rev. of Microbiol., 1978, 32,
464.
3. Huang, T. S. R.; Davies, J. Proc. 2nd Tokyo Symp. Microb.
Drug Resistance, in press.
4. Brzezinska, M.; Benveniste, R.; Davies, J.; Daniels,
P. J. L.; Weinstein, J. Biochemistry, 1972, 11, 761.
5. Biddlecome, S.; Haas, M.; Davies, J.; Miller, G. H.; Rane,
D. F.; Daniels, P. J. L. Antimicrob. Agents Chemother., 1976,
9, 951.
6. Kagan, S. A., unpublished observations.
7. Davies, J.; Kagan, S. A. "R-Factors: Their Properties and
Possible Control (Eds. Drews, J.; Høgenauer, G.) Spring-
Verlag, Wien-New York; 1977, p. 207.
8. Tai, P. C.; Wallace, B. J.; Davis, B. D. Proc. Natl. Acad.
Sci. USA, 1978, 75, 275.
9. Perzynski, S., unpublished observations.
10. Chang, F. N.; Flaks, J. G. Antimicrob. Agents Chemother.,
1972, 2, 294.
11. Bryan, L. E.; Van denElzen, H. M. Antimicrob. Agents
Chemother., 1977, 12, 163.
12. Høltje, J. V. Eur. J. Biochem. 1978, 86, 345.
13. Dowding, J. E.; Davies, J. Microbiology 1974, 1974, 179.
14. Courvalin, P.; Flandt, M.; Davies, J. Microbiology 1978,
1978, 262.
15. Courvalin, P.; Weisblum, B.; Davies, J. Proc. Natl. Acad.
Sci., 1977, 74, 999.
16. Shaw, W. V.; Hopwood, D. A. J. Gen. Microbiol., 1976, 94,
159.
17. Ogawara, H.; Horikawa, S.; Shimada-Hiyoshi, S.; Yasuzawa, K.
Antimicrob. Agents Chemother., 1978, 13, 865.

18. Graham, H. Y.; Weisblum, B. Microbiology 1978, 1978, 255.
19. Cundliffe, E. Nature, 1978, 272, 792.
20. Argoudelis, A. D.; Coats, J. H. J. Amer. Chem. Soc., 1969, 93, 534.
21. Okanishi, M.; Ohta, T.; Umezawa, H. J. Antibiot., 1970, 23, 45.
22. Akagawa, H.; Okanishi, M.; Umezawa, H. J. Gen. Microbiol., 1975, 90, 336.
23. Yagisawa, M.; Huang, T. S. R.,; Davies, J. J. Antibiot., 1978, 31, 809.
24. Hotta, K.; Okami, Y.; Umezawa, H. J. Antibiot., 1977, 30, 1146.
25. Kirby, R.; Wright, L. F.; Hopwood, D. A. Nature, 1975, 254, 265.
26. Ochi, K.; Katz, E. J. Antibiot., 1978, 31, 1143.
27. Shaw, P. D.; Piwowski, J. J. Antibiot., 1977, 30, 404.
28. Schrempf, H.; Goebel, W. Abstracts of 3rd Intl. Symp. Genet. Ind. Micro.; Madison, WI, 1978, Abs. 80.
29. Boronin, A. M.; Sadovnikova, L. G. Genetika, 1972, 8, 174.
30. Umezawa, H.; Okami, Y.; Hotta, K. J. Antibiot., 1978, 31, 99.
31. White, T. J., unpublished observations.
32. Nagaoka, K.; Demain, A. L. J. Antibiot., 1975, 28, 627.
33. Rinehart, K. L. Jr. Pure Appl. Chem., 1977, 49, 1361.
34. Guerola, R.; Ingraham, J. L.; Cerda-Olmedo, E. Nature, 1971, 230, 122.
35. Randazzo, R.; Ser monti, G.; Carere, A.; Bignami, J. J. of Bacteriol., 1973, 113, 500.
36. Bibb, M. J.,; Ward, J. M.; Hopwood, D. A. Nature, 1978, 274, 398.
37. Hopwood, D. A.; Wright, M. H.; Bibb, M. J.; Cohen, S. N. Nature, 1977, 268, 171.

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Biosynthesis and Mutasythesis of Aminocyclitol Antibiotics

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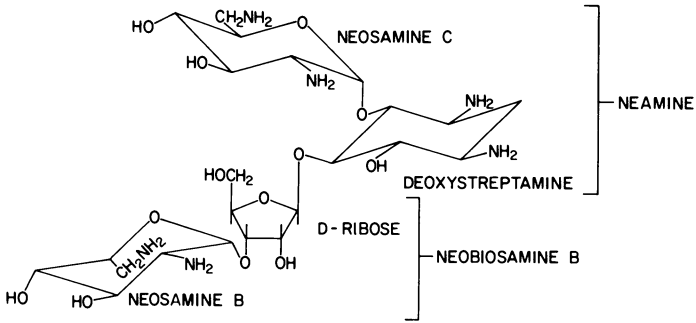
Aminocyclitol antibiotics constitute a large and diverse group of important, clinically useful compounds. As is appropriate to a class of unusual structure and important activity, considerable attention has been directed toward detecting and understanding the reactions involved in the synthesis of these antibiotics by the microorganisms which produce them. In the ensuing discussion, special attention will be directed toward the aminocyclitol rings themselves, the parent structural units of the antibiotic class, though biosyntheses of the other portions of the molecules will be described where known.

Thus far, most studies of biosynthesis have involved deoxystreptamine-containing antibiotics or streptomycin, which contains streptidine, and a few have involved spectinomycin, which contains actinamine. Biosynthetic studies have not been reported for antibiotics with other aminocyclitols. With each antibiotic or class of antibiotics, three points must be determined: first, the nature of the primary precursor of the carbon skeleton of the antibiotic; second, the intermediates between that primary precursor and the subunits found in the antibiotic; and third, the order of linking of the subunits in the antibiotic. The present report addresses these three points in turn for each class.

Deoxystreptamine Antibiotics

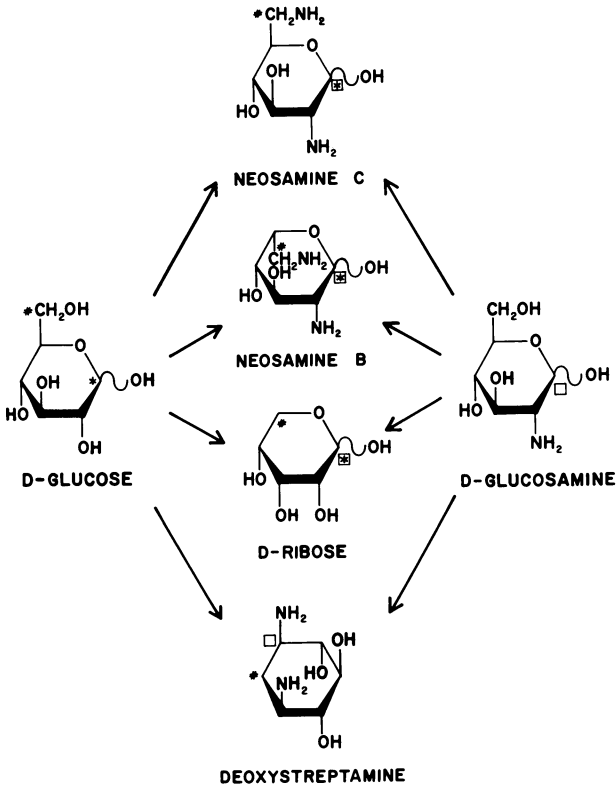
Studies on Neomycin. The most extensively studied biosynthesis in the deoxystreptamine class of aminocyclitols is that of the pseudotetrasaccharide neomycin, whose subunits are identified in Figure 1.

It was early established that uniformly labeled glucose, $[1-^{14}\text{C}]$ glucose, and $[6-^{14}\text{C}]$ glucose were all incorporated, to approximately the same extent, into neomycin, thus establishing that the primary precursor is glucose, all of whose carbons are converted to neomycin (1). Moreover, each of the four subunits of neomycin--deoxystreptamine, neosamines B and C, ribose--is labeled approximately equally by $[\text{U}-^{14}\text{C}]$, $[1-^{14}\text{C}]$, and $[6-^{14}\text{C}]$ -



Journal of Antibiotics

Figure 1. Neomycin B and its subunits (8)



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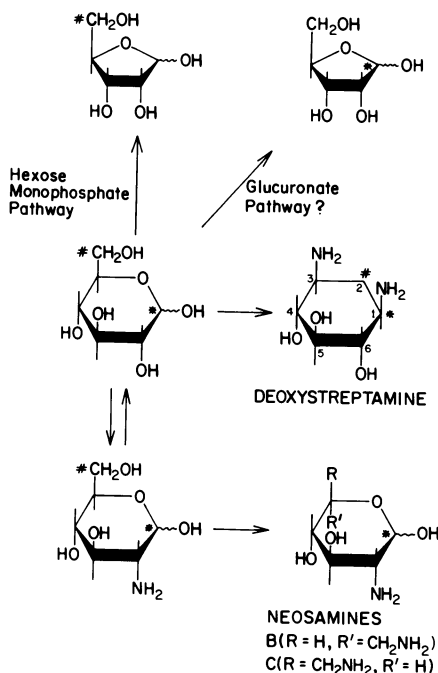
Figure 2. Specific labeling of neomycin subunits by D-glucose and D-glucosamine (8)

glucose (1), establishing that glucose is the source of all units of the antibiotic. The one exception to equal labeling is the subunit D-ribose, in that [U-¹⁴C]glucose labels ribose somewhat more extensively than [1-¹⁴C] or [6-¹⁴C]glucose (1). Degradation experiments during this work and later ¹³C NMR experiments demonstrated that [1-¹⁴C] (1) and [6-¹³C]glucose (2) label the four subunits specifically, in one carbon of each subunit (Figure 2).

Among potential intermediates between glucose and the neomycin subunits the first to be investigated was D-glucosamine, which is incorporated into neomycin to a somewhat greater degree than glucose (1). [1-¹³C]Glucosamine labeled specifically C-1 of neosamines B and C, ribose and deoxystreptamine, as shown in Figure 2 (2). The ¹³C-enrichment in the neosamines was higher from glucosamine than from glucose, while the ¹³C-enrichment in deoxystreptamine and ribose was lower from glucosamine, arguing that glucosamine was not an intermediate en route from glucose to deoxystreptamine (and, of course, not an intermediate en route from glucose to ribose). To confirm this, [¹⁵N]glucosamine was administered to *Streptomyces fradiae* and the ¹⁵N-label from this feeding was demonstrated to reside in the neosamines, with none being found in deoxystreptamine (3). Thus, glucosamine must go back through glucose in order to be converted to deoxystreptamine, as shown in Figure 3, which summarizes the pathways from glucose to the subunits.

Beyond glucosamine, the only labeled intermediates which have thus far been demonstrated to be incorporated into neomycin are two of the subunits themselves, [1-¹⁴C]deoxystreptamine and [1-¹⁴C]ribose, which specifically labeled the corresponding units in neomycin B (Figure 4) (1). However, neosamine C was not incorporated into neomycin (4) and neosamine B has not been tested. Lack of incorporation of neosamine C has been generally attributed to its not appearing underivatized on the biosynthetic pathway (4). Most modifications of sugars take place on nucleotide-bound substrates and it can be argued that glucosamine is converted to a glucosamine nucleoside diphosphate, which is then converted to a nucleotide-bound neosamine B or C, which is then attached to the deoxystreptamine or ribose subunit of neomycin.

In addition to studies employing labeled precursors and intermediates, other studies have made use of the mutant technique. Mutants of the microorganisms which produce deoxystreptamine-containing antibiotics have been sought in which a block has been introduced which prevents the biosynthesis of deoxystreptamine (5). These mutants have been labeled D⁻ mutants by our group (5) and, more generally, idiotrophs by Demain (6). They are recognized by their ability to produce neomycin or another deoxystreptamine-containing antibiotic in the presence of added deoxystreptamine coupled with their inability to produce the antibiotic in its absence. Once such a mutant has been prepared, it can be used in a number of ways.



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Figure 3. Proposed biosynthetic pathways from 1- and 6-labeled glucose and glucosamine to labeled neomycin B subunits (35)

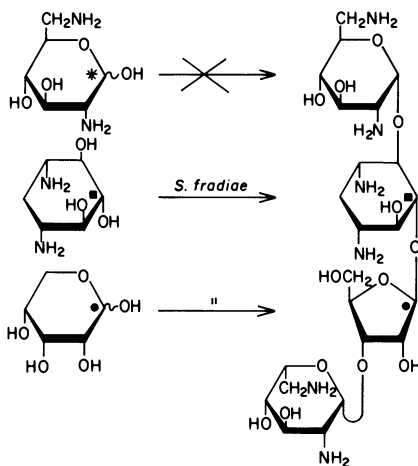


Figure 4. Incorporation into neomycin B of [1-¹⁴C]deoxystreptamine and D-[1-¹⁴C]ribose and lack of incorporation of [1-¹⁴C]neosamine C (35)

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One of these ways is illustrated by the work of Daum, *et al.* (7), who studied the biosynthesis of gentamicin C using a D⁻ mutant of *Micromonospora purpurea*. Using this D⁻ mutant these authors were able to demonstrate that gentamicin C could be produced in the presence of certain potential intermediates between glucose and deoxystreptamine, as well as in the presence of deoxystreptamine itself (7). These intermediates included a tetrahydroxycyclohexanone, a quercitol, and a tetrahydroxycyclohexene, as shown in Figure 5. The first of these, the ketone, had been suggested by us (8) to be a cyclization product derived from glucose and a direct precursor of deoxystreptamine, one which could undergo transamination to give a monoaminotetrahydroxycyclohexane. The quercitol could presumably be oxidized to the ketone and the tetrahydroxycyclohexene could perhaps be hydrated to the quercitol.

In further substantiation of the argument that a cyclohexanone is an intermediate between glucose and deoxystreptamine, transaminases have recently been found by Walker in *S. fradiae* and *M. purpurea* (9) which can aminate *scyllo*-inosose and which can use deoxystreptamine as a substrate for the transamination (Figure 6). The product of the transamination of deoxystreptamine must be a ketodeaminodeoxystreptamine and, since the reaction must be reversible, the latter compound should be on the biosynthetic pathway. Unfortunately, both the incorporation of the tetrahydroxycyclohexanone into gentamicin (7) and the transamination studies (9) were carried out on racemic compounds, so it has not been established which enantiomer is involved, though it was earlier argued (8) that the enantiomers shown are those utilized.

In an attempt to discover intermediates between glucose and tetrahydroxycyclohexanone, Byrne has recently demonstrated that [U-¹⁴C]glucose-dTDP is converted by a cell-free extract of *S. fradiae* 3535X into two radioactive compounds, identified as 4-keto-4,6-dideoxyglucose-dTDP and 6-deoxyglucose-dTDP (10), and has also demonstrated that the 4-keto nucleotide is the precursor of 6-deoxyglucose-dTDP (Figure 7). When the cell-free reaction was carried out employing a strain (*S. fradiae* 3535X-5X) chosen for its enhanced production of neomycin, a third compound (GM3, glucose metabolite 3) was produced which could also be shown to arise from 4-keto-4,6-dideoxyglucose-dTDP. This compound has not yet been identified but appears to be an acid or lactone. Whether any of these compounds derived from glucose-dTDP plays a biosynthetic role as an intermediate en route to deoxystreptamine is still unclear.

The intermediates between glucosamine and neomycin have not yet been determined. In an effort to identify them, Tadano has recently prepared radioactive glucosamine-dTDP, -UDP, -CDP, -ADP, and -GDP by the route shown in Figure 8 (11). The radioactive nucleotides were then administered to the same cell-free system from *S. fradiae* employed for glucose-dTDP, both without and with

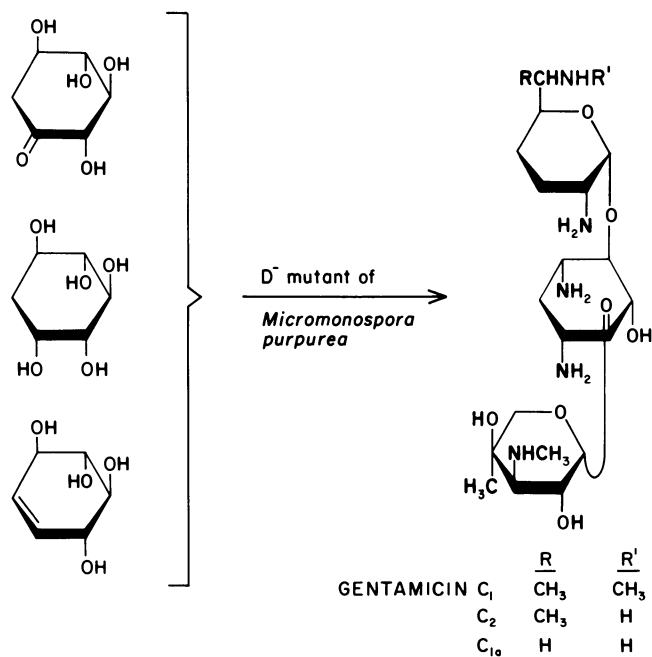


Figure 5. Incorporation of cyclic potential precursors to deoxystreptamine into gentamicin by a D^- mutant of *M. purpurea*

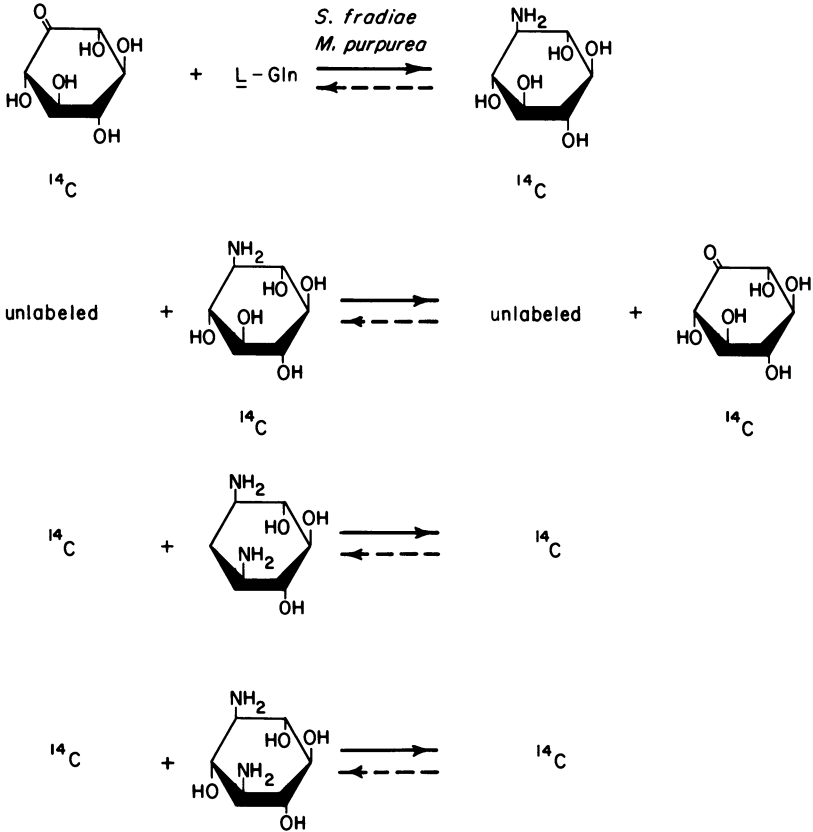


Figure 6. Transamination of aminocyclitols and cyclitols catalyzed by extracts of *S. fradiae* and *M. purpurea*

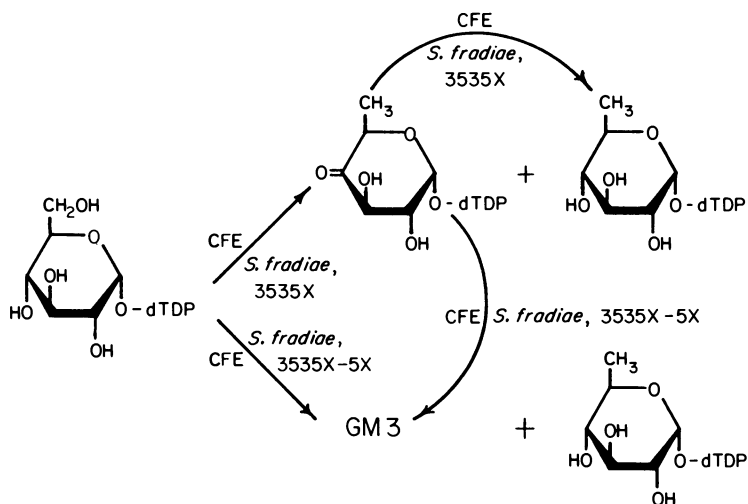


Figure 7. Bioconversion by cell-free extracts of *Streptomyces fradiae* of glucose-dTDP to 4-keto-4,6-dideoxyglucose-dTDP, 6-deoxyglucose-dTDP, and a third, unidentified glucose metabolite

added deoxystreptamine. Thus far, no neomycin intermediate (e.g., neosamine-NDP, paromamine, neamine) has been detected, but the studies are continuing. No other reports describing intermediates between glucose and the neomycin subunits have appeared.

The third point, dealing with the order of linking the subunits to make neomycin B, has been addressed in a number of laboratories. As shown in Figure 9, there are several possible alternative orders of linking the subunits, with the potential initial steps involving formation of neamine, neobiosamine, or ribosyl-deoxystreptamine, and the potential final steps involving attachment of neosamine B to ribostamycin, attachment of neosamine C to neobiosaminyl(B)-deoxystreptamine, or attachment of neobiosamine to neamine.

Intuitively, one feels that neamine should be the first pseudo-oligosaccharide biosynthesized. Neamine is found underivatized in varying amounts as a component (sometimes as the major component) of neomycin preparations (12) and when 2,5-dideoxystreptamine is administered to a D⁻ mutant, 5-deoxyneamine is the product (13, 14, 15). On the other hand, attempts to demonstrate the incorporation of neamine into neomycin using D⁻ mutants of *S. fradiae* were unsuccessful, as were attempts to incorporate paromamine, a pseudodisaccharide from paromomycin, into paromomycin (16). More recent studies employing a mutant of *S. paromomycinus*, however, have allowed the production of neomycin by a D⁻ mutant of *S. paromomycinus* when neamine was administered (Figure 10) (15). Moreover, when neamine was administered to a D⁻ mutant of *Streptomyces ribosidificus*, ribostamycin was produced (17). Thus, neamine appears to be a definite biosynthetic intermediate en route to neomycin.

Two alternative routes to neamine are, however, possible. One involves attaching neosamine C (presumably via its nucleoside diphosphate) directly to deoxystreptamine to give neamine, the other involves attaching glucosamine (again, presumably via a nucleoside diphosphate) to deoxystreptamine to give paromamine, which could be aminated to give neamine. A definite decision between these two possibilities is not yet possible, but a compelling argument has recently been made for the latter possibility. As summarized in Figure 11, an N⁻ mutant of *Bacillus circulans* (a mutant which cannot produce butirosin except in the presence of neamine) was shown to convert neamine to a mixture of ribostamycin, xylostasin, and butirosins A and B (18). The same mutant was able to convert paromamine to a mixture of compounds including the same antibiotics but also including 6'-deamino-6'-hydroxy(DAH)-ribostamycin, DAH-xylostasin, and DAH-butirosins A and B. From these results it appears that, at least by *Bacillus circulans*, paromamine can be converted to neamine.

A similar argument was adduced earlier for the gentamicins by Testa and Tilley (Figure 12), who argued that paromamine is

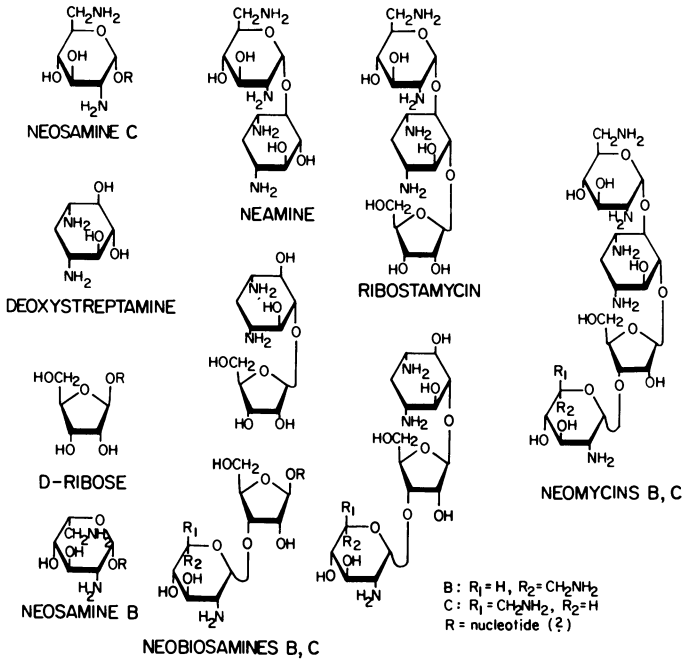


Figure 9. Neomycins B and C and their mono-, di-, and trisaccharide subunits

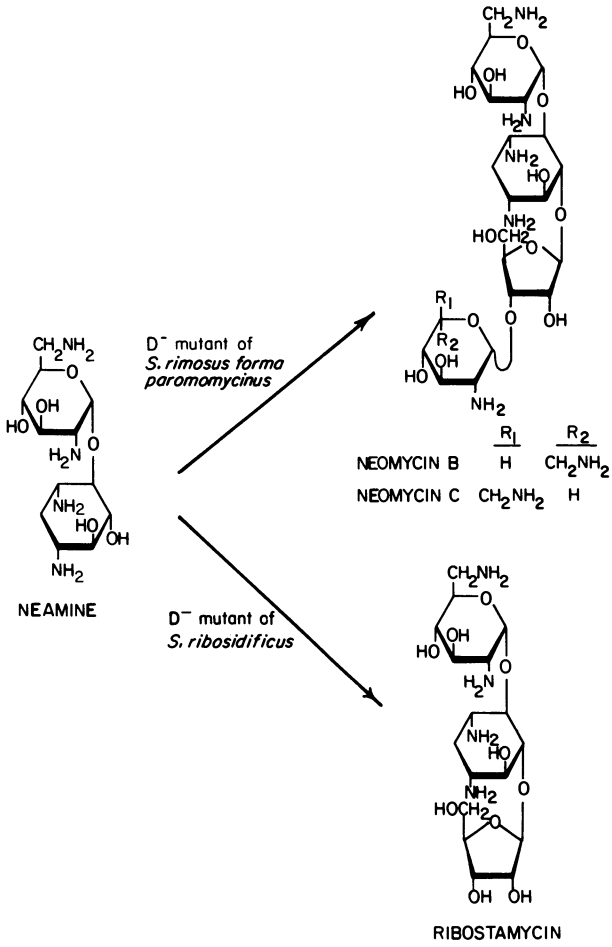


Figure 10. Bioconversion of neamine to neomycins and ribostamycin by *D*⁻ mutants

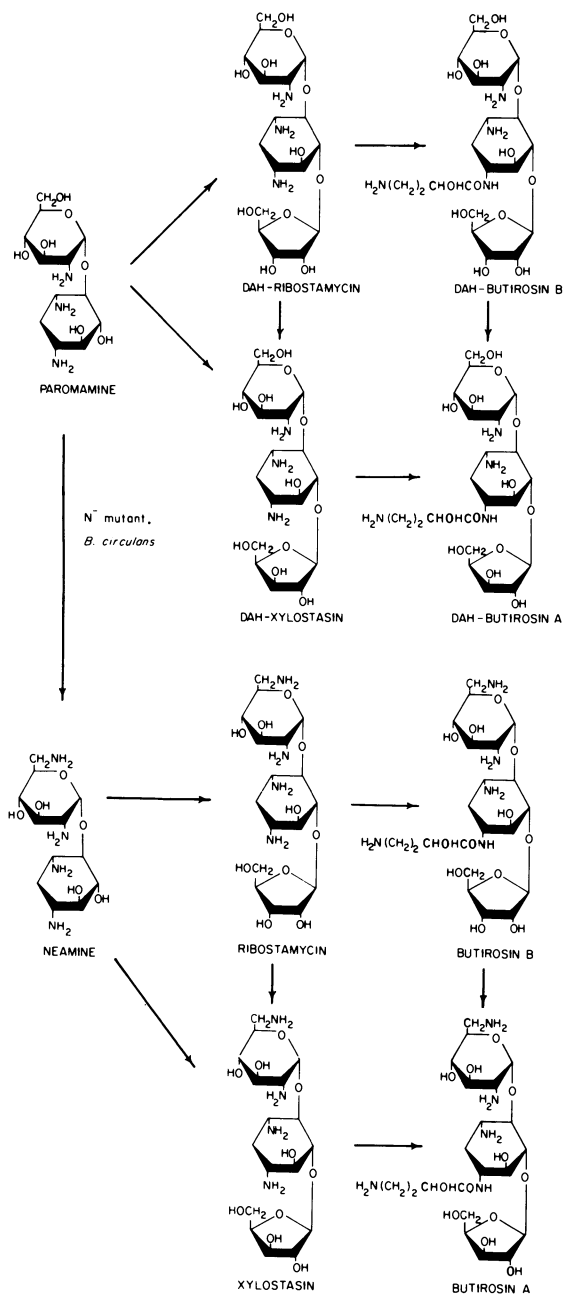


Figure 11. Conversion of paromamine to ribostamycin, xylostasin, and butirosins and their 6'-deamino-6'-hydroxy analogs

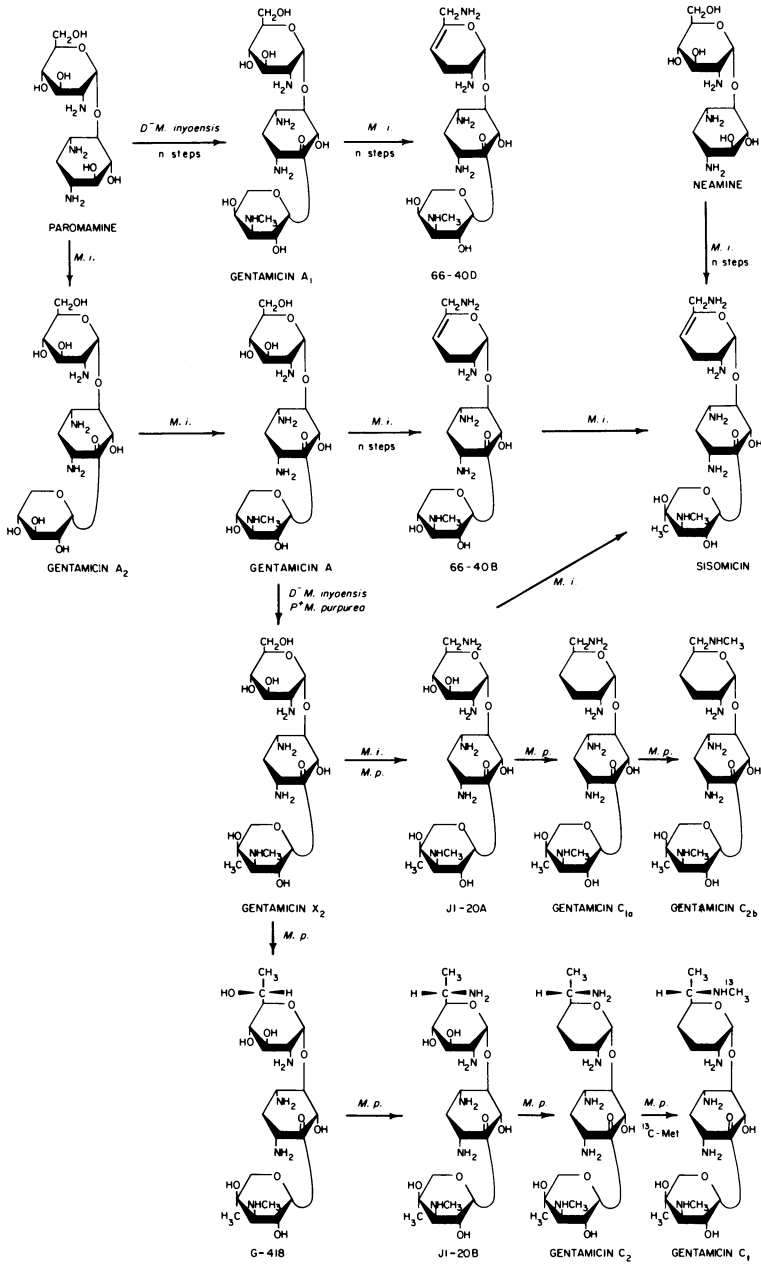


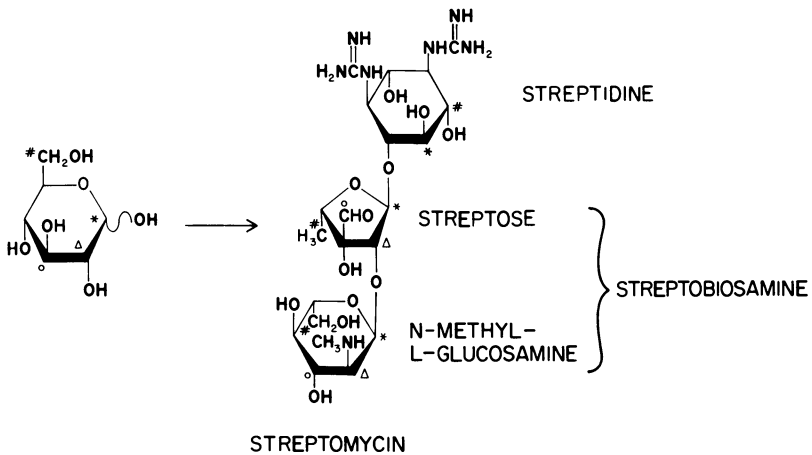
Figure 12. Conversion of paromamine to gentamicins and sisomicin

the first pseudo-oligosaccharide precursor on the biosynthetic pathway to the gentamicins (19) and sisomicin (20). This pathway is very complex and involves several branch points, but the argument runs that paromamine is first converted to two pseudo-trisaccharides (gentamicins A₁ and A₂) and that these then serve as intermediates in a series of conversions--aminations, deoxygenations, dehydrogenations, and N- and C-methylations, until the gentamicins, sisomicin, and related antibiotics are ultimately produced.

The argument for biological modification of monosaccharides while they are attached to deoxystreptamine runs counter to the usually accepted view that monosaccharides are modified while nucleotide-bound, but other secondary metabolites (steroids, alkaloids, etc.) are, of course, modified by organisms without the benefit of nucleotide binding. Indeed, deoxystreptamine may serve as a surrogate nucleotide in steering the sugars to the enzymes for modification. This proposition needs additional study.

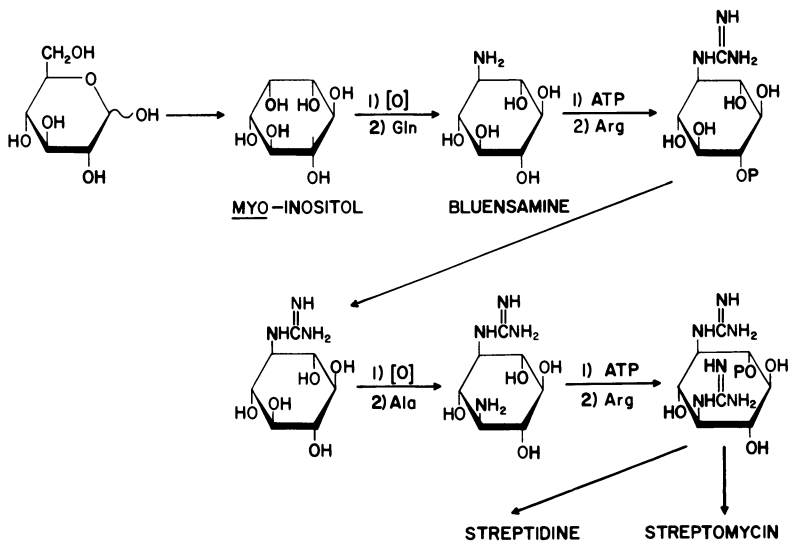
Streptomycin. The biosynthesis of streptomycin, like that of neomycin, has been quite extensively studied. Like neomycin, streptomycin is formed from glucose as a primary precursor (21). Also, like neomycin, specifically labeled glucose is converted into streptomycin in which each of the subunits is labeled at the corresponding carbon; this was established some years ago by several groups (Figure 13), notably those of Horner (22,23), of Rieder (24), of Baddiley (25), and of Weiner (26), with some points being confirmed by ¹³C-labeling in our own laboratory (27). However, there are differences between the biosyntheses of neomycin and streptomycin. First, although deoxystreptamine is incorporated into neomycin, streptomycin, the corresponding aminocyclitol of streptomycin, is not incorporated into streptomycin (25). Second, scyllo- and myo-inositols are incorporated into streptomycin (25) but not into neomycin. Finally, the labeling pattern from glucose for streptidine in streptomycin is different from the labeling pattern for deoxystreptamine in neomycin; although C-1 and C-6 of glucose label contiguous carbons in each aminocyclitol, the two carbons labeled are different for streptidine and deoxystreptamine (27).

As noted before, failure of scyllo-inositol to label deoxystreptamine reflects the fact that it does not appear on the biosynthetic pathway to deoxystreptamine; similarly, failure of streptomycin to be incorporated into streptomycin is a result of the failure of that compound to appear on the biosynthetic pathway to streptomycin. The comprehensive studies of Walker indicate why streptomycin is not incorporated (28). As shown in Figure 14, myo-inositol is converted to myo-inosose, which is transaminated to give aminodeoxy-scyllo-inositol (bluensamine), which is sequentially phosphorylated, carbamidinylated, dephosphorylated, oxidized, transaminated, phosphorylated and



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Figure 13. Carbons of streptomycin labeled by specifically labeled D-glucose (8)



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Figure 14. Biosynthetic pathway from D-glucose to streptidine (8)

carbamidinylated to give, ultimately, streptidine phosphate, which is incorporated into streptomycin. Thus, added streptidine can be converted to streptomycin via streptidine phosphate, but streptomine is found nowhere on the biosynthetic pathway and its failure to be incorporated into streptomycin is a direct result.

In keeping with the appearance of streptidine on the biosynthetic pathway, Demain was able to prepare a streptidine-negative (S^-) mutant of Streptomyces griseus which produced streptomycin only in the presence of added streptidine (6).

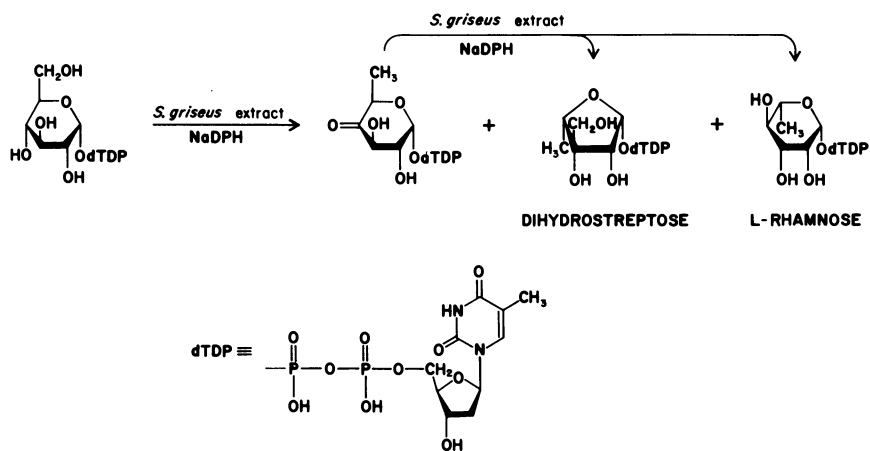
Some of the intermediates between glucose and dihydrostreptose also are now known as a result of the work of Grisebach (29), who demonstrated that [^{14}C]glucose-dTDP was converted to 4-keto-4,6-dideoxyglucose-dTDP, which in turn was converted to dihydrostreptose-dTDP (Figure 15). Partial purification of the enzymes involved in these bioconversions has been carried out (30), and the stereochemistry of the glucose-dTDP \rightarrow 4-keto-4,6-dideoxyglucose-dTDP conversion has been investigated recently by Floss (Figure 16) (30), who showed the C-4 to C-6 hydride transfer occurs with inversion at C-6.

Nothing is known yet regarding intermediates between glucose and N-methyl-L-glucosamine, in which every asymmetric center of glucose has been inverted. However, recent studies with streptidine 6-phosphate and dihydrostreptose-dTDP have provided evidence that it is the glycosidic bond between streptose and streptidine which is formed first in streptomycin (29).

Spectinomycin. The third type of aminocyclitol antibiotic whose biosynthesis has been investigated, by Mitscher, et al. (31), our group (32), and Floss, et al. (33) is spectinomycin. This pseudosaccharide was also shown to be derived from glucose, which specifically labeled individual carbons of both the actinamine and actinospectose moieties (Figure 17). Intermediates identified thus far include actinamine, which was incorporated by an actinamine-dependent (A^-) mutant into spectinomycin (Figure 18) (34). The same A^- mutant also converted 2-epistreptamine (N,N'-didemethylactinamine) to spectinomycin; thus, 2-epistreptamine and actinamine both are intermediates in the biosynthesis of spectinomycin.

Mutasynthesis

Studies of the biosynthesis of the aminocyclitol antibiotics demonstrated that deoxystreptomine is incorporated into neomycin and this, in turn, led to the development of mutants which were unable to produce neomycin except in the presence of added deoxystreptomine. Following this observation, it was a logical next step to inquire whether it would be possible to synthesize new antibiotics by employing aminocyclitols related to deoxystreptomine. This technique indeed was reported in 1969 by Shier, Rinehart, and Gottlieb (5), who prepared 2-hydroxyneomycin



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Figure 15. Conversion of dTDP-D-glucose by *S. griseus* extracts to dTDP-dihydrostreptose and related compounds (8)

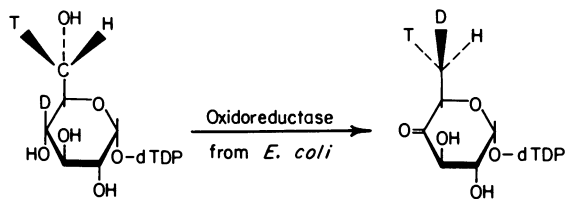
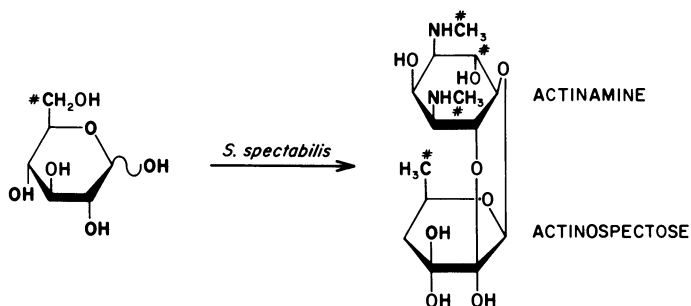
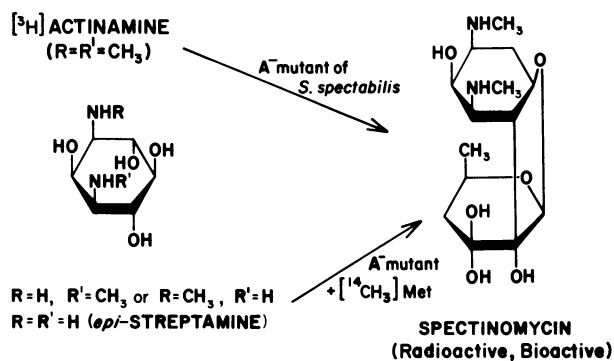


Figure 16. Stereochemistry of the conversion of D-glucose-dTDP to 4-keto-4,6-dideoxy-D-glucose-dTDP



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Figure 17. Carbon atoms of spectinomycin labeled by D -[6- ^{13}C]glucose. The N-methyl carbons are labeled via a multistep conversion of C-6 of glucose to the methyl carbon of methionine (8)



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Figure 18. Labeling of spectinomycin by radioactive actinamine and methionine, using an actinamine-requiring mutant of *Streptomyces spectabilis* (8)

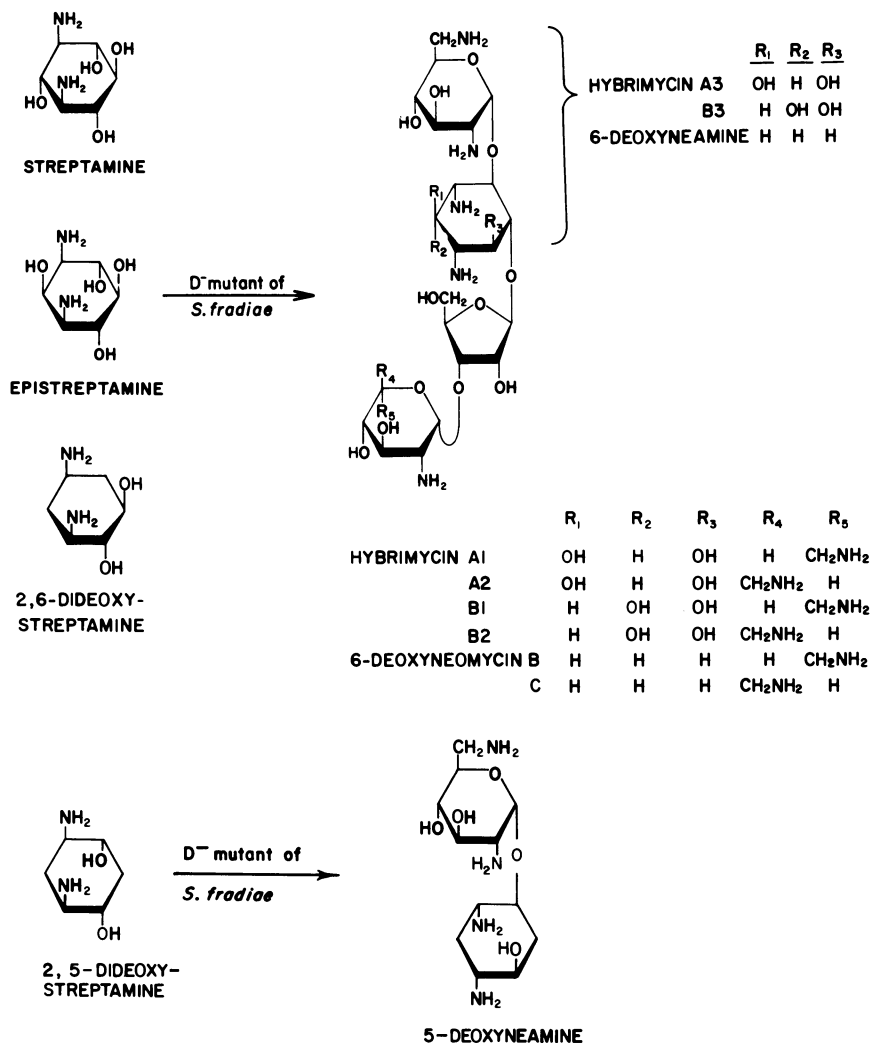
(hybrimycin A) and 2-epihydroxyneomycin (hybrimycin B) by adding streptomine and 2-epistreptomine, respectively, to the D⁻ mutant of *Streptomyces fradiae* (Figure 19). When the 5-hydroxyl group required for ribose substitution was lacking, as in 2,5-dideoxystreptomine, the corresponding neamine analog was the final product (13-15).

Since that time, analogs of nearly every clinically important aminocyclitol antibiotic have been prepared utilizing this technique (35). For example, as seen in Figure 20, 2-hydroxyparomomycin (hybrimycin C) was similarly prepared (16). More recently, 6-deoxyneomycin and 6-deoxyparomomycin have been prepared in other laboratories (36).

It must be emphasized at the outset that, during the mutation process, additional genetic modifications of the producing microorganisms may take place and it is necessary to characterize carefully the new antibiotics produced. The preferred procedures for doing this involve characterization by means of field desorption mass spectrometry and ¹³C NMR spectroscopy, as well as rotation and chromatographic behavior compared to the native antibiotics (35). The surrogate aminocyclitol added to the aminocyclitol-deficient mutant has been referred to as a mutasynton, the analog antibiotics as mutasyntetic antibiotics, and the procedure for their preparation as mutasyntesis (35).

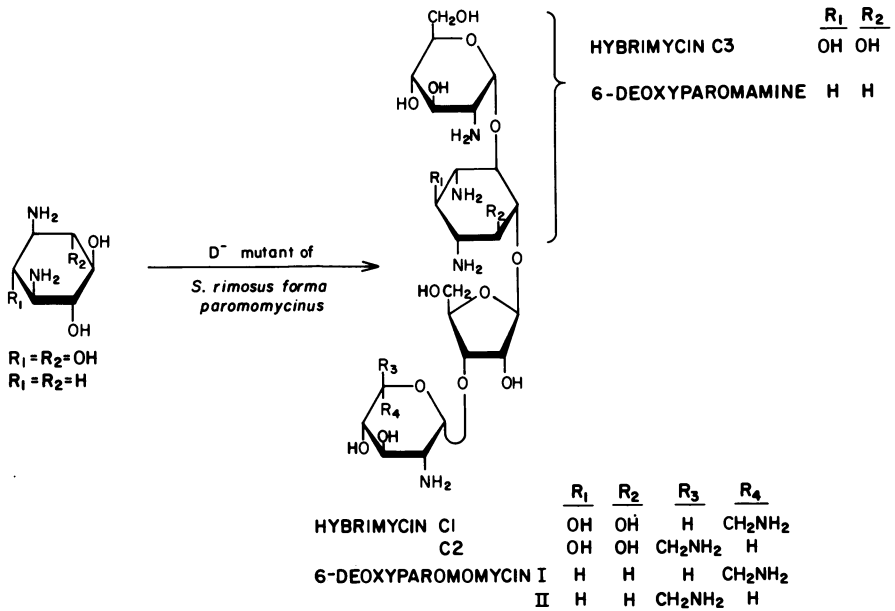
Another example of the use of mutasyntesis to prepare new antibiotics involved the preparation of analogs of ribostamycin from the D⁻ mutant of *S. ribosidificus* (17). Again, hydroxyl substitution at C-2 was allowed and, in this case, N-methylation at N-1. In addition, the technique was extended to produce new antibiotics using compounds related to neamine, such as 3',4'-dideoxynamine, as shown in Figure 21. Considerably more extensive modifications have been carried out employing D⁻ mutants of *Bacillus circulans* to produce the analogs of butirosin or butirosamine shown in Figure 22 (37, 38, 39, 40). The main feature of the recent work is the extensive modification in the amino sugar portion at C-4, which allowed preparation of antibiotics analogous to gentamicin, with N-methyl and C-methyl substitution at C-6 of the diaminoheptose unit.

Mutasyntesis has also been employed for preparation of analogs of kanamycin, as shown in Figure 23 (17). This preparation illustrates the necessity for carrying out careful structural investigation on the product, in that two modifications were observed relative to kanamycin. Not only was the deoxystreptomine unit replaced by a mutasynton but the 2'-amino-, 6'-amino- and 2',6'-diaminoglucose unit was replaced by glucose itself, resulting in a considerably less active antibiotic than kanamycin. In this case the structures were assigned to the mutasyntetic antibiotics by hydrolysis to the components, which included glucose instead of an aminoglucose.



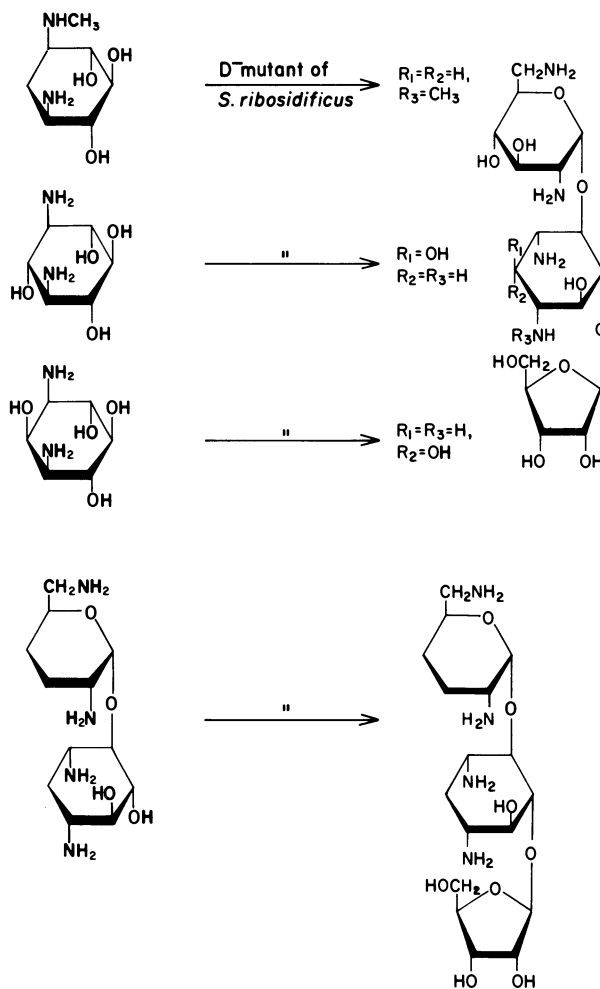
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Figure 19. Preparation of mutasynthetic antibiotics related to neomycin and neamine (35)



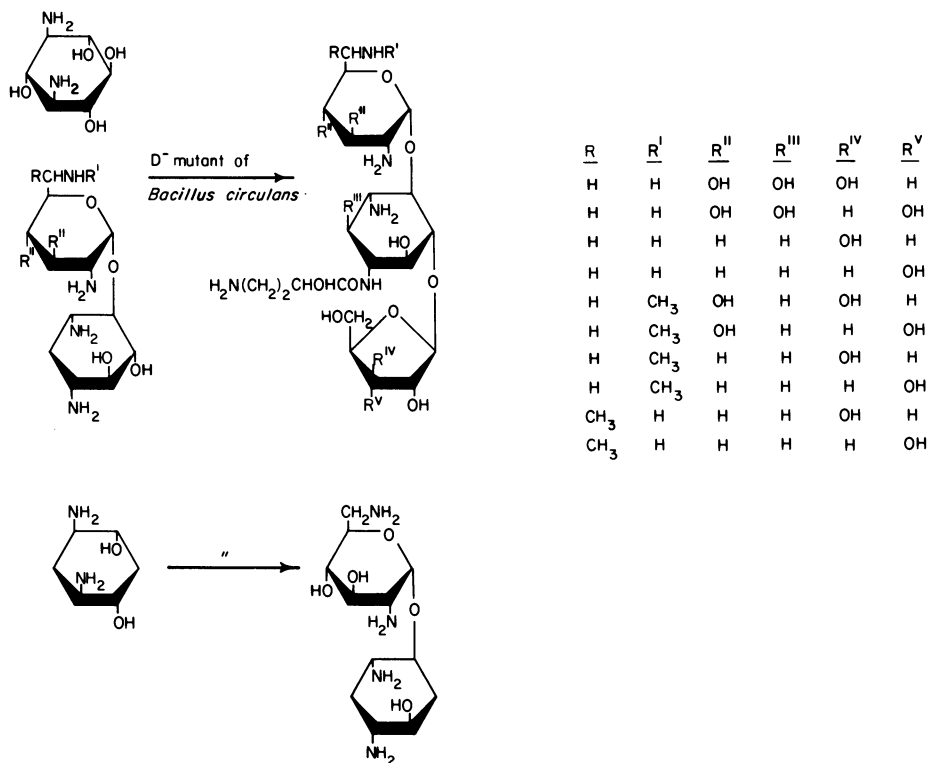
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Figure 20. Preparation of mutasynthetic antibiotics related to paromomycin (35)



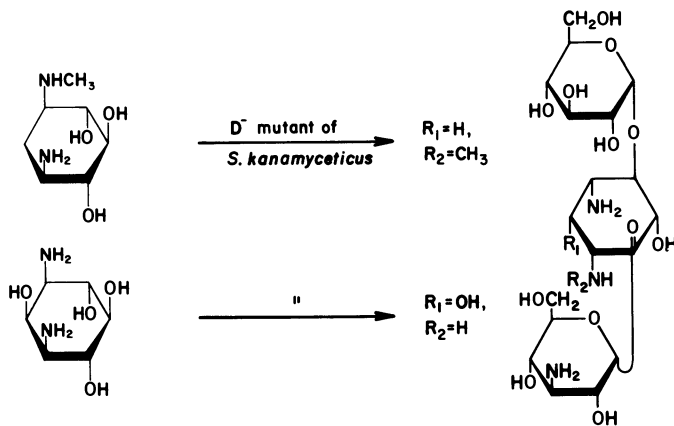
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Figure 21. Preparation of mutasynthetic antibiotics related to ribostamycin (8)



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Figure 22. Preparation of mutasynthetic antibiotics related to butirosin (35)



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Figure 23. Preparation of mutasynthetic antibiotics related to kanamycin. A glucose unit replaces the glucosamine-neosamine unit of kanamycin A. (35)

Another interesting variation on the mutasynthetic technique was provided by the work of Daum and Rosi (Figure 24), who administered as mutasynthons not only aminocyclitols such as streptomine and 2,5-dideoxystreptomine, but also precursors of streptomine such as *myo*-inosose, which yielded hydroxygentamicin (7). Indeed, a number of compounds related to the mutasynthon streptomine apparently gave the same mutasynthetic antibiotic.

Perhaps the most extensive work on mutasynthetic antibiotics is that by Daniels, *et al.*, summarized in Figure 25, which will be discussed elsewhere in this volume (41). Here again, the necessity for adequately characterizing the antibiotics produced is illustrated by the formation in one case of a gentamicin A analog (Mu 2a) rather than the expected sisomicin analog and the lack of a 3"-N-methyl group or its replacement by acetyl in Mu 1a and 1b.

Beyond the deoxystreptomine antibiotics, mutasynthesis has been employed but has proved less successful than with the deoxystreptomine compounds. The streptidine-negative mutant of *Streptomyces griseus* prepared by Nagaoka and Demain (6) has been reported to incorporate deoxystreptidine into a new antibiotic, but the antibiotic has not yet been identified.

A mutasynthetic compound related to spectinomycin has also been prepared (Figure 26). However, in this case the product proved to be inactive as an antibiotic and was only identified by its radioactivity when prepared in the presence of [methyl-¹⁴C]methionine (34). The presumption that the product was indeed 2-epispectinomycin was further substantiated by subsequent synthetic work carried out at Abbott Laboratories in which synthetic 2-epispectinomycin was prepared and, indeed, proved to be bioinactive (42).

As can be seen in Table I and as noted earlier in the present paper, mutasynthetic analogs of nearly all of the clinically important aminocyclitol antibiotics have been prepared. These mutasynthetic compounds have been evaluated both for their antimicrobial activity and their toxicity. Most of the mutasynthetic analogs have proved to be less active than their parent antibiotics (Table II), but some are as active or more active than their parents. The latter group (about as active or more active) includes 2-hydroxy- and 6-deoxyneomycins, 2-hydroxy- and 5-deoxygentamicins, 5-episisomicin and several ribostamycin and butirosin analogs.

However, the more important parameter for evaluation is the toxicity of the compounds, since aminocyclitols are toxic antibiotics. Although data are available for only a few of the analogs, 2-hydroxyneomycin and 2-hydroxygentamicin have proved to be considerably less toxic than their parents (Table III) (41, 43, 44). Indeed, 2-hydroxygentamicin and 5-episisomicin are regarded as candidates for clinical evaluation.

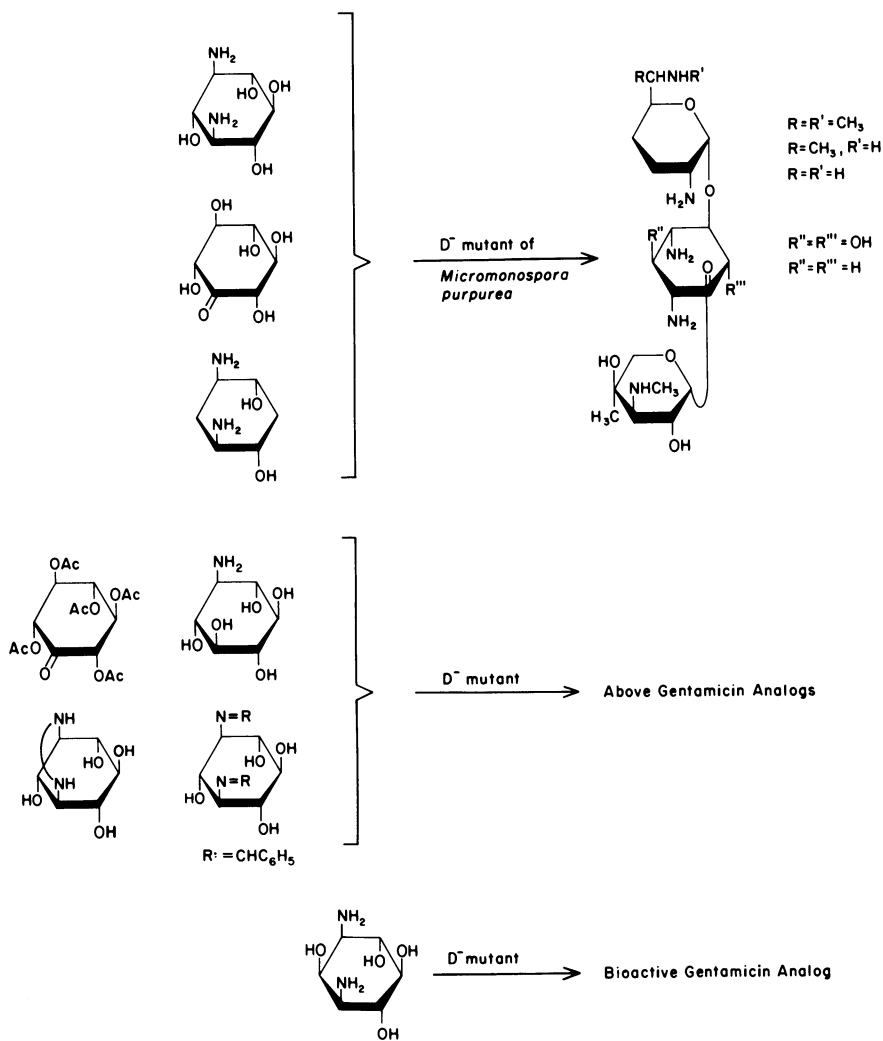


Figure 24. Preparation of mutasynthetic antibiotics related to gentamicin

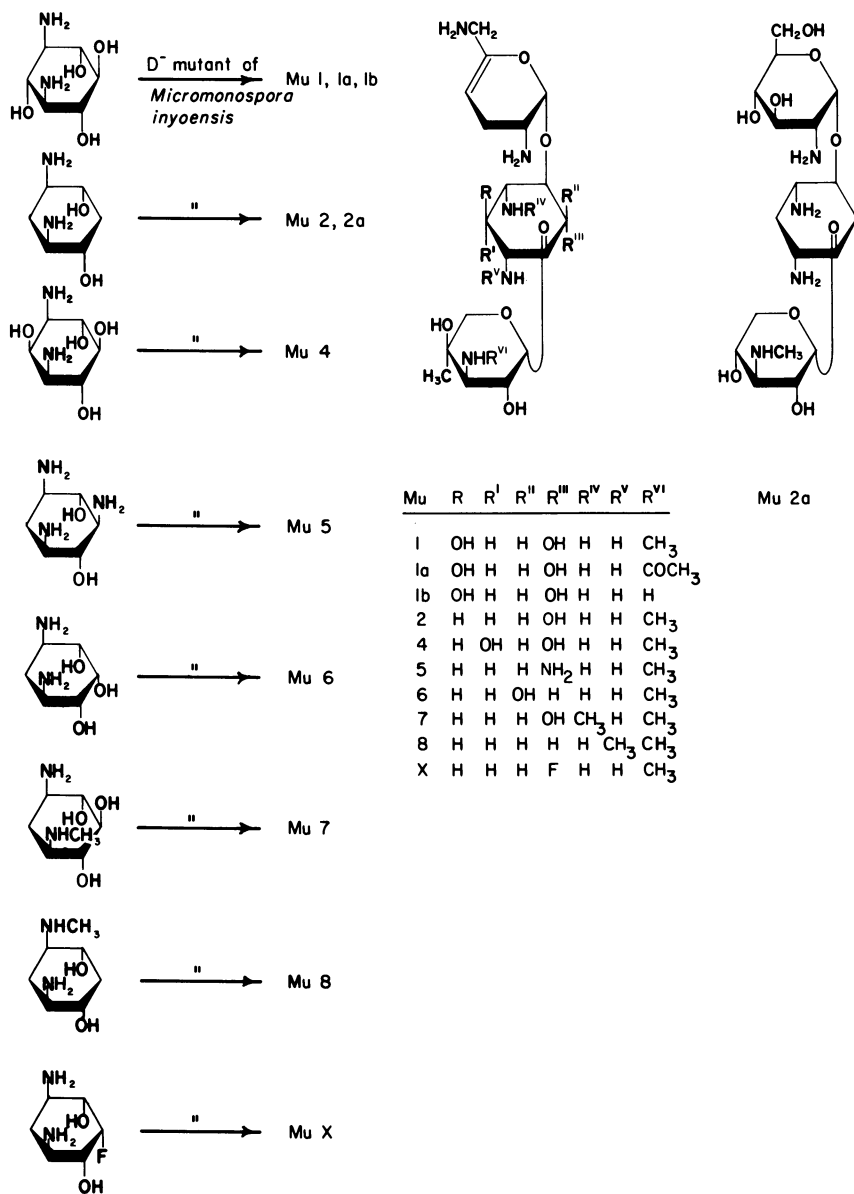
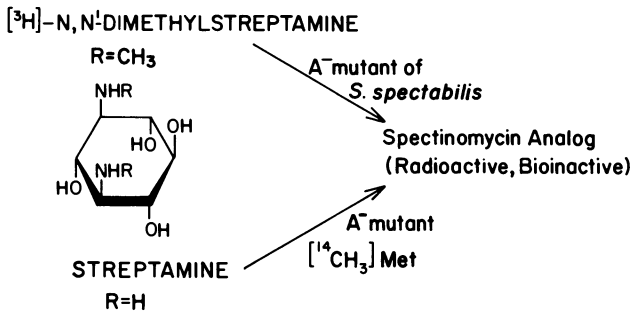


Figure 25. Preparation of mutasynthetic antibiotics related to sisomicin and gentamicin A (35)



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Figure 26. Formation of a bioinactive analog of spectinomycin using the mutasynthons dimethylstreptamine (2-epiactinamine) and streptamine and an A⁻ mutant of S. spectabilis (35)

Table I. Summary of Mutant Organisms, Mutasynthons and Mutasynthetic Antibiotics (35)

Mutant (Ref.)	Normal Antibiotic	Mutasynthon	Mutasynthetic Antibiotic
<i>S. fradiae</i> (D ⁻) (5, 36, 45)	Neomycin	Streptamine 2-Epistreptamine 2,6-Dideoxystreptamine 2,5-Dideoxystreptamine 6-O-Methyldeoxystreptamine 3-N-Methyldeoxystreptamine 2-Bromo-2-deoxystreptamine 6-Bromo-6-deoxystreptamine	2-Hydroxyneomycins B, C (hybrimycins A1, A2) 2-Epihydroxyneomycin (hybrimycins B1, B2) 6-Deoxyneomycins B, C Not isolated Not isolated Not isolated Not isolated
<i>S. rimosus</i> (D ⁻) (16, 36)	Paromomycin	Streptamine 2,6-Dideoxystreptamine	2-Hydroxyparomomycins I, II (hybrimycins C1, C2) 6-Deoxyparomomycins I, II
<i>S. ribosidificus</i> (D ⁻) (17)	Ribostamycin	Streptamine Epistreptamine 1-N-Methyldeoxystreptamine Gentamine C _{1a}	2-Hydroxyribostamycin 2-Epihydroxyribostamycin 1-N-Methylribostamycin 3',4'-Dideoxyribostamycin
<i>B. circulans</i> (D ⁻ , N ⁻) (37-40)	Butirosin	Streptamine 2,5-Dideoxystreptamine Gentamine C _{1a} 6'-N-Methylneamine 6'-N-Methylgentamine C _{1a} Gentamine C ₂	2-Hydroxybutirosins A, B 5-Deoxybutirosamine 3',4'-Dideoxybutirosins A, B 6'-N-Methylbutirosins A, B 3',4'-Dideoxy-6'-N-methylbutirosins A, B 3',4'-Dideoxy-6'-C-methylbutirosins A, B
<i>S. kanamyceticus</i> (D ⁻) (17)	Kanamycin	2-Epistreptamine 1-N-Methylstreptamine	6'-Hydroxy-6'-deamino-2-epihydroxykanamycin A 6'-Hydroxy-6'-deamino-1-N-methylkanamycin A
<i>M. purpurea</i> (D ⁻) (46-48)	Gentamicin	Streptamine Scyllo-inosose Scyllo-inosose pentaacetate 2,5-Dideoxystreptamine 4,6-Hydrazino- 1,3-cyclohexanediol 1,3-Di-N-benzylidene- 2,5-dideoxystreptamine Epistreptamine	2-Hydroxygentamicins C ₁ , C ₂ , C _{2a} 2-Hydroxygentamicin 2-Hydroxygentamicin 5-Deoxygentamicins C ₁ , C ₂ , C _{2a} Not isolated Not isolated Not isolated
<i>M. inyoensis</i> (D ⁻) (49-51)	Sisomicin	Streptamine 2,5-Dideoxystreptamine 2-Epistreptamine 5-Amino-2,5-dideoxy- streptamine 5-Epi-2-deoxystreptamine 3-N-Methyl-2-deoxy- streptamine 1-N-Methyl-2,5-dideoxy- streptamine 5-Epifluoro-2-deoxy- streptamine	2-Hydroxysisomicin (Mu 1) 3"-N-Demethyl-3"-N-acetyl-2-hydroxysisomicin (Mu 1a) 3"-N-Demethyl-2-hydroxysisomicin (Mu 1b) 5-Deoxysisomicin (Mu 2) 5-Deoxygentamicin A (Mu 2a) 2-Epihydroxysisomicin (Mu 4) 5-Amino-5-deoxysisomicin (Mu 5) 5-Episisomicin (Mu 6) 3-N-Methylsisomicin (Mu 7) 1-N-Methyl-5-deoxysisomicin (Mu 8) 5-Epifluorosomicin (Mu X)
<i>S. griseus</i> (S ⁻) (6)	Streptomycin	2-Deoxystreptidine	Not isolated (streptomutin A)
<i>S. spectabilis</i> (A ⁻) (34)	Spectinomycin	Streptamine N,N'-Dimethylstreptamine	Not isolated (bioinactive) Not isolated (bioinactive)

Table II. Relative Antibacterial Activities of Mutasynthetic Antibiotics and the Corresponding Antibiotics of the Wild Strains (35)

Mutasynthetic Antibiotic		Normal Antibiotic	Ratio [‡]	Remarks (Ref.)
2-Hydroxyneomycin	<	Neomycin	0.17-1.3	(5)
2-Hydroxyneamine	<	Neamine	0.06-0.25	(52)
2-Epihydroxyneomycin	<	Neomycin	0.04-0.67	(5)
2-Epihydroxyneamine	<	Neamine	0.02-0.25	(52)
6-Deoxyneomycin B	>	Neomycin B	-----	"Very similar" except vs. <i>E. coli</i> , <i>P. mirabilis</i> , <i>S. aureus</i> , and <i>S. typhimurium</i> (36)
6-Deoxyneomycin C	>	Neomycin C	-----	
2-Hydroxyparomomycin I	<	Paromomycin I	0.33-0.67	(16)
2-Hydroxyparomomycin II	<	Paromomycin II	0.10-0.67	(16)
2-Hydroxyparomamine	>	Paromamine	0.02-1.0	(16)
6-Deoxyparomomycin I	<	Paromomycin I	0.25	(36)
6-Deoxyparomomycin II	<	Paromomycin II	0.25	(36)
2-Hydroxyribostamycin	<	Ribostamycin	0.10	(17)
2-Epihydroxyribostamycin	<	Ribostamycin	<0.10	(17)
1-N-Methylribostamycin	<	Ribostamycin	0.25	(17)
3',4'-Dideoxyribostamycin	>	Ribostamycin	0.25-2.0	Active against kanamycin- and ribostamycin-resistant <i>P. aeruginosa</i> and <i>E. coli</i> (17)
2-Hydroxybutirosin	>	Butirosin	0.1-1.0	(37)
5-Deoxybutirosamine	>	Butirosamine	0.5-8.0	(37)
3',4'-Dideoxybutirosin A	=	Butirosin A	0.5-2.0	Active against butirosin-resistant <i>K. pneumoniae</i> , <i>E. coli</i> , <i>P. mirabilis</i> , and <i>P. aeruginosa</i> (53, 54)
3',4'-Dideoxybutirosin B	=	Butirosin B	0.25-2.0	
6'-N-Methylbutirosin A	=	Butirosin A	0.13-4.0	Active against some butirosin-kanamycin-dibekacin-gentamicin acetylating, phosphorylating or adenyating <i>E. coli</i> and <i>S. sonnei</i> strains (40)
6'-N-Methylbutirosin B*	=	Butirosin B	0.13-4.0	
3',4'-Dideoxy-6'-N-methylbutirosin A	=	Butirosin A	0.5-2.0	Active against some butirosin-kanamycin-dibekacin-gentamicin acetylating, phosphorylating or adenyating <i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. sonnei</i> , <i>P. rettgeri</i> , <i>P. stuartii</i> , and <i>P. aeruginosa</i> strains (39)
3',4'-Dideoxy-6'-N-methylbutirosin B*	≤	Butirosin B	0.5-2.0	
3',4'-Dideoxy-6'-C-methylbutirosin B*	=	Butirosin B	0.5-2.0	Active against some butirosin-kanamycin-dibekacin-gentamicin acetylating, phosphorylating, or adenyating <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i> strains (40)
6'-Hydroxy-6'-deamino-2-epihydroxykanamycin A	<<	Kanamycin A	-----	Weak bioactivity (17)
6'-Hydroxy-6'-deamino-1-N-methylkanamycin A	<<	Kanamycin A	-----	Weak bioactivity (17)
2-Hydroxygentamicin	≤	Gentamicin	0.25-2.0	Active against gentamicin-adenylating <i>E. coli</i> , <i>E. cloacae</i> and <i>K. pneumoniae</i> (47)
5-Deoxygentamicin	≥	Gentamicin	1.0-8.0	Active against gentamicin-acetylating <i>E. coli</i> and <i>P. aeruginosa</i> (47)
2-Hydroxysisomicin	≤	Sisomicin	0.1-1.0	Active against gentamicin-sisomicin-tobramycin adenyating <i>K. pneumoniae</i> and <i>E. coli</i> (49)
5-Deoxysisomicin	≤	Sisomicin	0.1-1.0	Active against gentamicin-sisomicin acetylating <i>P. aeruginosa</i> (49)
5-Amino-5-deoxysisomicin	=	Sisomicin	0.7-3.0	Same spectrum as sisomicin (50)
5-Episisomicin [†]	>	Sisomicin	0.5-8.0	Active against some gentamicin-tobramycin-amikacin acetylating, phosphorylating or adenyating <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. morganii</i> , <i>P. rettgeri</i> , <i>Providencia</i> , <i>P. aeruginosa</i> , <i>Serratia</i> , and <i>S. aureus</i> strains (51)
3-N-Methylsisomicin	<	Sisomicin	0.16-0.38	
1-N-Methyl-5-deoxysisomicin	=	Sisomicin	0.42-2.1	Active against sisomicin-gentamicin-tobramycin acetylating and adenyating strains (50)
5-Epifluorosomicin	>	Sisomicin		

*Compared to butirosin A. †Compared to gentamicin. ‡Always against sensitive strains. Special activities against some resistant strains.

Table III. Comparative Toxicities of Mutasynthetic Antibiotics and Their Normal Counterparts

Antibiotics	Toxicity ^a				
	Acute (ID ₅₀)		Nephro-	Vestibular	Oto-
	IV ^b	SC ^c			
Neomycin	1.00	1.00	1.00		
2-Hydroxy-	.90	.73	.46		
2-Epihydroxy-	.93	.86	1.25		
Gentamicin C	1.00		1.00	1.00	1.00
2-Hydroxy-	.58		.16	.25	.16
5-Deoxy-	2.51		2.00		
Butirosin A	1.00				
3',4'-Dideoxy-					
6'-N-methyl-	.83				

^aToxicity of normal antibiotic assigned as 1.00. ^bIntra-venous. ^cSubcutaneous.

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Literature Cited

1. Rinehart, K. L., Jr.; Schimbor, R. F.: "Neomycins." In Gottlieb, D., and Shaw, P. D., Ed., "Antibiotics. II. Biosynthesis." Springer-Verlag, New York, 1967; pp. 359-372.
2. Rinehart, K. L., Jr.; Malik, J. M.; Nystrom, R. S.; Stroshane, R. M.; Truitt, S. T.: "Biosynthetic Incorporation of [1-¹³C]Glucosamine and [6-¹³C]Glucose into Neomycin." *J. Am. Chem. Soc.*, 1974, 96, 2263-2265.

3. Rinehart, K. L., Jr.; Stroshane, R. M.; Shier, W. T.; Truitt, S. T.; Taniguchi, M.; Malik, J. M.; Schaefer, P. C.; Rolls, J. P.; Haak, W. J.: "Biosynthesis of Aminocyclitol Antibiotics and Application to Synthesis of New Antibiotics." Abstracts, 168th National Meeting of the American Chemical Society, Atlantic City, NJ, Sept. 1974; MICR 19.
4. Falkner, F. C.: "Studies on the Biosynthesis of Neomycin." Ph.D. Thesis, University of Illinois, Urbana, IL, 1969.
5. Shier, W. T.; Rinehart, K. L., Jr.; Gottlieb, D.: "Preparation of Four New Antibiotics from a Mutant of Streptomyces fradiae." Proc. Nat. Acad. Sci. U.S., 1969, 63, 198-204.
6. Nagaoka, K.; Demain, A. L.: "Mutational Biosynthesis of a New Antibiotic Streptomitin A, by an Idiotroph of Streptomyces griseus." J. Antibiot., 1975, 28, 627-635.
7. Daum, S. J.; Rosi, D.; Goss, W. A.: "Mutational Biosynthesis by Idiotrophs of Micromonospora purpurea. II. Conversion of Non-amino Containing Cyclitols to Aminoglycoside Antibiotics." J. Antibiot., 1977, 30, 98-105.
8. Rinehart, K. L., Jr.; Stroshane, R. M.: "Biosynthesis of Aminocyclitol Antibiotics." J. Antibiot., 1976, 29, 319-353.
9. Chen, Y.; Walker, J. B.: "Transaminations Involving Keto- and Amino-Inositols and Glutamine in Actinomycetes which Produce Gentamicin and Neomycin." Biochem. Biophys. Res. Commun., 1977, 77, 688-692.
10. Byrne, K. M.; Rinehart, K. L., Jr.: "Conversion of dTDP-Glucose to 4-Keto-4,6-dideoxyglucose and 6-Deoxyglucose by Cell-free Extracts of Streptomyces fradiae." J. Biol. Chem., submitted.
11. Tadano, K.-I.; Pearce, C. J.; Rinehart, K. L., Jr.: unpublished results.
12. Perlman, D.; O'Brien, E.: "Studies on Utilization of Lipids and Polysaccharides by Streptomyces fradiae." Bact. Proc., 1953, A25.
13. Pearce, C. J.: "Studies on the Biosynthesis of Neomycin." Ph.D. Thesis, University of Southampton, England, 1976.
14. Rubenstein, H. M.: "Biosynthetic and Mutasynthetic Studies of Neomycin." Ph.D. Thesis, University of Illinois, Urbana, IL, 1978.

15. Sepulchre, A. M.; Quiclet, B.; Colas, C.; Rolland, A.; Cleophax, J.; Pearce, C. J.; Gero, S. D.; "Cyclitols and Aminocyclitols: Potential Intermediates for Synthesis and Mutasynthesis of Aminoglycoside Antibiotics." Abstracts, 18th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, GA, Oct. 1-4, 1978; 172.
16. Shier, W. T.; Schaefer, P. C.; Gottlieb, D.; Rinehart, K. L., Jr.: "Use of Mutants in the Study of Aminocyclitol Antibiotic Biosynthesis and the Preparation of the Hybrimycin C Complex." Biochemistry, 1974, 13, 5073-5078.
17. Kojima, M.; Satoh, A.: "Microbial Semi-synthesis of Aminoglycosidic Antibiotics by Mutants of S. ribosidificus and S. kanamyceticus." J. Antibiot., 1973, 26, 784-786.
18. Takeda, K.; Aihara, K.; Furumai, T.; Ito, Y.: "Biosynthesis of Butirosins. I. Biosynthetic Pathways of Butirosins and Related Antibiotics." J. Antibiot., 1979, 32, 18-28.
19. Testa, R. T.; Tilley, B. C.: "Biotransformation, a New Approach to Aminoglycoside Biosynthesis: II. Gentamicin." J. Antibiot., 1976, 29, 140-146.
20. Testa, R. T.; Tilley, B. C.: "Biotransformation: A New Approach to Aminoglycoside Biosynthesis. I. Sisomicin." J. Antibiot., 1975, 28, 573-579.
21. Horner, W. H.: "Streptomycin." In Gottlieb, D.; Shaw, P. D., Ed., "Antibiotics. II. Biosynthesis." Springer-Verlag, New York, 1967; pp. 373-399, 447-448.
22. Horner, W. H.; Russ, G. A.: "Biosynthesis of Streptomycin. VII. Stereospecificity of the Enzymatic Dehydrogenation of 1-Guanidino-1-deoxy-scyllo-inositol." Biochim. Biophys. Acta, 1971, 237, 123-127.
23. Bruton, J.; Horner, W. H.; Russ, G. A.: "Biosynthesis of Streptomycin. IV. Further Studies on the Biosynthesis of Streptidine and N-Methyl-L-glucosamine." J. Biol. Chem., 1967, 242, 813-818.
24. Silverman, M.; Rieder, S. V.: "The Formation of N-Methyl-L-glucosamine from D-Glucose by Streptomyces griseus." J. Biol. Chem., 1960, 235, 1251-1254.
25. Candy, D. J.; Baddiley, J.: "The Biosynthesis of Streptomycin. The Origin of the C-Formyl Group of Streptose." Biochem. J., 1965, 96, 526-529.

26. Bruce, R. M.; Ragheb, H. S.; Weiner, H.: "Biosynthesis of Streptomycin: Origin of Streptidine from D-Glucose." Biochim. Biophys. Acta, 1968, 158, 499-500.
27. Munro, M. H. G.; Taniguchi, M.; Rinehart, K. L., Jr.; Gottlieb, D.; Stoudt, T. H.; Rogers, T. O.: "Carbon-13 Evidence for the Inositol Pathway in Streptomycin Biosynthesis." J. Am. Chem. Soc., 1975, 97, 4782-4783.
28. Walker, J. B.: "Biosynthesis of Aminocyclitols and Guanidinocyclitols." In "Cyclitols and Phosphoinositides." Academic Press, Inc., New York, 1978; pp. 423-438.
29. Grisebach, H.: "Biosynthesis of Sugar Components of Antibiotic Substances." Adv. Carbohydr. Chem. Biochem., 1978, 34, 81-126.
30. Snipes, C. E.; Brillinger, G.-U.; Sellers, L.; Mascaro, L.; Floss, H. G.: "Stereochemistry of the dTDP-glucose Oxidoreductase Reaction." J. Biol. Chem., 1977, 252, 8113-8117.
31. Mitscher, L. A.; Martin, L. L.; Feller, D. R., "The Biosynthesis of Spectinomycin." Chem. Commun., 1971, 1541-1542.
32. Stroshane, R. M.; Taniguchi, M.; Rinehart, K. L., Jr.; Rolls, J. P.; Haak, W. J.; Ruff, B. A.: "Spectinomycin Biosynthesis Studied by Carbon Magnetic Resonance Spectroscopy." J. Am. Chem. Soc., 1976, 98, 3025-3027.
33. Floss, H. G.; Chang, C.-j.; Mascaretti, O.; Shimada, K.: "Studies on the Biosynthesis of Antibiotics." Planta Medica, 1978, 34, 345-380.
34. Slechta, L.; Coats, J. H.: "Studies of the Biosynthesis of Spectinomycin." Abstracts, 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, Sept. 1974; 294.
35. Rinehart, K. L., Jr.: "Mutasythesis of New Antibiotics." Pure and Appl. Chem., 1977, 49, 1361-1384.
36. Cleophax, J.; Gero, S. D.; Leboul, J.; Akhtar, M.; Barnett, J. E. G.; Pearce, C. J.: "A Chiral Synthesis of D-(+)-2,6-Dideoxystreptamine and Its Microbial Incorporation into Novel Antibiotics." J. Am. Chem. Soc., 1976, 98, 7110-7112.
37. Taylor, H. D.; Schmitz, H.: "Antibiotics Derived from a Mutant of Bacillus circulans." J. Antibiot., 1976, 29, 532-535.

38. Takeda, K.; Okuno, S.; Ohashi, Y.; Furumai, T.: "Mutational Biosynthesis of Butirosin Analogs I. Conversion of Neamine Analogs into Butirosin Analogs by Mutants of Bacillus circulans." J. Antibiot., 1978, 31, 1023-1030.

39. Takeda, K.; Kinumaki, A.; Hayasaka, H.; Yamaguchi, T.; Ito, Y.: "Mutational Biosynthesis of Butirosin Analogs II. 3',4'-Dideoxy-6'-N-methylbutirosins, New Semisynthetic Aminoglycosides." J. Antibiot., 1978, 31, 1031-1038.

40. Takeda, K.; Kinumaki, A.; Okuno, S.; Matsushita, T.; Ito, Y.: "Mutational Biosynthesis of Butirosin Analogs III. 6'-N-Methylbutirosins and 3',4'-Dideoxy-6'-C-methylbutirosins, New Semisynthetic Aminoglycosides." J. Antibiot., 1978, 31, 1039-1045.

41. Daniels, P. J. L.; D. F. Rane, S. W. McCombie, R. T. Testa, J. J. Wright, and T. L. Nagabhushan, "Chemical and Biological Modification of Antibiotics of the Gentamicin Group." In Rinehart, K. L., Jr., Suami, T., Ed., "Aminocyclitol Antibiotics," ACS Symposium Series. American Chemical Society, Washington, D.C., 1979, pp.

42. Rosenbrook, W., Jr.; Carney, R. E.; Egan, R. S.; Stanaszek, R. S.; Cirovic, M.; Nishinaga, T.; Mochida, K.; Mori, Y.: "Spectinomycin Modification. II. 7-Epi-Spectinomycin." J. Antibiot., 1975, 28, 960-964.

43. Daum, S. J.: "New Gentamicin-Type Antibiotics Produced by Mutasynthesis." In Schlessinger, D., Ed., "Microbiology-1979." American Society for Microbiology, Washington, D.C., 1979, pp. 312-313.

44. Kikuchi, T.; Asahara, M.; Sekido, S.; Hiratsuka, R.; Iwasaki, M.; Kimura, G.: personal communication to KLR.

45. Goo, Y. M.; Rinehart, K. L., Jr.: unpublished results.

46. Rosi, D.; Goss, W. A.; Daum, S. J.: "Mutational Biosynthesis by Idiobionts of Micromonospora purpurea. I. Conversion of Aminocyclitols to New Aminoglycoside Antibiotics." J. Antibiot., 1977, 30, 88-97.

47. Daum, S. J.; Rosi, D.; Goss, W. A.; "Mutational Biosynthesis by Idiobionts of Micromonospora purpurea. II. Conversion of Non-amino Containing Cyclitols to Aminoglycoside Antibiotics." J. Antibiot., 1977, 30, 98-105.

48. Daum, S. J.; Rosi, D.; Goss, W. A.: "Production of Antibiotics by Biotransformation of 2,4,6/3,5-Pentahydroxycyclo-

hexanone and 2,4/3,5-Tetrahydroxycyclohexanone by a Deoxy-streptamine-negative Mutant of Micromonospora purpurea." J. Am. Chem. Soc., 1977, 99, 283-284.

49. Testa, R. T.; Wagman, G. H.; Daniels, P. J. L.; Weinstein, M. J.: "Mutamicins; Biosynthetically Created New Sisomicin Analogues." J. Antibiot., 1974, 27, 917-921.

50. (a) Daniels, P. J. L.; Rane, D. F.: "Synthetic and Mutasynthetic Antibiotics Related to Sisomicin." In Schlessinger, D., Ed., "Microbiology-1979." American Society for Microbiology, Washington, D.C., 1979, pp. 314-317; (b) Daniels, P. J. L.: "Synthetic and Mutasynthetic Antibiotics Related to Sisomicin." Symposium on Mutasynthesis of Antibiotics, 18th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Atlanta, GA, October 1-4, 1978.

51. Waitz, J. A.; Miller, G. H.; Moss, Jr., E.; Chiu, P. J. S.: "Chemotherapeutic Evaluation of 5-Episisomicin (Sch 22591), a New Semisynthetic Aminoglycoside." Antimicrob. Agents Chemother., 1978, 13, 41-48.

52. Shier, W. T.; Rinehart, Jr., K. L.; Gottlieb, D.: "Preparation of Two New Aminoglycoside Antibiotics." J. Antibiot., 1970, 23, 51-53.

53. Saeki, H.; Shimada, Y.; Ohashi, Y.; Tajima, M.; Sugawara, S.; Ohki, E.: "Synthesis of 3',4'-Dideoxybutirosin A, Active Against Butirosin Resistant Bacteria." Chem. Pharm. Bull. Japan, 1974, 22, 1145-1150.

54. Ikeda, D.; Tsuchiya, T.; Umezawa, S.; Umezawa, H.; Hamada, M.: "Synthesis of 3',4'-Dideoxybutirosin B." J. Antibiot., 1973, 26, 307-309.

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Chemical and Biological Modification of Antibiotics of the Gentamicin Group

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The aminocyclitol antibiotic gentamicin was first reported in 1963 by Weinstein and co-workers (1). Since 1968, clinical use of the antibiotic has increased dramatically and gentamicin is now the most extensively used aminocyclitol antibiotic in the world. Gentamicin is produced by fermentation of species of the genus Micromonospora and is used as a complex of three components, C_1 , C_2 and C_{1a} , which differ from one another only in the extent of methylation of the 2,6-diaminosugar portion of the molecule. Since the discovery of gentamicin, a substantial number of other antibiotics have been isolated from fermentation of Micromonospora species, both in our own laboratories and by others (2). Among many such antibiotics, sisomicin (3) has been of particular interest to us. Sisomicin, structurally an unsaturated derivative of gentamicin C_{1a} (4), is now also in clinical use in a number of countries. In the past few years, a relatively large number of novel aminocyclitols related to gentamicin and sisomicin have been prepared in our laboratories and some of these studies form the subject matter of this paper.

The initial objective of our program was the preparation of compounds which would be unable to serve as substrates for the modifying enzymes which give rise to clinical resistance to aminocyclitol antibiotics (5). Such mechanisms have proliferated in the past few years, concomitant with the increased use of these agents. The presently known modes of bacterial enzymatic inactivation of compounds of the gentamicin-kanamycin group are shown in Figure 1. The mechanisms involve N-acetylation, O-phosphorylation, and O-nucleotidylation of the substrate antibiotics. The enzymes are referred to by the shorthand nomenclature shown in the Figure. Thus, AAC(3) designates aminoglycoside N-acetyl transferase at the 3-position, ANT(2'') represents aminoglycoside O-nucleotidyltransferase at the 2'' position and APH, aminoglycoside O-phosphotransferase. Of the mechanisms depicted in the Figure, the one occurring most frequently involves phosphorylation of a 3'-hydroxyl group. The most important inactivation

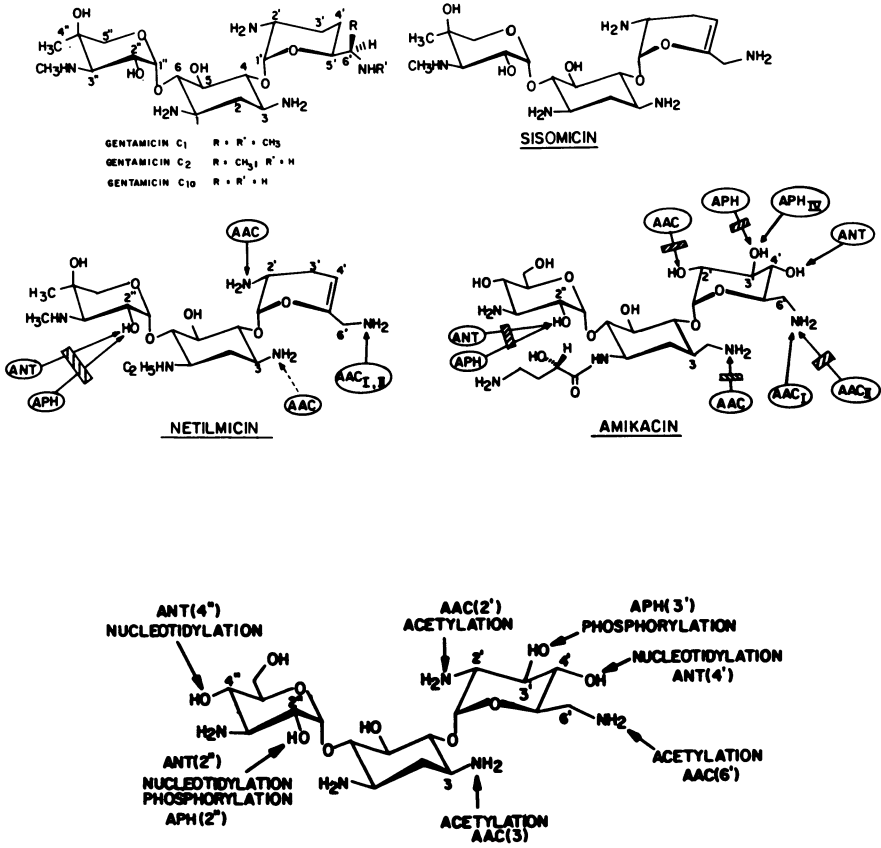


Figure 1. Aminoglycoside-modifying enzymes

mechanisms among compounds devoid of this functionality involve 2''-O-nucleotidylation and 3-N-acetylation. ANT(2'') enzymes occur in a number of organisms, particularly *Klebsiella* species. AAC(3) enzymes are found also in several bacterial genera, but are particularly common in strains of *Pseudomonas*. Enzymatic acetylation at the 2'-position [AAC(2')] is restricted to strains of *Providencia* and indole-positive *Proteus*, whereas AAC(6') enzymes are found predominantly in *Pseudomonas* and *Serratia*. Enzymes involved in 4'-O-nucleotidylation and 2'-O-phosphorylation have been found only in *Staphylococcus* and are relatively less important, since these strains are not normally treated with aminoglycosides (6).

Many of the problems associated with aminocyclitol resistance were resolved with the discovery of the semisynthetic antibiotic amikacin [BB-K8] by the Bristol-Banyu group (7). This antibiotic proved to be a non-substrate for the most important aminoglycoside-modifying enzymes, and consequently was active against strains harboring these enzymes. The spectrum of amikacin, in terms of resistance mechanisms, is shown by the blocked arrows in the structural formula. Since the advent of amikacin, the objectives of our studies have changed somewhat and, although aminocyclitols with very broad spectra of activity are still of interest, our chief focus has been the preparation of compounds having reduced toxicity compared to agents in current use. The side-effects associated with aminocyclitol therapy are nephrotoxicity, usually reversible when the drug is withdrawn, and ototoxicity which tends to be irreversible. The preparation of safer compounds comprises, in our opinion, the most important goal in aminocyclitol antibiotic research today. A subsidiary goal of all work in this area is that candidate compounds should have high potency. This goal is simply economic, since semi-synthetic aminocyclitols are expensive and the unit dose cost can become prohibitive for compounds of low potency.

Our work along these objective lines has involved modification of every functionality of gentamicin and sisomicin; this report, however, is restricted to modifications of the aminocyclitol part of the molecule. Compounds in this series were made both chemically and microbiologically by the process of mutasynthesis (8).

Among the aminocyclitol modifications we have carried out, that leading to netilmicin (9) is now the best known. Netilmicin, the 1-N-ethyl derivative of sisomicin, is a potent broad-spectrum compound (10) which is refractory to 2''-O-modifying and certain 3-N-acetylating enzymes, as indicated in the structural formula. More importantly, however, netilmicin is markedly less nephrotoxic and ototoxic than gentamicin in all species of laboratory animals so far tested (11). Netilmicin is now in phase III clinical trials and efficacy in man has been clearly demonstrated. Definitive studies, designed to assess accurately its relative toxicity in man, are in progress.

The original synthesis of netilmicin is shown in Figure 2. This synthesis depended upon the prediction that the relative reactivity of the amino groups of sisomicin towards reductive alkylation, using an aldehyde and a hydride reducing agent, would be pH dependent. Under conditions of low pH, the 1-amino function proved to be most reactive and was alkylated fairly selectively to give netilmicin in one step in 25% yield (9). Recently, however, a substantial advance in the preparation of netilmicin, and in selective reactions of aminoglycosides in general, has been made in our laboratories. It was recognized that the presence of several types of vicinal and non-vicinal amino-hydroxy group pairs in aminoglycoside molecules might allow selective complexing with divalent transition metal cations, leaving other more weakly complexed amino functions in the molecule available for selective protection (12). This concept led to an extensive series of studies, as a result of which it is now possible to carry out selective reactions at any amino group in an aminoglycoside molecule in high yield. For example, sisomicin can be selectively acylated at the 3, 2' and 6' positions via a cobaltous complex of the type shown in Figure 3. This reaction is essentially quantitative and chromatography is unnecessary to isolate pure product. Reductive alkylation then proceeds at the less hindered primary amino function and, after de-N-protection, netilmicin can be isolated in 60% overall yield from sisomicin.

Our work on modification of the aminocyclitol unit of antibiotics did not start with the synthesis of netilmicin, however, but with experiments in mutasynthesis (8). For this purpose mutants of the organism Micromonospora inyoensis were chosen since the wild-type strain produces predominantly only the single antibiotic component, sisomicin. A mutant of M. inyoensis was prepared, following the methods described by Shier, et al. (13), as outlined in Figure 4. M. inyoensis mutant 1550F produced no antibiotic when fermented alone, but gave sisomicin when fermented in the presence of 2-deoxystreptamine (14). Our first experiment with this mutant was to feed the aminocyclitol streptamine, which gave a new antibiotic complex comprising antibiotics Mu-1, Mu-1a and Mu-1b (originally named mutamicins, Figure 5). The structure of Mu-1, the major component of this fermentation, proved to be the expected streptamine analog of sisomicin, i.e., 2-hydroxysisomicin. It was, however, necessary to prove this point since, in a number of cases, the products of mutasynthesis have been shown not to be the expected products of simple aminocyclitol replacement. The structures of all mutasynthetic antibiotics described in this work were proven simply, but rigorously, using mass spectrometry and proton and carbon magnetic resonance spectroscopy at both acidic and basic pH.

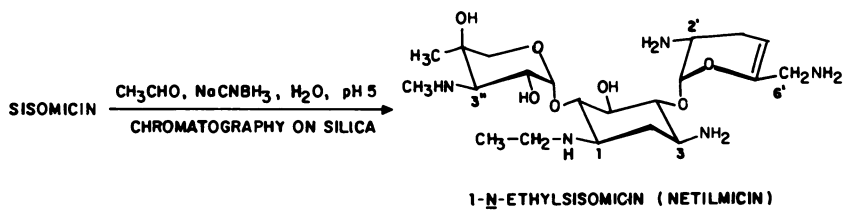


Figure 2.

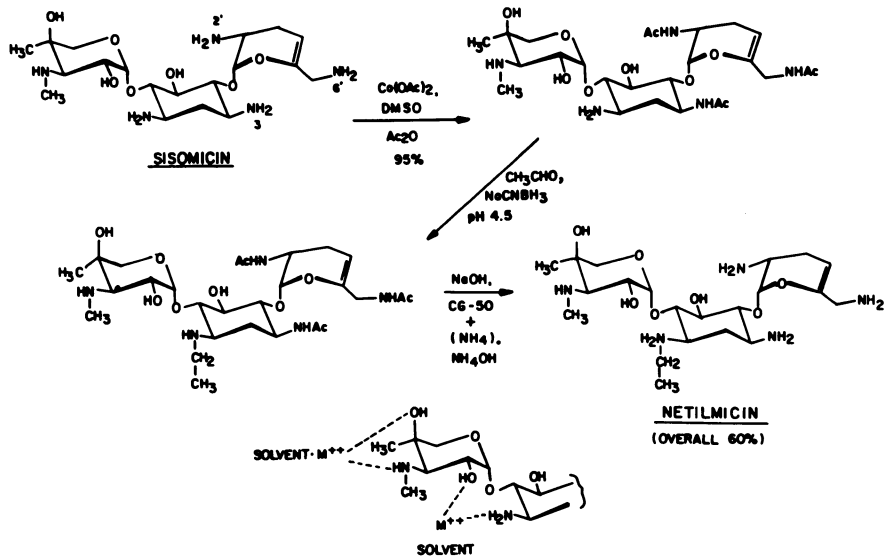


Figure 3.

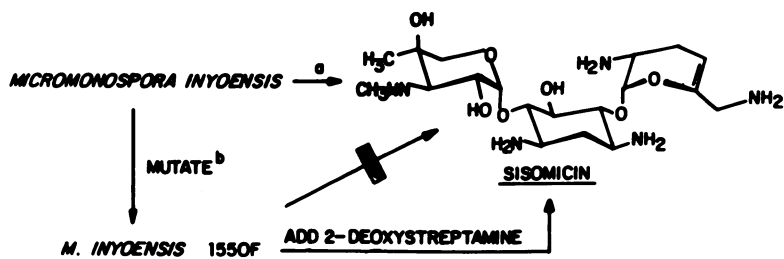


Figure 4. a (Ref. 3); b (Ref. 14)

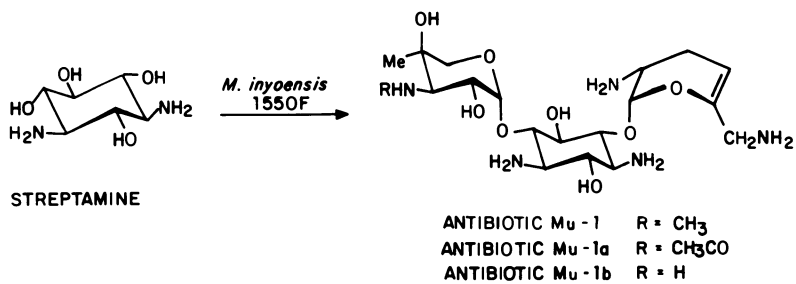


Figure 5.

Incorporation of 2,5-dideoxystreptamine by *M. inyoensis* 1550F afforded antibiotic Mu-2 complex (Figure 6). Chromatography afforded a minor, less polar component, antibiotic Mu-2, and a major, more polar component, Mu-2a. Mu-2 was shown to be the expected 5-deoxysisomicin, however, Mu-2a, the major component of the fermentation, proved to be 5-deoxygentamicin A, an unexpected product. The *in vitro* antibacterial activities of antibiotics Mu-1 and Mu-2 have been published (14). Both compounds are excellent broad spectrum antibiotics; Mu-1 is somewhat less potent than sisomicin but has improved activity against sisomicin-resistant strains containing ANT(2'') enzymes, whereas Mu-2 is more potent than Mu-1 and shows greater activity than sisomicin against bacterial strains containing AAC(3) enzymes. In a limited study, antibiotic Mu-1 was compared to sisomicin for toxicity (15). At a dose of 40 mg/kg/day, antibiotic Mu-1 produced no ataxia or elevated blood urea nitrogen (BUN) after 22 days. Under the same regimen, sisomicin produced ataxia on average on day 15, with substantial prior increase in BUN. This study was terminated due to lack of compound. However, reduced toxicity of Mu-1 compared to sisomicin was clearly demonstrated. Similar findings have been reported by Daum, *et al.* (16) for the analogous 2-hydroxygentamicins, also prepared by mutasynthesis.

Attempts to prepare sufficient antibiotic Mu-2 for toxicology studies using fermentation techniques were unsuccessful due to inefficient bioconversion of the aminocyclitol substrate. We therefore turned to chemical methods of deoxygenation, in particular that developed by Barton and McCombie (17), involving reduction of thioesters with tri-*n*-butylstannane. As shown in Figure 7, a protected derivative of sisomicin can be converted into its 5-*O*-thioformyl derivative and reduced with tri-*n*-butylstannane in a modification of the procedure previously reported (17). Removal of the protecting groups using sodium in ammonia, followed by base, afforded 5-deoxysisomicin [Mu-2] in approximately 60% overall yield (18). This deoxygenation method, *via* thioformates, proceeds well only for hindered secondary alcohols, in which the initial adduct radical undergoes C-O bond homolysis rather than quenching by Bu_3SnH . Since the completion of this work, other reports of the synthesis of 5-deoxyaminoglycosides have appeared (19, 20, 21).

The availability of a convenient method for preparing 5-deoxyaminoglycosides made possible the preparation of further analogs of these compounds, particularly their 1-*N*-substituted derivatives. This was accomplished using the selective transition metal blocking procedure previously described for the synthesis of netilmicin. As shown in Figure 8, 5-deoxysisomicin is acylated selectively at the 3, 2', and 6' positions via a $\text{Cu}^{++}/\text{Ni}^{++}$ complex of the type shown. Yields in this process were in excess of 90%. Selective reductive alkylation then yielded the 1-*N*-alkyl-5-deoxysisomicins illustrated in the Figure.

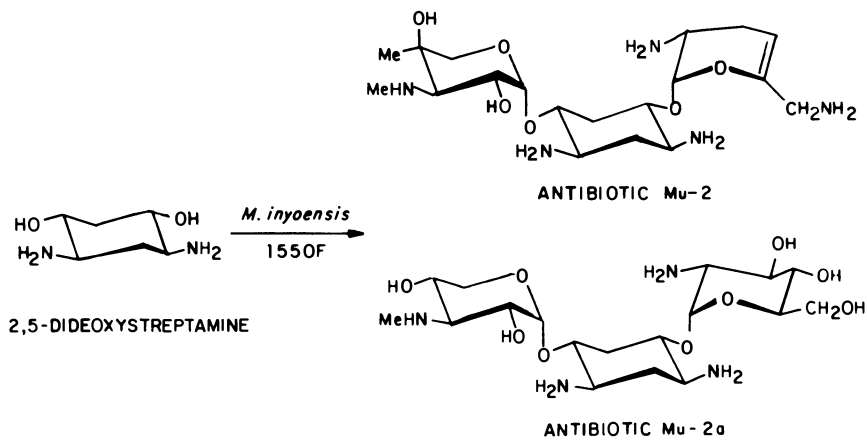


Figure 6.

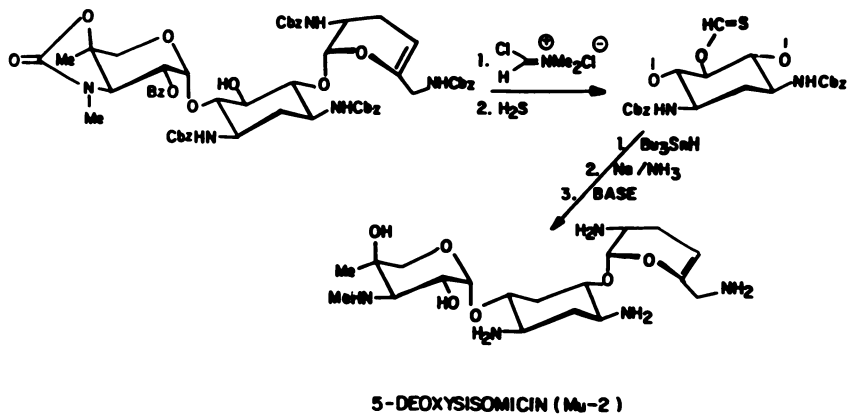


Figure 7.

The antibacterial activity of these compounds unfortunately proved to be quite similar to that of their 5-oxygenated precursors. The 1-N-ethyl derivative, 5-deoxynetilmicin, in studies in the rat showed the low chronic nephrotoxicity typical of netilmicin. In extended studies, however, this series of compounds did not show sufficient advantage to warrant further development. Our conclusion, therefore, is that deoxygenation at the 5-position offers only minor improvements in aminocyclitol antibiotics of this group.

Returning to mutasynthesis, 2-epistreptamine was incorporated by M. inyoensis 1550F to give antibiotic Mu-4 complex (Figure 9). This proved to be a relatively poor fermentation and the products were characterized only by their in vitro antibacterial activities. Antibiotic Mu-4 showed activity predominantly against sisomicin-sensitive organisms, with only slight activity against resistant strains. Because of problems in preparing this antibiotic in suitable yield, and in view of the spectrum and potency of the crude product, no further work was done with this complex. Incorporation of 1,3,5-triamino-1,2,3,5-tetradeoxy-scyllo-inositol (5-amino-2,5-dideoxystreptamine) by the 1550F mutant produced antibiotic Mu-5 complex (Figure 9). Once again, the yield in this fermentation was poor and the products were characterized only by disc assay, which showed in vitro activity solely against sisomicin-sensitive bacterial strains. Synthesis of the putative antibiotic Mu-5 later confirmed this spectrum of activity (vide infra).

2-Deoxy-5-epistreptamine was incorporated by M. inyoensis 1550F to give antibiotic Mu-6. This proved to be a relatively efficient conversion, at least on a small scale, and so far only one mutasynthetic antibiotic has been isolated from the fermentation (Figure 10). The product was shown to be 5-episisomicin by the usual spectroscopic techniques, and also by synthesis. The compound has now been extensively evaluated under the code designation Sch 22591. The synthesis of 5-episisomicin can be carried out quite efficiently (22) and is shown in Figure 11. The fully protected 5-O-mesylate, prepared in near quantitative yield by selective protection of sisomicin, on displacement with tetra-n-butylammonium acetate, followed by removal of the protecting groups, afforded 5-episisomicin in 60% overall yield from sisomicin. A number of papers have described the activity of this compound (23, 24, 25, 26,). It is remarkably potent, with greater activity than gentamicin or tobramycin against Pseudomonas aeruginosa, Providencia spp., Proteus rettgeri and other organisms. Representative minimal inhibitory concentrations (MIC) of this compound are shown in Table 1. In terms of aminoglycoside-resistance mechanisms, 5-episisomicin has excellent activity toward all strains possessing 2"- and 2'- modifying enzymes, as well as many strains harboring 3-N-acetylating enzymes (see arrows, Figure 10).

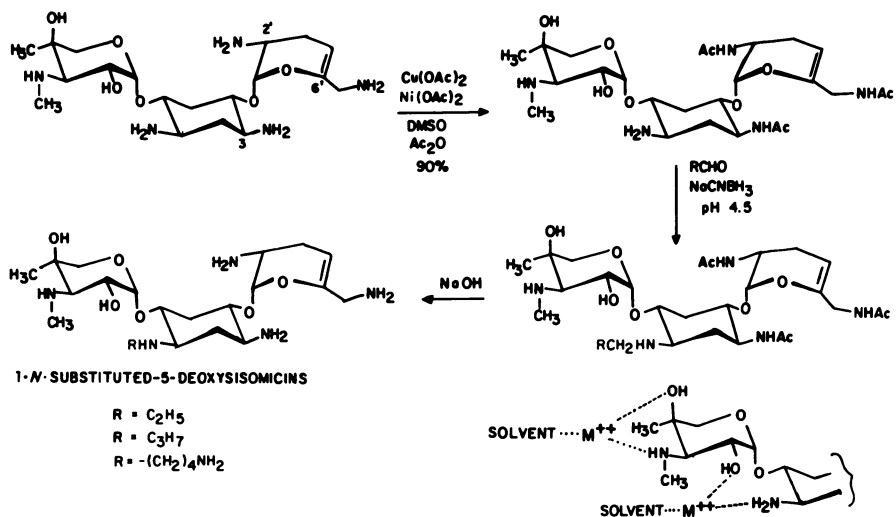


Figure 8.

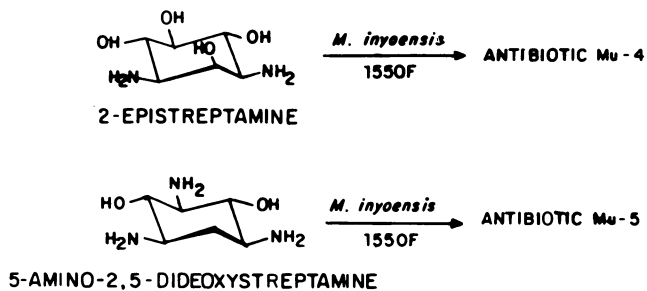


Figure 9.

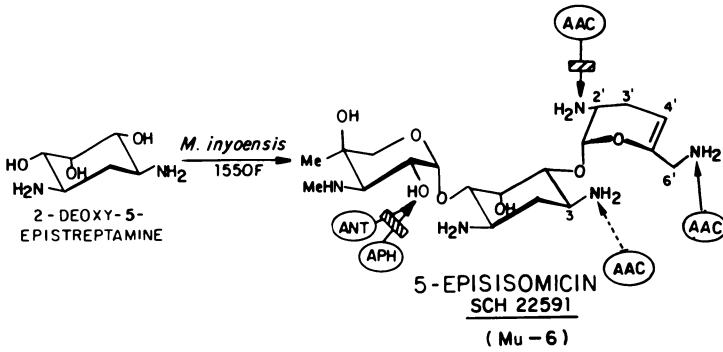


Figure 10.

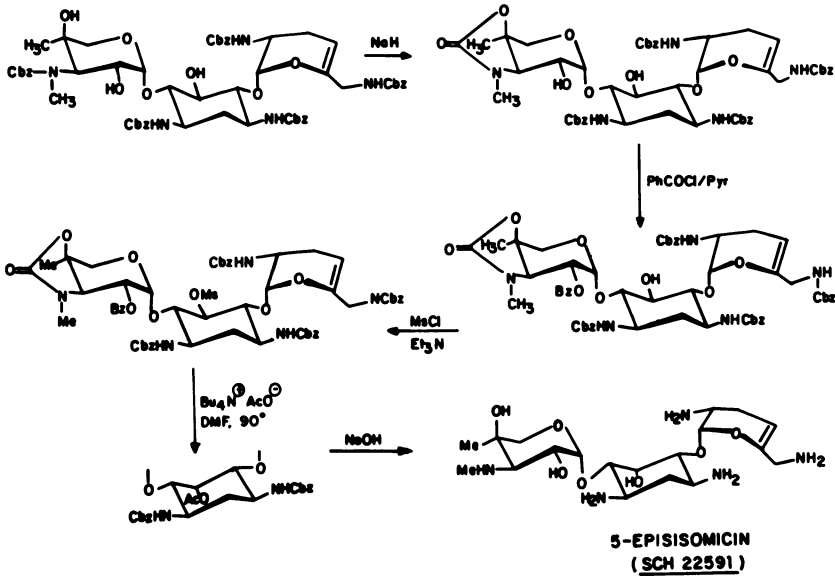


Figure 11.

Table I. In Vitro Sensitive Strains

ORGANISM	N*	MEDIAN MIC (mcg/ml)	
		SCH 22591	GENTAMICIN
<i>Bacillus subtilis</i>	1	<0.03	<0.03
<i>Staphylococcus aureus</i>	15	0.125	0.125
<i>Enterococcus</i>	9	4.0	8.0
<i>Acinetobacter</i>	1	2.0	8.0
<i>Citrobacter</i>	2	1.0	1.0
<i>Enterobacter</i>	10	0.25	0.5
<i>Escherichia coli</i>	43	0.5	0.5
<i>Klebsiella pneumoniae</i>	23	0.25	0.25
<i>Proteus mirabilis</i>	16	1.0	1.0
<i>Proteus morgani</i>	6	0.5	1.0
<i>Proteus rettgeri</i>	15	0.5	2.0
<i>Proteus vulgaris</i>	3	1.0	2.0
<i>Providencia</i>	14	0.5	4.0
<i>Pseudomonas</i>	33	0.25	1.0
<i>Serratia</i>	21	0.5	1.0

*N = number of strains.

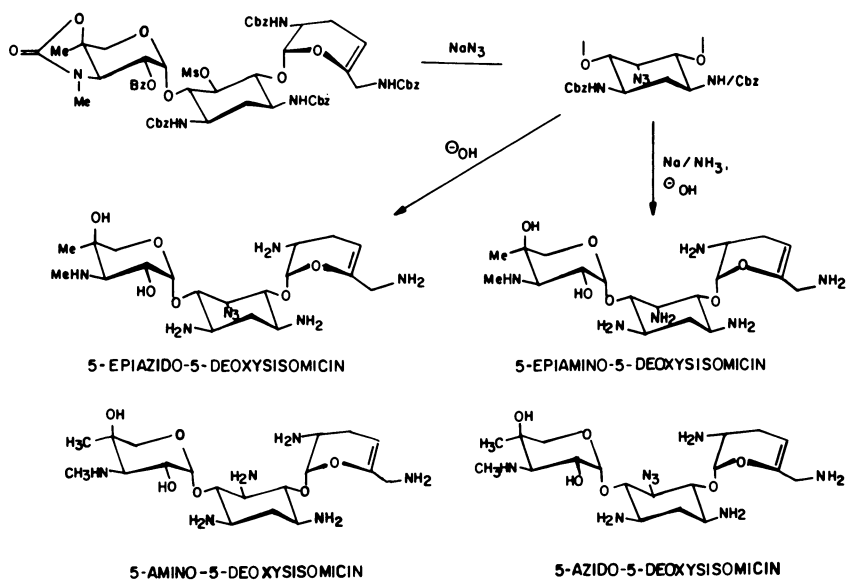


Figure 12.

The excellent activity and broad spectrum of 5-episisomicin stimulated the synthesis of many analogous compounds. Figure 12 shows the straightforward synthesis of 5-epiazido-5-deoxysisomicin and 5-epiamino-5-deoxysisomicin. The corresponding equatorially substituted 5-amino-5-deoxy and 5-azido-5-deoxy derivatives are prepared in similar fashion starting with 5-episisomicin (Figure 12). The *in vitro* antibacterial activities of these compounds are compared to sisomicin in Table 2. From these data it is clear that the equatorially substituted amino and azido compounds have basically the same antibacterial spectrum as sisomicin, although the azido compound is less potent. Note that 5-amino-5-deoxysisomicin is the putative antibiotic Mu-5, expected from incorporation of 5-amino-2,5-dideoxystreptamine by the DOS⁻ mutant (Figure 9). The antibacterial spectrum of the pure compound was identical to that of the material obtained in the mutasynthetic experiment. Both the 5-epiamino and 5-epiazido compounds, on the other hand, have excellent activity against sisomicin resistant strains, the amino compound again being substantially more potent than its azido substituted precursor. Overall, the spectrum and potency of 5-epiamino-5-deoxysisomicin are closely comparable to those of 5-episisomicin.

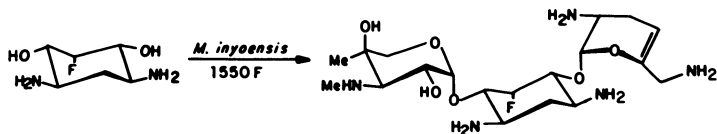
Incorporation of 5-epifluoro-2,5-dideoxystreptamine by the DOS⁻ mutant gave a single mutasynthetic antibiotic having the expected composition (Figure 13). The structure of the product was confirmed by the synthesis shown in Figure 14. Replacement of the 5-hydroxyl group by a fluorine atom was accomplished by reaction with diethylamino sulfur trifluoride (27), which proceeded with clean inversion, as demonstrated by the ¹³C NMR spectrum of the product, part of which is reproduced in the Figure. The small value of ³J between carbons 1 and 3 and fluorine clearly showed the gauche arrangement of these atoms and hence the axial disposition of the fluoro substituent. The epimeric 5-fluoro-5-deoxysisomicin was prepared in an analogous manner from 5-episisomicin. Once again the reaction proceeded with clean inversion, evidenced by the 3-bond coupling constants shown in the Figure. The antibacterial activity of the fluoro-substituted aminocyclitols is shown in Table 3 in comparison to 5-episisomicin. Against aminoglycoside-sensitive organisms, all three compounds showed similar activity. Against aminoglycoside-resistant strains, the 5-epifluoro compound was more potent than its equatorially substituted counterpart, but somewhat less potent than 5-episisomicin. Compared to sisomicin, the 5-epifluoro compound was at least as potent, with a substantially broader spectrum.

The examples cited of aminocyclitol antibiotics modified at position 5 illustrate what we now recognize to be a generality, which is that 5-axially substituted aminocyclitols, at least in the sisomicin series, are more active than their equatorially substituted isomers. In particular, the spectrum of activity is broader due to the inability of 5-axially substituted compounds

Table II. Minimal Inhibitory Concentrations (mcg/mL) in Mueller-Hinton Broth¹

ORGANISM AND RESISTANCE MECHANISM	SISOMICIN	5-EPIAMINO-5-DEOXY-SISOMICIN	5-EPIAZIDO-5-DEOXY-SISOMICIN	5-AMINO-5-DEOXY-SISOMICIN	5-AZIDO-5-DEOXY-SISOMICIN
SENSITIVE					
STAPHYLOCOCCUS AUREUS	< 0.06	< 0.01	0.075	0.03	0.06
BACILLUS SUBTILIS	< 0.06	< 0.01	< 0.01	0.03	0.125
ESCHERICHIA COLI	0.25	0.075	0.075	0.3	1.0
PSEUDOMONAS AERUGINOSA	0.25	0.03	0.75	0.3	4.0
KLEBSIELLA PNEUMONIAE	0.25	0.03	0.3	0.075	2.0
PROTEUS MIRABILIS	0.5	0.3	0.075	0.75	2.0
SALMONELLA TYPHIMURIUM	0.5	0.075	3.0	0.75	8
ANT(2'')					
ESCHERICHIA COLI	8	0.3	3.0	> 25	> 16
KLEBSIELLA PNEUMONIAE	8	0.3	3.0	17.5	> 16
AAC(3)-I					
ESCHERICHIA COLI	8	3.0	0.3	> 25	8
PSEUDOMONAS AERUGINOSA	128	0.3	3.0	> 25	> 16
AAC(3)-III					
PSEUDOMONAS AERUGINOSA	128	> 25	> 25	> 25	> 16
AAC(2')					
PROVIDENCIA	128	0.075	17.5	> 25	> 16
AAC(6')-I					
ESCHERICHIA COLI	4.0	0.75	0.3	7.5	> 16
AAC(6')-II					
PSEUDOMONAS AERUGINOSA	128	> 25	> 25	> 25	> 16

*Representative data given; M.I.C's not determined in same run.



5-EPIFLUORO-5-DEOXY-SISOMICIN

Figure 13.

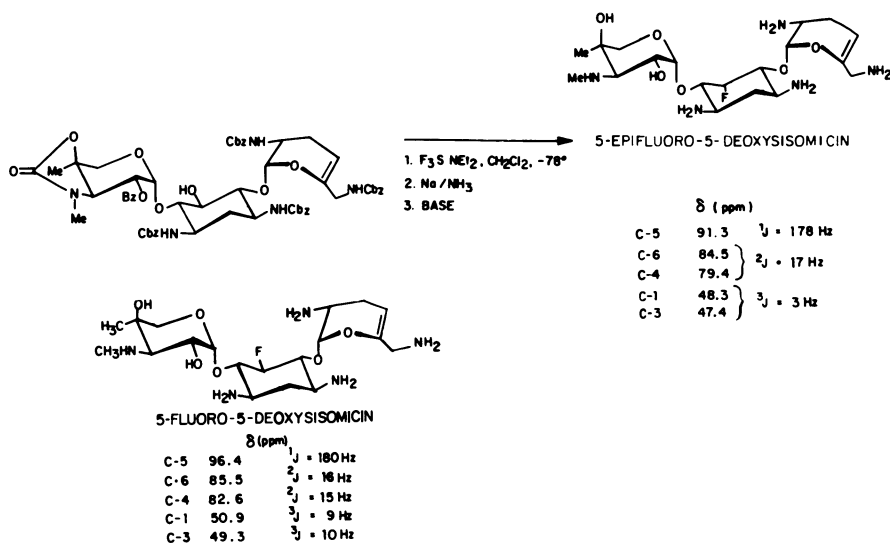


Figure 14.

Table III. Minimal Inhibitory Concentrations (mcg/mL) in Mueller-Hinton Broth

ORGANISM	RESISTANCE MECHANISM	5-EPI-SISOMICIN	5-EPIFLUORO-5-DEOXYISISOMICIN	5-FLUORO-5-DEOXYISISOMICIN
STAPHYLOCOCCUS AUREUS	} SENS.	0.03	0.03	0.125
ESCHERICHIA COLI		0.25	0.25	0.25
PROTEUS MIRABILIS		1.0	0.5	1.0
PROTEUS RETTGERI		0.5	0.5	1.0
PSEUDOMONAS AERUGINOSA		0.125	0.25	0.5
SALMONELLA TYPHIMURIUM		0.25	0.5	0.5
ESCHERICHIA COLI	} ANT(2 ^{III})	1.0	2.0	2.0
KLEBSIELLA PNEUMONIAE		0.5	2.0	4.0
PSEUDOMONAS AERUGINOSA		0.125	1.0	2.0
ESCHERICHIA COLI	} AAC(3)-I	0.25	0.5	2.0
PSEUDOMONAS AERUGINOSA		0.06	0.25	1.0
PSEUDOMONAS AERUGINOSA	AAC(3)-Ia	0.25	>16	>16
PSEUDOMONAS AERUGINOSA	AAC(3)-III	>16	>16	>16
PROTEUS RETTGERI	AAC(2) ^I	1.0	4.0	>16
ESCHERICHIA COLI	AAC(6 ^I)-I	8.0	16	16
PSEUDOMONAS AERUGINOSA	AAC(6 ^I)-II	>16	>16	>16
PSEUDOMONAS AERUGINOSA	PERMEABILITY	8	>16	>16

to serve as good substrates for aminoglycoside-inactivating enzymes. Carbon magnetic resonance studies have shown the conformation of 5-axially substituted antibiotics to be different from that of their equatorially substituted counterparts. It is reasonable to propose that this conformational difference, involving rotation around the C₄-glycosidic oxygen bond, differentiates the axially-substituted compounds from the natural isomers in their ability to serve as enzyme substrates. In most cases, inversion of stereochemistry at position 5 in antibiotics of this class provides a reliable means of improving antibacterial spectrum.

Attempts to prepare *N*-substituted aminocyclitol antibiotics by mutasynthesis have met with limited success in our hands. (+)-2-Deoxy-*N*-ethylstreptamine, for example, was not incorporated by *M. inyoensis* 1550F to give netilmicin. (+)-2-Deoxy-*N*-methylstreptamine, however, was transformed into antibiotic complex Mu-7 (Figure 15). The bioconversion in this process was poor, activity of the complex was low and pure components were not isolated. The observed low activity is likely related to the fact that the predicted products, 3-*N*-alkyl substituted antibiotics, have been confirmed to have low potency by unambiguous chemical semisynthesis (9, 28). (+)-2,5-Dideoxy-*N*-methylstreptamine was also bioconverted, albeit with very low efficiency, into antibiotic complex Mu-8 (Figure 15). The activity of this complex by disc assay indicated good potency against both sisomicin-sensitive and resistant strains. In view of the very poor yields obtained in the mutasynthetic process, however, we were compelled to prepare the expected products, i.e. 1-*N*-alkyl-5-deoxyaminoglycosides, using the synthetic method already outlined in Figure 8.

In view of our limited success in the efficient preparation of 1-*N*-substituted antibiotics by mutasynthesis, we explored this desired group of compounds via chemical semisynthesis. We especially concentrated on derivatives of 5-episisomicin (Mu-6) from which many derivatives have now been made. Once again these syntheses, shown in Figure 16, were accomplished by the selective transition metal blocking procedure described previously. Some of the range of compounds made are shown in the Figure. All were excellent antibiotics with high potency and broad antibacterial spectra. Of particular interest are 1-*N*-ethyl-5-episisomicin and 1-*N*-(*S*-3-amino-2-hydroxypropionyl)-5-episisomicin. The in vitro antimicrobial activity of these compounds is shown in the tables. In Table 4, the activity of 1-*N*-ethyl-5-episisomicin, identified by the code designation Sch 22703, is compared to gentamicin and netilmicin. Sch 22703 has very broad spectrum antibacterial activity and is superior in this regard to netilmicin and gentamicin. Especially noteworthy is the activity of this compound against organisms containing *N*-acetylating enzymes. Against these strains the compound is superior to netilmicin, and against AAC(6') containing bacteria is superior to amikacin (data not shown). In terms of potency, Sch 22703 is, on average,

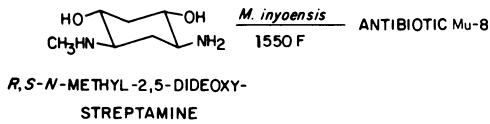
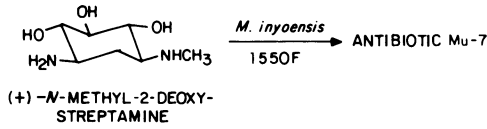


Figure 15.

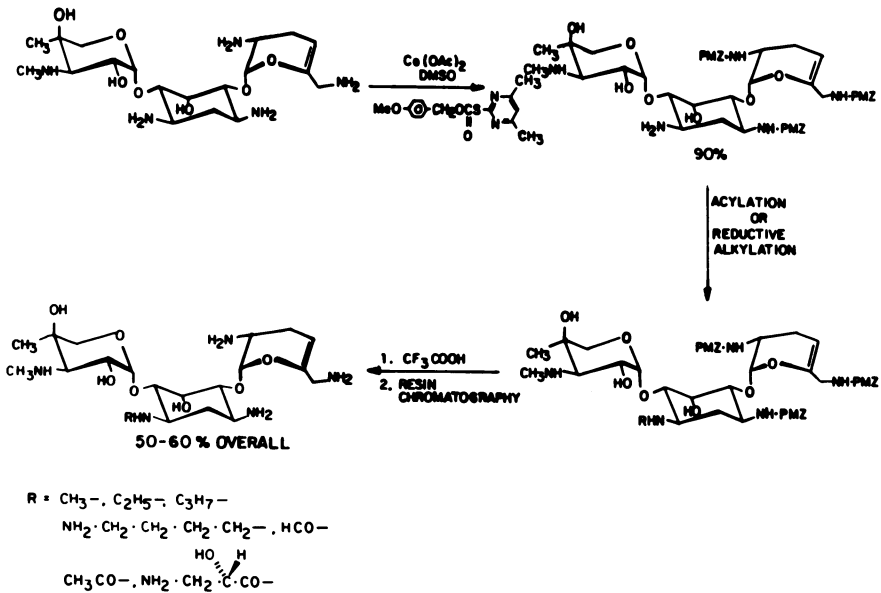


Figure 16.

Table IV. In Vitro Antibacterial Activity of 1-N-Ethyl-5-episomicin (SCH 22703) Compared to Gentamicin and Netilmicin

ORGANISM	RESISTANCE MECHANISM	MIC (ugm/ml) IN MUELLER-HINTON AGAR			
		GENTAMICIN	NETILMICIN	SCH 22703	
ENTEROBACTER, SPP	} SENS	0.25	0.25	0.25	
ESCHERICHIA COLI		0.25	0.25	0.25	
KLEBSIELLA PNEUMONIAE		0.25	0.25	0.25	
PROVIDENCIA, SPP		4.0	4.0	0.5	
PSEUDOMONAS AERUGINOSA		1.0	2.0	1.0	
PROTEUS MIRABILIS		0.25	0.25	0.25	
PROTEUS RETTGERI	} ANT(2')	1.0	0.5	0.25	
ENTEROBACTER CLOACAE		16	0.25	0.25	
ESCHERICHIA COLI		2.0	1.0	1.0	
KLEBSIELLA PNEUMONIAE		32	0.5	0.25	
PSEUDOMONAS AERUGINOSA		AAC(3)-I	16	1.0	0.5
PSEUDOMONAS AERUGINOSA		AAC(3)-1a	64	64	1.0
PSEUDOMONAS AERUGINOSA	AAC(6')-II*	64	64	4.0	
SERRATIA MARCESCENS	AAC(6')-I**	1.0	32	8.0	
PROVIDENCIA, SPP	} AAC(2')	16	16	1.0	
PROTEUS RETTGERI		32	32	2.0	
PSEUDOMONAS AERUGINOSA		PERMEABILITY	64	64	64

*Amikacin sensitive.

**Amikacin resistant.

Table V. In Vitro Activity of 1-N-(S-3-Amino-2-hydroxypropionyl)-5-episomicin (SCH 27082) Compared to Gentamycin

ORGANISM	RESISTANCE MECHANISM	MIC (ugm/ml) IN MUELLER-HINTON AGAR		
		GENTAMICIN	SCH 27082	
ENTEROBACTER, SPP	} SENS	0.25	0.25	
ESCHERICHIA COLI		0.25	0.125	
KLEBSIELLA PNEUMONIAE		0.25	0.125	
PROTEUS MIRABILIS		0.5	1.0	
PSEUDOMONAS AERUGINOSA		1.0	1.0	
ENTEROBACTER CLOACAE		32	0.25	
ESCHERICHIA COLI	} ANT(2'')	64	0.125	
KLEBSIELLA PNEUMONIAE		16	0.125	
PSEUDOMONAS AERUGINOSA		AAC(3)-I	64	2.0
PSEUDOMONAS AERUGINOSA		AAC(3)-1a	128	0.5
SERRATIA MARCESCENS		AAC(3)-II	>128	2.0
PSEUDOMONAS AERUGINOSA		AAC(6')-II	128	2.0
SERRATIA MARCESCENS	AAC(6')-I*	2.0	1.0	
STAPHYLOCOCCUS AUREUS	ANT(4')*	0.5	1.0	
STAPHYLOCOCCUS AUREUS	APH(3')-IV*	0.5	1.0	
PSEUDOMONAS AERUGINOSA	PERMEABILITY*	32	32	

*Amikacin resistant.

two-fold more potent than netilmicin and two to four-fold more potent than amikacin (data not shown). Preliminary experiments have shown Sch 22703 to have about the same nephrotoxic potential in animals as netilmicin or amikacin (29). The greater potency of Sch 22703, however, would lead one to predict that this compound should have an improved therapeutic index compared to these latter antibiotics. In Table 5, the *in vitro* antimicrobial activity of 1-N-(S-3-amino-2-hydroxypropionyl)-5-episisomicin, also designated Sch 27082, is shown in comparison to gentamicin. Against sensitive strains this compound is at least as active as gentamicin; against resistant strains, however, Sch 27082 is remarkable, exhibiting excellent activity against all aminoglycoside-resistant strains containing inactivating enzymes. These findings have been confirmed in extended studies using over 200 recent clinical isolates (30). The only bacterial resistance to Sch 27082 that we have found involves strains impermeable to aminoglycosides. Studies are currently underway to determine the chronic toxicity of this compound.

To complete the discussion of our work on modification of the aminocyclitol unit of compounds in the gentamicin group, mention should be made of 1-N-(S-3-amino-2-hydroxypropionyl)-gentamicin B, also known as Sch 21420 (Figure 17). We recently described this antibiotic (31) as a very broad spectrum compound, not susceptible to inactivation by the most important aminoglycoside-modifying enzymes. The antibacterial spectrum of Sch 21420 is identical to that of amikacin and the potency of the two compounds is very similar. Of greater interest, however, the nephrotoxicity of Sch 21420 in animal tests to date, has proved significantly lower than amikacin (32). Sch 21420 is, therefore, promising as a safer drug.

In Figure 18, other structural modifications involving the aminocyclitol unit, which have been carried out in our laboratories, are summarized. Modifications of N-3 are of particular interest, since this is an important position of enzymatic modification. Unfortunately, the molecule is intolerant to change at this position and all modified compounds listed on the left hand side of Figure 18 are devoid of useful antibacterial activity. These modifications include N-alkylation (9), epimerization, or replacement of the amino group with a hydroxyl function (33). On the other hand, fairly substantial modifications at N-1 of the aminocyclitol unit can be accomplished whilst maintaining, or enhancing antibiotic properties. Thus N-alkylation, as already discussed, epimerization, or replacement of the amino group with hydroxy (34) produced compounds with antibacterial activity similar to, or better than the parent compounds.

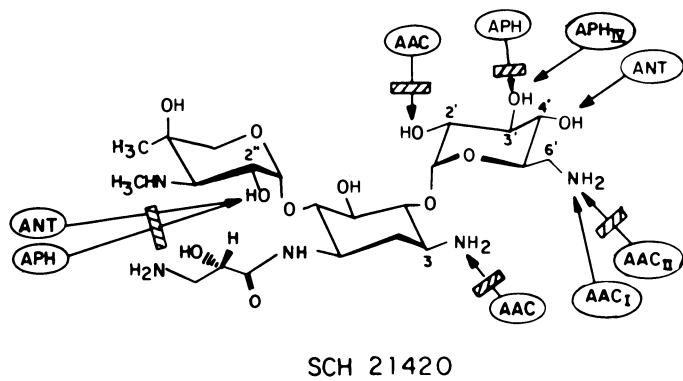


Figure 17.

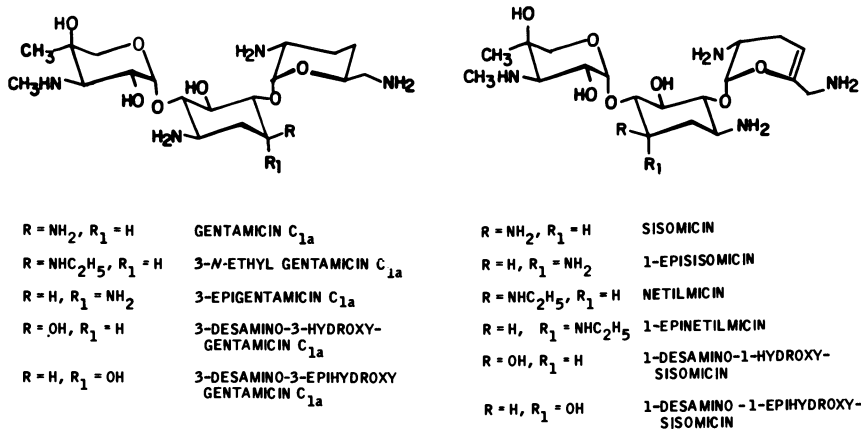


Figure 18.

Acknowledgement

The antibacterial activities of the compounds described in this work were determined by Drs. J.A. Waitz, G.H. Miller and co-workers. Animal nephrotoxicity referred to was determined by Drs. P.J.S. Chiu, G.H. Miller and co-workers. The assistance of Mr. A.S. Yehaskel is gratefully acknowledged.

Literature Cited

1. Weinstein, M.J., Luedemann, G.M., Oden, E.M. and Wagman, G.H., Antimicrobial Agents and Chemotherapy-1963, (1964), 1.
2. Nara, T., Kawamoto, I., Okachi, R. and Tetsue, O., J. Antibiot., (1977), 30, S-174.
3. Weinstein, M.J., Marquez, J.A., Testa, R.T., Wagman, G.H., Oden, E.M. and Waitz, J.A., J. Antibiot., (1970), 23, 551.
4. Reimann, H., Cooper, D.J., Mallams, A.K., Jaret, R.S., Yehaskel, A., Kugelman, M., Vernay, H.F. and Schumacher, D., J. Org. Chem., (1974), 39, 1451.
5. Davies, J. and Smith, D.I., Ann. Rev. of Microbiol., (1978), 32, 464.
6. Miller, G.H., Sabatelli, F.J., Hare, R.S. and Waitz, J.A., Developments in Industrial Microbiology, (1980), 21, (in press).
7. Kawaguchi, H., Naito, T., Nakagawa, S. and Fujisawa, K., J. Antibiot., (1972), 25, 695.
8. Rinehart, Jr., K.L., Pure Appl. Chem., (1977), 49, 1361.
9. Wright, J.J., J. Chem. Soc. Chem. Commun., (1976), 206.
10. Miller, G.H., Arcieri, G., Weinstein, M.J. and Waitz, J.A., Antimicrob. Agents Chemother., (1976), 10, 827.
11. Luft, F.C., J. Int. Med. Research, (1978), 6, 286.
12. Nagabhushan, T.L., Cooper, A.B., Turner, W.N., Tsai, H., McCombie, S., Mallams, A.K., Rane, D., Wright, J.J., Reichert, P., Boxler, D.L. and Weinstein, J., J. Am. Chem. Soc., (1978), 100, 5253.
13. Shier, W.T., Rinehart, Jr., K.L. and Gottlieb, D., Proc. Natl. Acad. Sci. USA., (1969), 63, 198.
14. Testa, R.T., Wagman, G.H., Daniels, P.J.L. and Weinstein, M.J., J. Antibiot., (1974), 27, 917.
15. Waitz, J.A., unpublished observations.
16. Daum, S.J., Microbiology 1979, (1979), 312.
17. Barton, D.H.R. and McCombie, S.W., J. Chem. Soc. Perkin Trans. 1., (1975), 1574.
18. Daniels, P.J.L. and McCombie, S.W., U.S. Patent 4,053,591; Chem. Abstr., (1978), 88, 2338x.
19. Hayashi, T., Iwaoka, T., Takeda, N. and Oki, E., Chem. Pharm. Bull. Jpn., (1978), 26, 1786.

20. Suami, T., Nishiyama, S., Ishikawa, Y. and Umemura, E., Bull. Chem. Soc. Jpn., (1978), 51, 2354.
21. Kavadias, G., Dextraze, P., Masse, R. and Belleau, B., Can. J. Chem., (1978), 56, 2086.
22. Daniels, P.J.L., U.S. Patent 4,000,261; Chem. Abstr., (1977), 86, 5776t.
23. Waitz, J.A., Miller, G.H., Moss, Jr., E. and Chiu, P.J.S., Antimicrob. Agents Chemother., (1978), 13, 41.
24. Sanders, C.C., Sanders, Jr., W.E. and Goering, R.V., Antimicrob. Agents and Chemother., (1978), 14, 178.
25. Fu, K.P. and Neu, H., Antimicrob. Agents Chemother., (1978), 14, 194.
26. Kabins, S.A. and Nathan, C., Antimicrob. Agents Chemother., (1978), 14, 391.
27. Middleton, W.J., J. Org. Chem., (1974), 40, 574.
28. Nakagawa, S., Toda, S., Abe, Y., Yamashita, H., Fujisawa, K., Naito, T. and Kawaguchi, H., J. Antibiot., (1978), 31, 675.
29. Chiu, P.J.S. and Miller, G.H., unpublished observations.
30. Miller, G.H. and co-workers, unpublished observations.
31. Nagabhushan, T.L., Cooper, A.B., Tsai, H., Daniels, P.J.L. and Miller, G.H., J. Antibiot., (1978), 31, 681.
32. Miller, G.H., Chiu, P.J.S. and Waitz, J.A., J. Antibiot., (1978), 31, 688.
33. McCombie, S.W., Abstracts, 9th International Symposium on Carbohydrate Chemistry, London, (1978), Abstract B39.
34. Mallams, A.K., Davies, D.H., Boxler, D.L., Vernay, H.F. and Reichert, P., Abstracts, 17th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, (1977), Abstract 251.

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Synthesis and Mutasynthesis of Pseudosaccharides Related to Aminocyclitol-Glycoside Antibiotics

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The aminocyclitol glycosides enumerated in Table 1, are produced mainly by Streptomyces species but also by Micromonospora, Bacillus and even Pseudomonas species (1, 2, 3). They constitute a very important class of clinically used antibiotics and provide a cover for the pathogens most commonly found in the hospital environment. To varying degrees all the amino-glycosides are toxic (oto and nephrotoxicity) and therefore their administration is strictly controlled.

It was first noticed in 1965 (4) that some pathogenic bacteria became resistant to these antibiotics, and in subsequent years, it was shown that the major resistance mechanism was an R-factor mediated enzymatic inactivation, resulting in O-phosphorylation, O-nucleotidylation or N-acetylation of the antibiotics at different positions (5, 6, 7). The discovery and explanation of these enzymatic inactivations led to an increased effort to find from natural sources (through soil screening programs) and through chemical modification procedures new products effective against resistant organisms.

Chemical modification of already existing naturally occurring antibiotics, either by removal of certain functional groups subject to inactivating enzymes or by substitution (acylation or alkylation) of the 1-amino group of the 2-deoxystreptamine moiety, led to semi-

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TABLE 1 MAJOR DISCOVERIES OF AMINOGLYCOSIDE
ANTIBIOTICS (1944-1977)

YEAR	ANTIBIOTIC	PRODUCING ORGANISM
1944	STREPTOMYCIN	S. GRISEUS
1949	NEOMYCIN	S. FRADIAE
1957	KANAMYCIN	S. KANAMYCETICUS
1959	PAROMOMYCIN	S. RIMOSUS F. PAROMOMYCINUS
1961	SPECTINOMYCIN	S. SPECTABILIS
1963	GENTAMICIN C	M. PURPUREA
1965	KASUGAMYCIN	S. KASUGAENSIS
1968	TOBRAMYCIN	S. TENEBRARIUS
1970	RIBOSTAMYCIN	S. RIBOSIDIFICUS
1970	SISOMICIN	M. INYOENSIS
1971	LIVIDOMYCIN	S. LIVIDUS
1971	BUTIROCIN	B. CIRCULANS
1973	APRAMYCIN	S. TENEBRARIUS
1974	MINOSAMINOMYCIN	ACTINOMYCES SP
1975-77	SELDOMYCIN	S. HOFUENSIS
1976	SORBISTIN	P. SORBICINII
1977	FORTIMICIN	M. OLIVOASTEROSPORA

M = Micromonospora ; S = Streptomyces ; B = Bacillus ;
P = Pseudomonas.

synthetic derivatives [dibekacin (8), amikacin (9), netilmicin (10), UK 18892 (11) and Sch 21420 (12), Fig. 1], which were active against aminoglycoside resistant bacteria.

In our laboratory we have pursued two different but complementary approaches : mutasynthesis and total chemical synthesis and we will report here our efforts to produce pseudosaccharides related to aminoglycoside antibiotics.

Let us first examine the structural features of the pseudodisaccharide moiety of the aminoglycosides which exhibits antibacterial activity. Except in fortimicin B, in which the aglycone is a novel 1,4-diaminocyclitol named fortamine, the other pseudodisaccharides presented in Figure 2, contain 2-deoxystreptamine which is assymmetrically α -glycosylated at position 4 by a variety of aminohexopyranosides which differ from each other by the presence or absence of amino, hydroxyl and double bond functions.

The prerequisite for structure-activity relationship studies is the readily availability of cyclitols or amino-cyclitols and their α -glycosides. They might be obtained by either mutasynthesis or total chemical synthesis.

Meso 2-deoxystreptamine and meso 2,5-dideoxystreptamine can be obtained by hydrolysis of natural antibiotics or by chemical synthesis respectively (13, 14, 15) but they were considered unsuitable for practical chiral synthesis. Quinic acid, on the other hand, possesses functional groups and an absolute configuration amenable to our coveted goals and therefore was chosen as starting material.

In this article we deal briefly with the preparation of 2,6-di- and 2,5,6-trideoxystreptamines and 3,5-

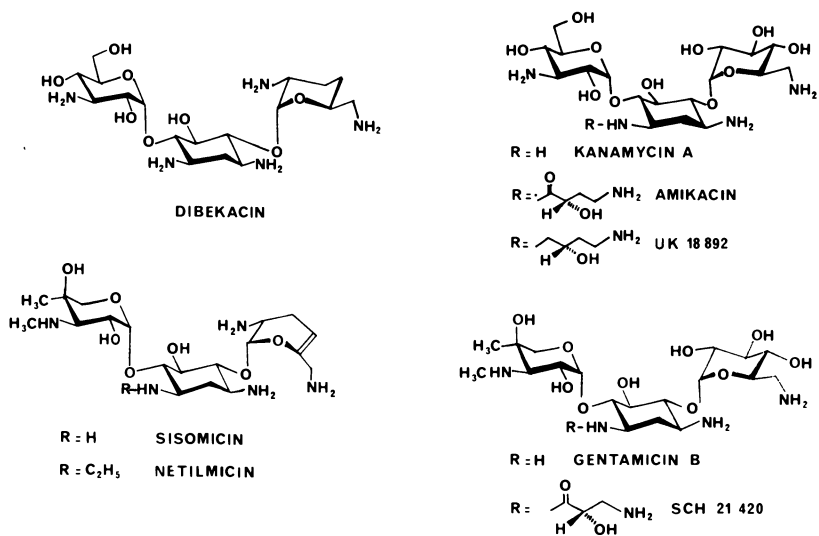


Figure 1.

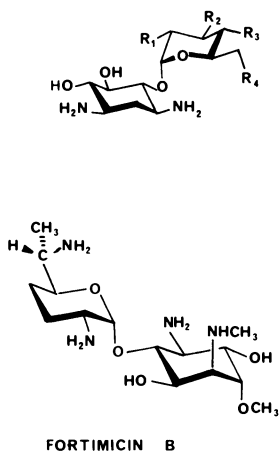


Figure 2.

dideoxyfortamine and an acid catalysed α -glycosylation procedure leading to hitherto unknown pseudodisaccharides.

SYNTHESIS OF CYCLITOL AND AMINOCYCLITOL DERIVATIVES FROM QUINIC ACID

- Preparation of D-2,6-dideoxystreptamine 6 and D-2,5,6-trideoxystreptamine 10.

In view of the importance of preparing analogs of 4-substituted or 4,5-disubstituted 2-deoxystreptamine antibiotics, it seemed attractive to prepare chiral 2,6-dideoxystreptamine, 2,5,6-trideoxystreptamine and their precursors for use in microbial transformation or for total synthesis.

Quinic acid 1 was readily transformed as we described recently (16) in excellent overall yield, to the 3,4-0-cyclohexylidene 3,4/5-trihydroxycyclohexanone 2. The latter was quantitatively reduced with lithium borohydride to a mixture of two epimeric diols 3 and 4. The trans diol (4), on acidic hydrolysis followed by selective tosylation, gave the ditosylate 5, which was transformed by azidolysis followed by hydrogenation using Adam's catalyst into 2,6-dideoxystreptamine 6 (17) (Fig. 3).

The 2,5,6-trideoxystreptamine 10 was also obtained from the ketone 2 in the following way : treatment of the ketone 2 with p-toluenesulfonyl chloride in pyridine gave the α,β unsaturated ketone 7 which was catalytically reduced to the saturated ketone 8. The latter in turn, was regiospecifically converted to a cyclohexane-triol derivative which after deprotection and selective tosylation furnished the ditosylate 9. From 9, the re-

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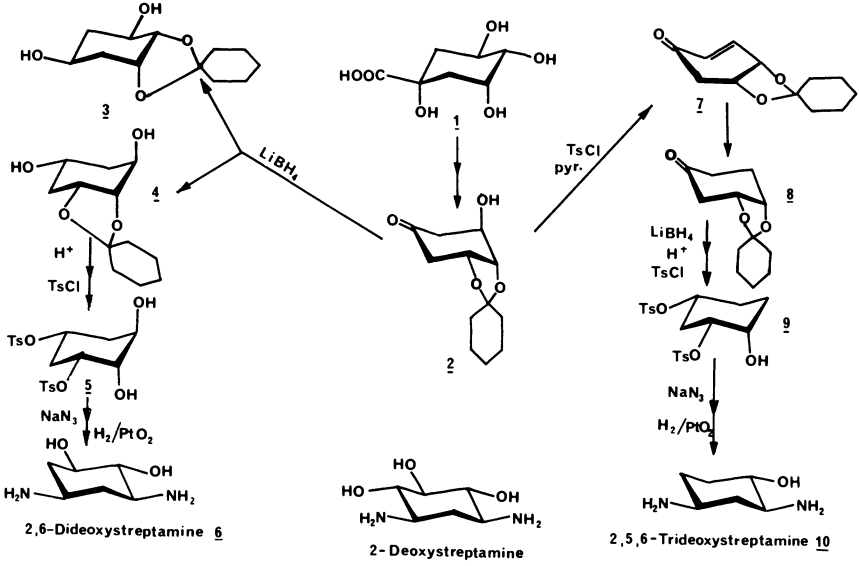


Figure 3.

quired 2,5,6-trideoxystreptamine 10 was obtained by treatment with sodium azide followed by Adam's catalyst reduction of the diazide formed.

A priori, the mode of synthesis of 6 and 10 outlined on Fig. 3, from quinic acid 1, should lead to chiral products possessing the same absolute configuration as the 2-deoxystreptamine aglycone in the natural products. Indeed these compounds exhibited optical rotations and their ^{13}C NMR data were consistent with the structures proposed. Racemic 2,6-dideoxy-streptamine and 2,5,6-trideoxystreptamine have been reported (18).

- Synthesis of 3,5-dideoxyfortamine

Recently, a completely new type of aminocyclitol glycoside antibiotic, fortimicins A and B, have been isolated from Micromonospora species (19). These pseudodisaccharides contain the 6-epi-purpurosamine subunit which is α -linked to the hitherto unknown chiral aglycon fortamine. The isolation of this antibiotic complex is a major event in the development of novel aminoglycoside antibiotics because it contains the chiro-1,4-diaminocyclohexanetetrol and not the usually encountered 1,3-diaminocyclohexanetriol (2-deoxystreptamine). Additionally, they are comparatively simple molecules being pseudodisaccharides and therefore within easy reach for the synthetic chemist.

We were interested in 1,4-diaminocyclohexanols for our mutasynthetic and total chemically synthetic studies. The synthesis of the meso and chiral fortamine derivatives utilized ditosyl 0-cyclohexylidene cyclohexanetetrol 11 derived also from quinic acid (1) as previously described (16). A very brief exposure of the ditosylate 11 to sodium azide in dimethylformamide gave the monoazide 12. Hydrolysis removed the cyclohexylidene

group and treatment of the diol 13 with sodium methoxide in methanol furnished the epoxide derivative 14 which was readily converted to its benzoate 15 (Fig. 4).

Azidolysis of 14 afforded the meso 1,4-diazido derivative 16 in high yield (85 %) whereas similar treatment of its benzoate 15 gave a mixture of 1,4 and 1,3 diazido compounds 17 and 18 in a ratio of 4:6, respectively. Reduction of the diazides 16 and 17, by the usual method yielded the meso (19) and chiral (20) 3,5-dideoxyfortamine derivatives as was evident from their optical rotation and ^{13}C NMR data. The ^{13}C NMR spectra of the meso 19 and the chiral 20 compounds exhibited four and six signals respectively.

MUTASYNTHESIS

Having in hand this variety of aminocyclitols, we attempted to incorporate them into novel antibiotics using idiotrophs of antibiotic-producing strains (Fig. 5). Using the mutasynthetic method of Rinehart (20) we were successful in incorporating 2,6-dideoxystreptamine 6 and 2,5,6-trideoxystreptamine 10 into antimicrobial products : 6-deoxyneomycin complex (16) and 5,6-dideoxyneamine (21), using the idiotroph of Streptomyces fradiae (22) as indicated in fig. 5. We were unable to transform 3,5-dideoxy-fortamine 19 into bioactive product using the same mutant. Positive result experienced previously with neamine (23) - using idiotroph of S.rimosus forma paromomycinus (24) - encouraged us to attempt the bioconversion of some of our synthetic pseudodissacharides depicted in Figure 9, but until now, no bioactive products could be obtained.

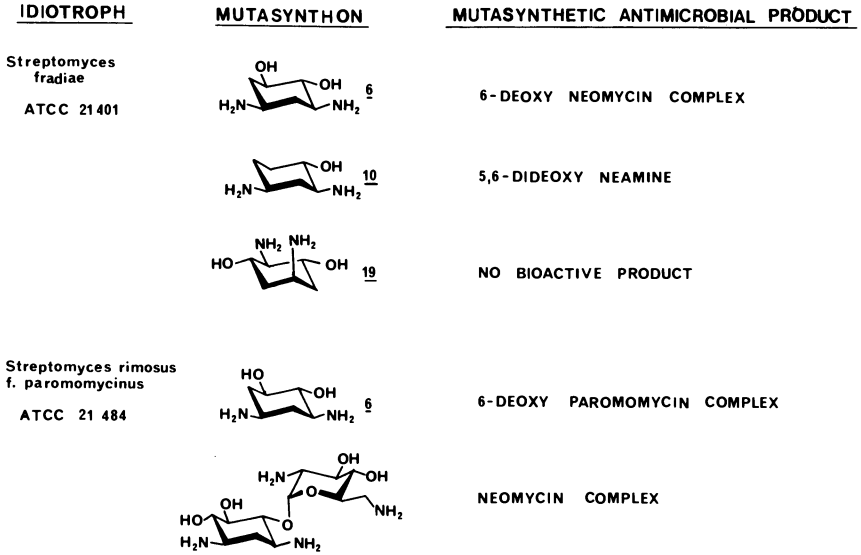
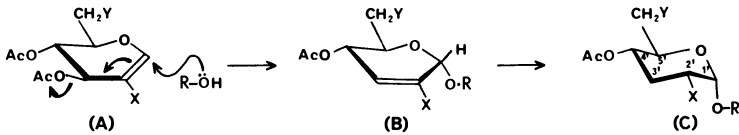


Figure 5.



X = OAc, H, F

Y = OAc, OTs

R = CYCLITOL and AMINOCYCLITOL

Figure 6.

TOTAL SYNTHESIS OF PSEUDODISACCHARIDES RELATED TO AMINOGLYCOSIDE ANTIBIOTICS

All of the clinically important aminoglycoside antibiotics are produced either by fermentation or by chemical modification of the natural products (Fig.1).

To produce pseudosaccharides differing greatly from the fermentation products, we decided, having a selection of potentially useful aglycon handles, to construct α -glycosides related to pseudosaccharides units of the natural products, by strictly synthetic means.

The long standing problem of 1,2-cis-glycoside synthesis (25, 26, 27, 28) was, at the outset of our work, our major concern. It was essential that any synthetic approach adopted should produce an α -glycoside linkage with high stereospecificity. The method developed in our laboratory and described here not only fulfils this requirement but in addition simultaneously yielded deoxygenated products at the C-3' position, a feature that is necessary for the avoidance of a major pathway of enzymatic inactivation, as well as for enhanced biological activity.

The scheme in figure 6 sets out the basic two step reaction sequence : the first step is a "quasi SN_2 ," reaction consisting of an acidcatalysed addition of an alcohol (R-OH) to the glycal (A) with allylic rearrangement ; this type of reaction has been extensively studied using simple alcohols (29, 30). The second step involves the hydrogenation of the double bond in compound (B). If (B) could be reduced with high regiospecificity from the β face, the resulting product (C) would be the required 3'-deoxy α -glycoside having the natural D-ribo-configuration.

For our purpose, the group R in the scheme needs to be a suitably protected aminocyclitol unit or some easily modified precursor of such a molecule. The chiral ditosyl-cyclohexanetetrol 5 and ditosyl-cyclohexanetriol 9 are such precursors. In particular 5 can be selectively substituted at either hydroxyl group. The reaction of 5 with benzoyl chloride in the presence of imidazole gave the benzoate 21, while with t-butyl dimethyl silyl chloride the silyl ether 22 was obtained.

As a typical example, we give here details of the synthesis of 5-O-(3'-deoxy- α -D-ribohexopyranosyl)2,6-dideoxy streptomycin 30 (31) using compound 21 and the glycol 23 (Fig. 7 and 8). A dichloroethane solution of compound 23 is added to a dichloroethane solution of 21 (1 equiv.) containing a catalytic amount of boron-trifluoride-ether at -20°C over 15 min. The reaction mixture is maintained at -15°C for another 2 h. After extraction a mixture of two products is obtained in 94 % yield. The major component (82 %) was isolated by a single crystallisation from alcohol. ^1H NMR data suggested that this compound was the α -glycoside 24 ($J_{1'-3'}$, 0.5, $J_{4'-5'}$, 9 Hz). The β anomer 25 was formed in 12 % yield ($J_{1'-3'}$, 0.6 ; $J_{4'-5'}$, 4.5 Hz). The α -glycoside 24 was regiospecifically hydrogenated or deuteriated in quantitative yield using 10 % palladium on carbon in ethylacetate in the presence of a trace of glacial acetic acid to compounds 26 and 26' respectively. As indicated by the ^1H NMR data obtained for 26 ($J_{1'-2'}$, 5 Hz) and especially for the dideuterio compound 26' ($J_{3'-4'}$, 10 Hz), the reduction occurred exclusively from the β face of the molecule, there was no evidence for the formation the D-arabino isomer. In contrast, catalytic reduction of the β -glycoside 25 using the same conditions, proceeded sluggishly yielding two products in poor yield which were

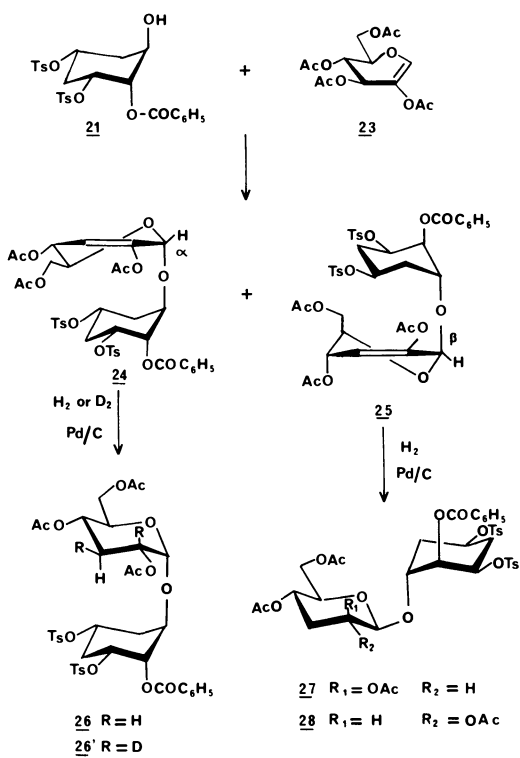


Figure 7.

characterised as compounds 27 (30 %) ($J_{1,-2}$, 1.5 Hz) and 28 (18 %) ($J_{1,-2}$, 9 Hz). In addition some hydrogenolysed products were formed which were not examined.

Azidolysis of 26, using sodium azide in N,N-dimethyl formamide at 110°C over 2h gave a mixture of three products in 81 % yield which were separated by silica gel chromatography. The major component (51 %) was identified as 29. The two minor components arose by elimination of toluene-p-sulphonic acid. Saponification of 29 followed by reduction in the presence of PtO₂ in methanol-water (1:1) gave compound 30. (Fig. 8)

Using a variety of glycals and cyclitol derivatives, pseudodisaccharides depicted in Fig. 9 have been prepared. These products are related to the 4-0-substituted 2-deoxystreptamine glycosides (Fig. 2) which represented the minimum requirement for antimicrobial activity. The yield of the α -glycosylation procedure varies between 65-90 % and depends on the nature of the glycals and aglycones used in the reaction. Especially, pseudodisaccharides with a range of groups at the strategic 2' and 6' positions including 2',3' di-deoxy and 2'-fluoro pseudodisaccharides have been synthesised (16, 31, 32).

The extension of this α -glycosylation procedure for the synthesis of a pseudotrisaccharide 33 (33) (Fig. 10), related to ribostamycin and the butirosins was also investigated. Since there are many effective antibiotics of this class containing a β -D-ribose group at the 5 position ; we first prepared the 5-0-D-ribose derivative 31 from compound 5 by condensation with tri-0-benzoyl- β -D-ribofuranosyl chloride in the presence of mercury (II) bromide and molecular sieves (4Å) under reflux over 8 hours (33). The latter on treatment with glycal 23 under the usual conditions

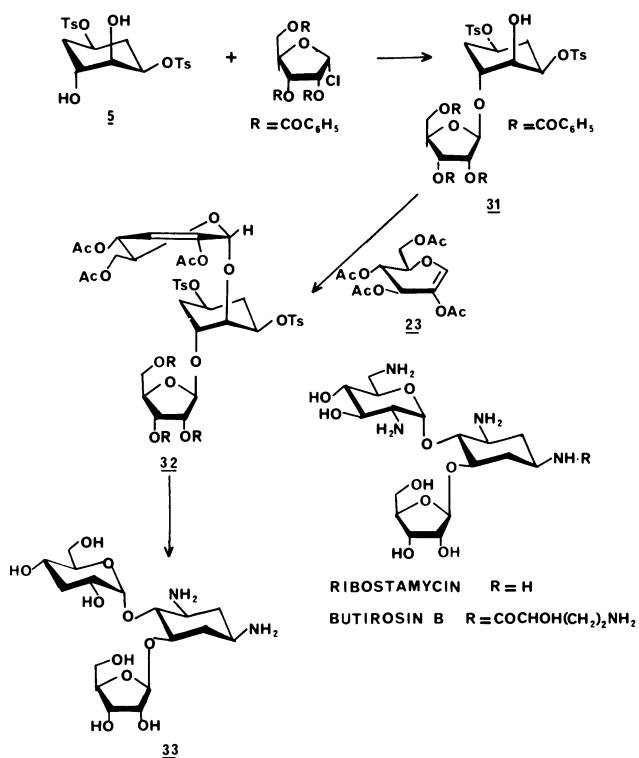


Figure 10.

afforded the unsaturated pseudotrisaccharide $\tilde{\tilde{32}}$, in 62 % yield. Using well established methods ; $\tilde{\tilde{32}}$ was converted to the trisaccharide $\tilde{\tilde{33}}$.

We feel that the discovery of novel and more efficient antibiotics for clinical use might be obtained by the combination of the mutasynthetic and the total synthetic methodology.

Despite that the yield of biotransformation using idiotrophs is extremely low, mutasynthesis might provide a rapid information concerning the impact exerted by the mutasynton on the antibacterial activity. The results of our studies indicated that the removal of hydroxy groups at C-6 or at C-5 and C-6, does not affect greatly the biological properties. The microbial spectra of 6-deoxyneomycins and 5.6-dideoxyneamine were very similar to that of neomycins and neamine, respectively.

Total chemical synthesis allows the introduction of a variety of functional groups as summarized in Figure 9 and we hope that our methodology will lead to novel type of bioactive substances.

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Literature Cited

- 1 Umezawa, S. ; Advan.Carbohyd.Chem.Biochem., 1974, 30, 111.
- 2 Cox, D.A. ; Richardson, K. ; Ross, B.C. ; Topics in Antibiotic Chemistry, Sammes P.G. Ed., Ellis Horwood Limited, England, 1977, 1, 1.

- 3 Nara, T., Annual Reports on Fermentation Processes, Perlman, D., Academic Press 1977 ; 1, 299.
- 4 Okamoto, S., Suzuki, Y., Nature, 1965, 108, 1301.
- 5 Umezawa, H., Advan.Carbohyd.Chem.Biochem., 1974, 30 183.
- 6 Umezawa, H., Drug Action and Drug Resistance in Bacteria, Mitsuhashi, S., Ed. University Park Press, Tokyo, 1975, 2, 211.
- 7 Davies, J., Smith, D.J., Ann.Rev. of Microbiol., 1978, 32, 464.
- 8 Umezawa, H. ; Umezawa, S., Tsuchiya, T., Okazaki, Y., J. Antibiotics, 1971, 24, 485.
- 9 Kawaguchi, H. ; Naito, T. ; Nakagawa, S., Fujisawa, K., J. Antibiotics, 1972, 25, 695.
- 10 Wright, J.J. ; J.C.S. Chem. Comm., 1976, 206.
- 11 Richardson, K.; Jevons, S., Moore ; J.W., Ross ; B.C., Wright, J.R. J.Antibiotics, 1977, 30, 843.
- 12 Nagabhushan, T.L. ; Cooper, A.B., Tsai, H. ; Daniels P.J.L., Miller G.H. ; J.Antibiotics, 1978, 31, 681
- 13 Kavadias, G. ; Velkof, S., Belleau, B. ; Can.J.Chem. 1978, 56, 404.
- 14 Suami, T. ; Ogawa, S. ; Uchino, H. ; Funaki, Y. ; J.Org.Chem., 1975, 40, 456.
- 15 Ogawa, S., Veda, T., Funaki, Y., Hongo, Y., Kasuga, A., Suami, T., J.Org.Chem., 1977, 42, 3083
- 16 Cleophax, J. ; Géro, S.D. ; Leboul, J. ; Akhtar, M. ; Barnett, J.E.G. ; Pearce, C.J., J.Amer.Chem.Soc., 1976, 98, 7110.
- 17 Rolland, A. ; Thesis, University Paris-Sud, Orsay, 1978.
- 18 Ogawa, S., Hongo, Y., Fujimori, H., Iwata, K., Kasuga, A., Suami, T., Bull.Soc.Chem.Japan, 1978, 51, 2957.

- 19 Egan, R.S. ; Stanaszek, R.S. ; Cirovic, M. ; Mueller, S.L. ; Tadanier, J. ; Martin, J.R. ; Collum, P. ; Goldstein, A.W. ; De Vault, R.L. ; Sinclair, A.C. ; Fager, E.E. ; Mitscher, L.A. ; J.Antibiotics, 1977, 30, 552.
- 20 Rinehart, Jr., K.L., Pure Appl.Chem., 1977, 49, 1367.
- 21 Sepulchre, A.M. ; Quiclet, B. ; Colas, C.; Rolland, A. ; Cleophax, J., Pearce, C.J., Géro, S.D., Abst 18th Int.Conf.Antimicrob.Agents Chemother. Atlanta, 1978, N°172.
- 22 Shier, W.T. ; Rinehart, Jr., K.L. ; Gottlieb, D., Proc.Nat.Acad.Sci., U.S.A., 1969, 63, 198.
- 23 Pearce, C.J. ; Barnett, J.E.G. ; Anthony, C. ; Akhtar, M. ; Géro, S.D., Biochem.J., 1976, 159, 601.
- 24 Shier, W.T. ; Schaefer, P.C. ; Gottlieb, D., Rinehart, Jr., K.L., Biochem., 1974, 13, 5073.
- 25 Lemieux, R.U., James, K., Nagabhushan, T.L., Can. J.Chem., 1973, 51, 42.
- 26 Wulff, G., Röhle, G., Angew.Chem.Int.Ed., 1974, 13, 157.
- 27 Lemieux, R.U., Hendriks, K.B., Stick, R.V., James, K., J.Amer.Chem.Soc., 1975, 97, 4056.
- 28 Pougny, J.R., Nassr, M.A.M., Naulet, N., Sinaÿ, P., Nouv.J.Chim., 1978, 2, 389.
- 29 Ferrier, R.J., Advan.Carbohydr.Chem.Biochem., 1969, 24, 199
- 30 Ferrier, R.J., Fortschr.Chem.Forsch., 1970, 14, 389.
- 31 Cleophax, J., Do Khac Duc, Delaumény, J.M., Géro, S.D., Rolland, A., J.C.S.Chem.Comm., 1978, 771.
- 32 Vass, G., Rolland, A., Cleophax, J., Mercier, D., Quiclet, B., Géro, S.D., J.Antibiotics, 1979, 32, 670.
- 33 Cléophax, J., Delaumény, J.M., Géro, S.D., Rolland, A., Rolland, N., J.C.S.Chem.Comm., 1978, 773.

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Multiple Interactions of Aminoglycoside Antibiotics with Ribosomes

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Streptomycin (Str) has been studied longer than any other antiribosomal antibiotic, yet until relatively recently the mechanism of its bactericidal action has been quite unclear. One reason is that this antibiotic exerts an unusually wide variety of effects, some of which are mutually exclusive. In addition, for many years protein synthesis could be analyzed *in vitro* only with synthetic polynucleotide messengers, which bypass physiological initiation; and though this system was able to reveal the key actions of those antibiotics that act on chain elongation (e.g., puromycin, chloramphenicol, tetracycline), it failed to reproduce the blockade of protein synthesis by Str that is observed in cells. Only after physiologically initiating messenger became available, in the form of the RNA of small RNA phages, could this blockade be achieved *in vitro* and its mechanism studied. Finally, the isolation of purified polysomes, free of initiation factors, made possible reliable comparison of effects on initiating and on non-initiating, chain-elongating ribosomes; and the different effects of Str on these two systems explained earlier contradictions in its actions.

In this long development studies on Str have shed light on many features of the ribosome; they have revealed several mechanisms that have served as models for the action of other antibiotics. 1) First, aminoglycosides were found to affect the accuracy as well as the rate of translation; and the recognition of this misreading effect had broad reverberations: it showed that an antibiotic can act by distorting the ribosome and not simply by blocking an active site, and this knowledge then led to the recognition that mutations can increase or decrease translational fidelity. 2) Str was also the first antibiotic for which a mutation to resistance was traced to the ribosome, and then to a specific ribosomal protein (S12). 3) Str also selects for dependent (str^D) mutants, which can grow only in the presence of Str; and this remarkable phenomenon (later extended to other antibiotics) was explained as a mutational distortion of the ribosome that could be compensated for by the opposite distorting effect

of the antibiotic. 4) Studies with Str focused attention on the different range of conformations of free (and hence initiating) ribosomes, compared with ribosomes carrying the ligands involved in chain elongation: Str was the first antibiotic shown to block only initiating ribosomes. This finding was extended to other classes of antibiotics besides aminoglycosides (kasugamycin, spectinomycin, erythromycin), but aminoglycosides were found to be unique in having an additional set of actions on chain-elongating ribosomes. 5) The subtlety of ribosome-antibiotic interactions was further shown by the finding that the interference with initiating ribosomes is not restricted to inhibition of initiation complex formation (as is observed with kasugamycin); Str and certain other antibiotics bind to free ribosomes, allow them to form initiation complexes, and then prevent these from continuing into the cycles of chain elongation. 6) The initiation complexes blocked by various antibiotics are all unstable: they spontaneously release the ribosomes from the mRNA after a few minutes, in contrast to the striking stabilization of polysomes by inhibitors of chain elongation; and the released ribosome can form a blocked initiation complex again. 7) This cyclic polysomal blockade is of particular interest because it accounts for the dominance of sensitivity over resistance, in heterozygotes containing both sensitive and resistant ribosomes.

We shall consider briefly some of the key findings, which have been reviewed in greater detail elsewhere (1,2,3,4). We shall also note that Str, the prototype aminoglycoside, is atypical in having only a single binding site. Other aminoglycosides, with a larger number of cationic groups, have multiple sites; and gentamicin has the interesting paradoxical effect that binding to the second site decreases the inhibitory effect of first-site binding and increases the misreading.

The Misreading Effect

The action of Str and aminoglycosides in promoting misreading on the ribosome was discovered through the ability of these antibiotics to cause phenotypic suppression of certain auxotrophic mutations, i.e. to cause errors in translation that reverse or compensate for genetic errors, and thus restore, at a low level, synthesis of the missing enzyme (5). The inferred misreading was soon verified in vitro with a synthetic homopolynucleotide messenger, poly U (6), and it has recently been directly demonstrated in cells by showing the incorporation into flagellin of a normally absent amino acid, cysteine (7).

This subject has been extensively reviewed, and we shall note only a few key points. The induced errors were found to involve incorporation of amino acids whose codons differed from the correct one by a single base (8). Moreover, in the cell most phenotypic suppression by Str involves correction of a termination (= nonsense) mutation within a gene (9), i.e., the distortion

of the ribosome by the antibiotic favors the reading of a termination codon by an incorrect aminoacyl-tRNA, rather than by the correct protein termination factor. Curiously, though phenotypic suppression by Str was discovered in a Str-resistant (Str^R) strain, the misreading of polynucleotides in vitro could be demonstrated only with sensitive (Str^S) ribosomes (5,6,8), which are evidently more severely distorted by Str than are Str^R ribosomes.

The misreading effect of Str first aroused interest in the study of factors affecting the fidelity of translation. This aspect of information transfer has become a topic of deep interest, closely paralleling the study of errors in DNA replication (mutations) and in transcription. As in the latter areas, it is clear that evolution does not select for maximal accuracy, which is evidently too expensive: it is possible to select not only for mutants with increased ribosomal ambiguity (ram), which mimic the action of Str (10), but also for mutants (str^R, str^D) that have a more restricted rather than a looser codon-anticodon fit, resulting in a decrease in background misreading of polynucleotides. This model of closer or looser codon-anticodon fit is supported by the finding that ram mutations can replace the addition of Str in supporting the growth of str^D mutants; moreover, the double mutation is associated with less ambiguity than ram alone (9).

Though the misreading effect of Str thus revealed profound new features of ribosome-antibiotic interaction, it did not provide the key to the complete, irreversible inhibition of protein synthesis that is associated with its characteristic bactericidal action on cells. The formation of error-filled proteins clearly is not the basis for this action: ram mutations, with a high error frequency, are not lethal; and in the presence of puromycin, which prevents accumulation of long chains (but allows ribosome turnover on mRNA), the bactericidal action of Str not only is not prevented but is even enhanced.

The Blockade of Initiation Complexes

As was noted above, the blockade of protein synthesis by Str in vitro was first achieved with ribosomes initiating on viral RNA (11). However, further studies showed that the antibiotic does not prevent formation of initiation complexes. Instead, it interacts with free ribosomes (or their initiating subunits) in a way that allows initiation complexes to form; but these complexes are distorted, and they not only fail to continue into chain elongation, but they spontaneously dissociate, with a half-life at 37°C of 3-5 min (12,13).

In cells the ribosomes released from the blocked initiation complexes rapidly reinitiate. Thus cells killed by Str, and no longer synthesizing protein, nevertheless maintain a significant level of polysomes; and these turned out to be not ordinary polysomes, carrying chains of varying length: they are unstable,

cyclically renewed, blocked polyinitiation complexes (14). Thus these polysomes could be labeled with the initiating amino acid methionine, but not with other amino acids. Moreover, the mRNA was constantly turning over, as shown by pulse-labeling with [³H]uracil. Turnover was also shown by disappearance of the polysomes when the renewal of RNA was blocked by rifampicin, or when the formation of fMet-tRNA was blocked by trimethoprim.

Because the blockade of initiation by Str is cyclic (i.e., the attachment of the Str-ribosome complex is not permanent), a heterozygote will always have sensitive ribosomes available to block any new mRNA. The resultant interference with the resistant ribosomes explains (14) why sensitivity to Str is dominant over resistance, in terms not only of inhibition of growth (15) but also of bactericidal action (16).

The Effects on Chain-elongating Polysomes

The double action of Str on cells -- misreading at low concentrations but complete inhibition at high concentrations -- was thus paralleled in vitro. Yet there was a paradox: with synthetic messenger only misreading was observed over a wide concentration range, and with initiating natural messenger only inhibition, over the same range. The two effects in cells thus could not be readily interpreted in terms of the amount bound per ribosome. Moreover, in vitro studies revealed binding of only one molecule per ribosome.

The paradox was resolved by developing a system that could complete already initiated chains, without reinitiation, on natural mRNA. Such a system was developed on the basis of the known distribution of the initiation factors (IF) in bacterial lysates. In the macrocycle of protein synthesis free 70S ribosomes are released, at termination of a polypeptide; these ribosomes dissociate into a 30S and a 50S subunit, by complexing the former with three IFs; and these "native" subunits form initiation complexes at specific ribosome binding sites at the beginning of a gene transcript in mRNA. The IFs are found only on the 30S native ribosomal subunits: during formation of the initiation complexes they are released and rapidly reattach to the 30S subunits of the free ribosomes released at the termination of a round of translation. The polysomes in bacterial lysates could thus be freed from IFs, and hence prevented from reinitiating, by being carefully separated from the native subunits, either by zonal centrifugation or by gel filtration. Such purified polysomes were found to carry out protein synthesis briskly but only for a few minutes, during which they completed and released their nascent chains but did not reinitiate (17).

This chain elongation was slowed (but not halted) by Str, to a degree that decreased markedly with Mg²⁺ concentration but was constant over a very wide range of Str concentration (18). This behavior closely paralleled the translation of synthetic messen-

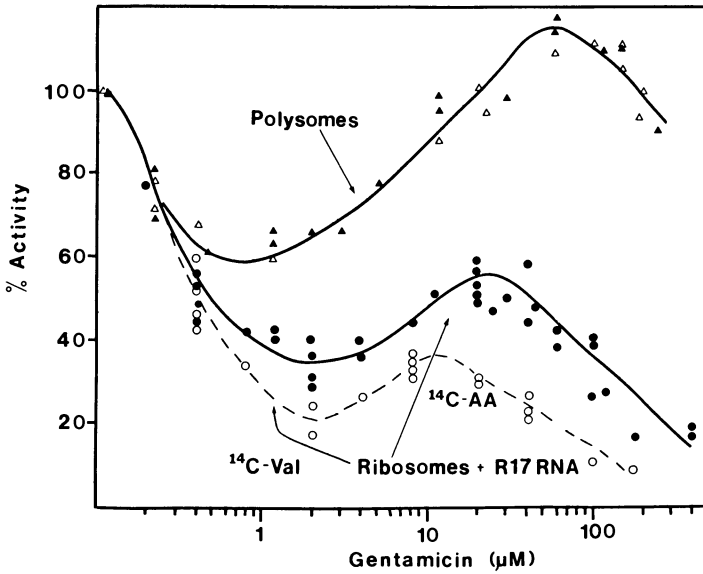
gers; and the slowing clearly showed that the Str was interacting with the chain-elongating ribosomes. It thus seemed possible that Str might be distorting these ribosomes, though less drastically than free ribosomes, causing slowing and increased error frequency but not cessation of protein synthesis. This hypothesis was readily confirmed by providing purified, non-initiating polysomes with an incomplete set of amino acids (1), or even better by achieving absolute deprivation of glutamyl-tRNA, by use of extracts of a mutant with a temperature-sensitive glutamyl-tRNA synthetase (19): in the absence of Str these incomplete systems virtually ceased protein synthesis, while in the presence of Str the synthesis was nearly as extensive and rapid as in the similarly treated complete system.

These findings have several implications. By reproducing the misreading effect with a physiological system they increase confidence in the relevance of the earlier findings with synthetic messengers such as poly U, which function only at abnormally high Mg^{2+} concentrations. Moreover, they have closed an earlier gap in the evidence by demonstrating misreading with Str^R as well as with Str^S ribosomes (19). But most important is that we finally have an explanation for the paradox of both misreading (which requires continued synthesis) and blockade of synthesis by an antibiotic that can bind only one molecule per ribosome. Evidently at high enough concentrations Str soon blocks all the initiation sites; while at low concentrations most free ribosomes have not contacted Str and initiate normally. Under the latter circumstances Str will encounter chain-elongating ribosomes much more frequently than free ribosomes, since the former constitute about 90% of the total population of ribosomes in cells in steady-state growth. In a word, Str exerts two mutually incompatible effects, at different concentrations, by binding predominantly to one or another of two different pools of ribosomes: free and complexed.

Triphasic Concentration Effects of Gentamicin

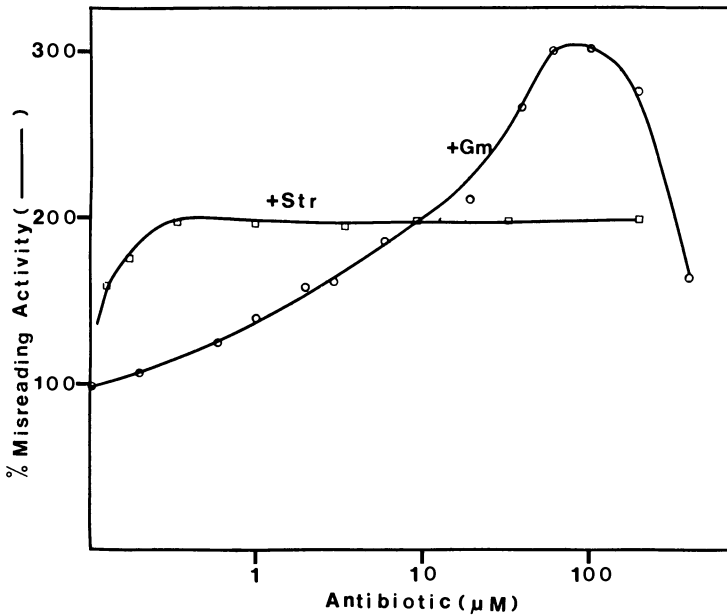
Because Str binds only one molecule per ribosome, the mutually exclusive effects of low and of high concentrations long presented an interesting paradox. However, the binding of only one molecule turned out to be atypical among aminoglycosides. Moreover, in recent studies with natural messengers (21,22) the multiple binding of gentamicin has been found to lead to another paradox: a triphasic concentration-action curve, in which the degree of inhibition (of either initiating or non-initiating systems) rises, falls, and rises again with increasing concentrations (Figure 1). Moreover, in the range where the inhibition decreases the frequency of misreading increases (Figure 2).

While the concentration-action curve for Str fits the mass law applied to a single site, the broad concentration range of both effects in the first two phases of gentamicin action clearly implies binding to several sites. The first causes mostly in-



Biochemistry

Figure 1. Effect of gentamicin (Gm) concentration on protein synthesis. For chain elongation on endogenous polysomes the reaction mixtures contained purified IF-free polysomes, with either 20 ^{14}C -labeled amino acids (\blacktriangle) or [^{14}C]valine and 19 other unlabeled amino acids (\triangle). For the translation of phage R17 RNA the mixtures contained ribosomes, IF, R17 RNA, and either 20 ^{14}C -labeled amino acids (\bullet) or [^{14}C]valine plus unlabeled amino acids (\circ) as above, with Gm as indicated (21).



Biochemistry

Figure 2. Stimulation of misreading by Gm. The reaction mixtures were as in Figure 1 (with purified polysomes) except that S100 from a mutant with temperature-sensitive Glu-tRNA synthetase was used, with Gm (○) or Str (□) at the concentration indicated (21).

hibition, while the second partly reverses the inhibition but has a strong misreading effect. (The third phase, at very high concentrations, does not alter the misreading, which suggests that it may involve a non-specific effect, perhaps even on components of the system other than the ribosome.) Furthermore, a mutation in ribosomal protein L6 yields ribosomes that retain the inhibitory effect but have lost the misreading effect (22). A triphasic effect on protein synthesis is similarly observed, though less distinctly, with neomycin, kanamycin, and paromomycin (our unpublished data). Furthermore, Zierhut et al. (23) have recently observed a similar effect with additional aminoglycosides that also contain a diglycosylated 2-deoxystreptamine.

The existence of multiple binding sites for gentamicin had been suggested earlier by the observation, with synthetic messenger, that its misreading effect varies over a broader concentration range than that of Str; it also causes more intense misreading (8). Moreover, multiple binding sites may also explain why gentamicin, unlike Str, does not select for 1-step mutants with high-level resistance (8). S. Perzynski and J. Davies (personal communication) have recently demonstrated cooperative binding of 3-5 molecules of radioactive gentamicin to each ribosome.

Remaining Problems

The recognition of the different effects of aminoglycosides on free and on complexed (polysomal, chain-elongating) ribosomes has considerably clarified the complex actions of these antibiotics. Since a single mutation to Str resistance markedly reduces both major effects it appears that both depend on binding to the same site, but that in chain-elongating ribosomes the conformational restrictions imposed by the other ligands reduce the distortion of the ribosome by the antibiotic, perhaps by reducing the accessible fraction of the site. Identification of the binding sites for aminoglycosides offers an interesting challenge: all the members of this group have similar actions (including both misreading during chain elongation and a blockade of initiation complexes), yet their binding sites must have distinctive features, since most members do not show cross-resistance.

Initiation complexes can evidently be blocked in different ways by different antibiotics. Thus spectinomycin (an aminocyclitol but not an aminoglycoside) also binds to 30S subunits, but it does not cause misreading, and its blockade of initiation complexes is reversible (i.e., its action on the cell is bacteriostatic and not bactericidal) (24). Erythromycin binds to the 50S rather than the 30S subunit (25). The details of these several blocks somewhere between initiation and chain elongation remain to be elucidated.

Early studies showed that Str not only inhibits protein synthesis but causes early impairment of the integrity of the cell

membrane (26). The basis for this effect, and the mechanism of uptake of Str, are still unclear; but the effect on the membrane is evidently linked to action on the ribosome, since the damage is prevented by Str^R mutations that alter a ribosomal protein. It may now be worthwhile to reinvestigate the problem of membrane damage with the many new technics that are now available for studying membrane structure. In addition, the recent demonstration that bacteria have functionally significant membrane-bound ribosomes, involved in the synthesis of membrane proteins and secreted proteins (27), suggests a possible basis for an effect of Str on the membrane via attached ribosomes.

One of the special features of Str is the selection of 1-step mutants with a very high level of resistance (increased 100-fold or more). A similar effect has been observed with several other ribosomal antibiotics (erythromycin, spectinomycin, and kasugamycin), but not with many others (e.g., tetracycline, chloramphenicol, sparsomycin). Curiously, the former group all block only initiating ribosomes, while the latter can block chain-elongating ribosomes. This correlation suggests an important difference in the binding sites of the two classes: an antibiotic that blocks chain elongation must find its binding site on some conformation of the ribosome within the limited range permitted by the ligands present during chain elongation, while an antibiotic that acts only on free (initiating) ribosomes clearly binds to a site made available in some additional, "optional" conformation. If the former conformation is essential for function a mutation that eliminated it would inactivate the ribosome and hence be lethal; while mutations that eliminate an optional conformation evidently do not destroy function and hence can be recovered.

Recent advances in studies of ribosomal structure have provided information not only on the shape of the organelle and the topography of exposed regions of its component molecules, but also on some specific contacts between these molecules (28). The greatest further challenge in such studies would appear to be definition of the conformational changes (including changes in contacts) undergone by the ribosome during its cycle. The use of different antibiotics to fix the ribosome in different conformations will probably prove to be a useful tool for this purpose, especially in the light of the finding that Str makes the ribosome more rigid (29).

Literature Cited

1. Davis, B. D., Tai, P.-C, and Wallace, B. J., in "Ribosomes," Cold Spring Harbor Laboratory, 1974, p. 771.
2. Wallace, B. J., Tai, P.-C, and Davis, B. D., in Hahn, F. (ed.), "Antibiotics: Mechanism of Action." Springer, New York, 1979.
3. Pestka, S., in "Molecular mechanisms of protein biosynthesis." Weissbach, H. and Pestka, S. (eds.) Academic Press, New

- York, 1977, p. 467.
4. Vazquez, D. "Inhibitors of Protein Biosynthesis." Springer, New York, 1979.
 5. Gorini, L., and Kataja, E. Proc. Natl. Acad. Sci. USA (1964) 51:487.
 6. Davies, J., Gilbert, W., and Gorini, L. Proc. Natl. Acad. Sci. USA (1964) 51:883.
 7. Edelman, P., and Gallant, J. Cell (1977) 10:131.
 8. Davies, J., Gorini, L., and Davis, B. D. Molec. Pharmacol. (1965) 1:93.
 9. Gorini, L., in "Ribosomes," Cold Spring Harbor Laboratory, 1974, p. 791.
 10. Rosset, R., and Gorini, L. J. Mol. Biol. (1969) 39:95.
 11. Anderson, P., Davies, J., and Davis, B. D. J. Mol. Biol. (1967) 29:203.
 12. Modolell, J., and Davis, B. D. Proc. Natl. Acad. Sci. USA (1970) 67:1148.
 13. Lelong, J. C., Cousin, M. A., Gros, D., Greenberg-Manago, M., and Gros, F. Biochem. Biophys. Res. Comm. (1971) 42:530.
 14. Wallace, B. J., and Davis, B. D. J. Mol. Biol. (1973) 75:377.
 15. Lederberg, J. J. Bacteriol. (1951) 61:549.
 16. Sparling, P. F., and Davis, B. D. Antimicrob. Agents Chemother. (1972) 1:252.
 17. Tai, P.-C., Wallace, B. J., Herzog, E. L., and Davis, B. D. Biochemistry (1973) 12:609.
 18. Wallace, B. J., Tai, P.-C., Herzog, E. L., and Davis, B. D. Proc. Natl. Acad. Sci. USA (1973) 70:1234.
 19. Tai, P.-C., Wallace, B. J., and Davis, B. D. Proc. Natl. Acad. Sci. USA (1978) 75:275.
 20. Chang, F. N., and Flaks, J. G. Antimicrob. Agents Chemother. (1972) 2:294.
 21. Tai, P.-C., and Davis, B. D. Biochemistry (1979) 18:193.
 22. Kuhberger, R., Piepersberg, W., Petzet, A., Buckel, P., and Bock, A. Biochemistry (1979) 18:187.
 23. Zierhut, G., Piepersberg, W., and Bock, A. Eur. J. Biochem. (1979), in press.
 24. Wallace, B. J., Tai, P.-C., and Davis, B. D. Proc. Natl. Acad. Sci. USA (1974) 71:1634.
 25. Tai, P.-C., Wallace, B. J., and Davis, B. D. Biochemistry (1974) 13:4653.
 26. Anand, N. and Davis, B. D. Nature (1960) 185:22.
 27. Smith, W. P., Tai, P.-C., Thompson, R. C., and Davis, B. D. Proc. Natl. Acad. Sci. USA (1977) 74:2830.
 28. Wittmann, H. G., in "Concepts of chemical recognition in biology." Chapeville, F., and Haenni, A. L. (Eds.) Springer-Verlag, Heidelberg, 1979, in press.
 29. Miskin, R., and Zamir, A. J. Mol. Biol. (1974) 87:135.

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