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RECENT ADVANCES IN THE SYNTHESIS OF AMINOGLYCOSIDE ANTIBIOTICS

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Abstract - Aminoglycoside antibiotics have established an important position in medicine and synthetic chemistry in this field has recently been developed. A review is provided of general synthetic schemes which we have developed for the total synthesis of streptomycin, dihydrostreptomycin, kanamycins, butirosin B, neomycin C, and others. Resistant bacteria are a serious subject in present chemotherapy and, the mechanisms of resistance have recently been revealed by medical microbiologists. In the light of these mechanisms, an effort has been made to synthesise compounds, active against resistant bacteria, by extension of the above mentioned synthetic schemes and by derivation from natural antibiotics.

INTRODUCTION

The chemistry of aminoglycoside antibiotics originated with the isolation of streptomycin from the culture of Streptomyces griseus by Waksman and his co-workers in 1944. Streptomycin was remarkably active against gram-negative bacteria and this observation was especially encouraging since penicillin and sulfonamide drugs were chiefly effective against gram-positive bacteria. Furthermore, streptomycin was remarkably effective in the treatment of tuberculous patients. This landmark was followed by the discovery of many allied antibiotics. Micro-organisms of Streptomyces spp. have been the most productive and the aminoglycosides from this source include neomycins (1949), kanamycins (1957), paromomycins (1959), spectinomycin (1961) and others. Furthermore, aminoglycosides have been obtained from cultures of Micromonospora, Nocardia, and bacteria; gentamicins (1963), sisomicin (1970), and fortimicins (1977) were obtained from Micromonospora, and butirosins (1971) were isolated from a Bacillus.

This large group of antibiotics is classified as aminoglycosides because they generally contain several amino groups in their glycosidic moieties.

Various aminoglycoside antibiotics have been introduced into and have become established in chemotherapy, being especially useful for the treatment of serious gram-negative infections, although aminoglycoside antibiotics are potentially toxic to varying degrees.

The study of aminoglycoside antibiotics and other sugar-containing antibiotics has provided some of the most 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 ^{1}H- and ^{13}C-n.m.r. and mass spectroscopic techniques. During the past ten years, remarkable progress has been seen in the chemistry and biochemistry of aminoglycoside antibiotics.

(1) The challenge offered by the total synthesis of complex aminoglycoside antibiotics has been overcome, although many new structures attractive to synthetic organic chemists still remain to be synthesised.

(2) The biological mechanisms of inactivation of aminoglycoside antibiotics by resistant bacteria have been revealed.

(3) As a result of progress in (1) and (2) the way has been opened to the synthesis of new and improved antibiotics which are remarkably effective against micro-organisms resistant to the natural aminoglycoside antibiotics.

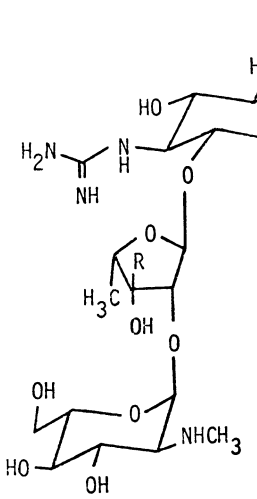
(4) Fermentation has been the major source of new aminoglycosides and many new structures have recently been revealed, including butirosins, validamycins (1971), apramycin (1973), minosaminomycin (1974), sorbistins (1976) and fortimicins. In addition, biotransformation using a "blocked mutant" is proving to be another fruitful source of structural variants of the aminoglycoside antibiotics (Ref. 1).

(5) Representatives of this group of antibiotics have also received attention from the viewpoint of biosynthesis (Ref. 2).

This paper is concerned with synthetic aspects. Since several excellent reviews (Refs. 3-6) of the chemistry of aminoglycoside antibiotics have recently appeared, I will emphasise our recent advances.

TOTAL SYNTHESIS OF AMINOGLYCOSIDE ANTIBIOTICS

In order to better appreciate the kind of challenges presented by aminoglycoside synthesis, let us examine several representative aminoglycoside antibiotics. The streptomycin group of antibiotics e.g. streptomycin (1) and dihydrostreptomycin (2), is characterised by the presence of L-streptose or its reduced form, L-dihydrostreptose. The aglycone is an aminocyclitol streptidine. These antibiotics contain 2-deoxy-2-methylamino-α-L-glucopyranoside moieties.



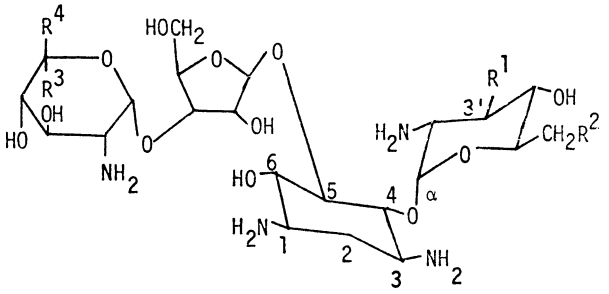
streptidine

streptose (R = CHO)

		R
(1)	Streptomycin	CHO
(2)	Dihydrostreptomycin	CH_2OH

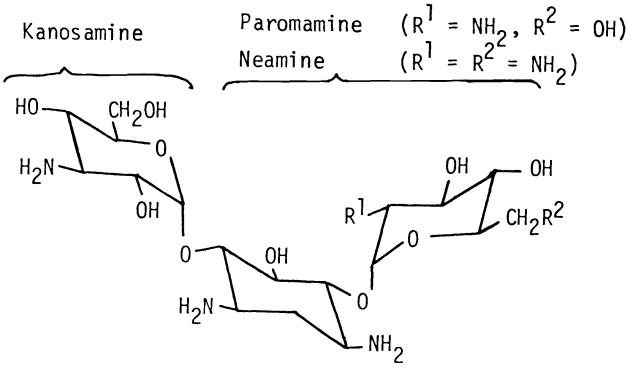
N-methyl-L-glucosamine

The neomycins (3,4), paromomycins (5,6), and lividomycin B (7) are closely related antibiotics. Neomycin was the first antibiotic of this group to be discovered. The neomycins differ in the configuration of position 5 in one of the 2,6-diamino-2,6-dideoxyhexopyranosyl moieties. Paromomycins differ from neomycins by the substitution of an amino group by a hydroxyl group. Lividomycin B corresponds to the 3'-deoxy derivative of paromomycin I . In these antibiotics, the two 2,6-diamino-2,6-dideoxyhexopyranosyl moieties are linked with the substituents at positions 1 and 2 in cis relationship (1,2-cis-pyranosides).



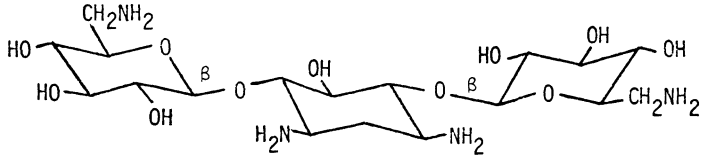
		R^1	R^2	R^3	R^4
(3)	Neomycin B	OH	NH_2	CH_2NH_2	H
(4)	Neomycin C	OH	NH_2	H	CH_2NH_2
(5)	Paromomycin I	OH	OH	CH_2NH_2	H
(6)	Paromomycin II	OH	OH	H	CH_2NH_2
(7)	Lividomycin B	H	OH	CH_2NH_2	H

In the kanamycins (8-10), the two amino sugars are attached to non-vicinal hydroxyl groups, namely at positions 4 and 6 of the 2-deoxystreptamine by α-D linkages. Kanamycins vary in the positions and number of the amino groups. The number of structurally elucidated aminoglycoside antibiotics of microbial origin is now about one hundred, and, among them, total syntheses of about fifteen have been accomplished.

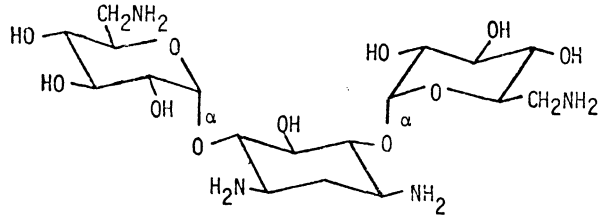


		R^1	R^2
(8)	Kanamycin A	OH	NH_2
(9)	Kanamycin B	NH_2	NH_2
(10)	Kanamycin C	NH_2	OH

My interest in this field of antibiotics dates back to structural studies of kanamycins in 1958. Since then, my associates and students have been interested in the synthesis of aminoglycosides in order to learn more about the structure-activity relationships. Initially we prepared the β,β-glycoside (11), an analog of kanamycin, from 6-amino-6-deoxy-D-glucose and 2-deoxystreptamine via the usual Koenigs-Knorr condensation, but this compound showed no antibacterial activity. This result suggested that the presence of the α-glycoside linkages in kanamycin might be essential for antibiotic activity. Several years later we (Ref. 7) synthesised the corresponding α,α-glycoside (12) which had fairly strong antibacterial activity.



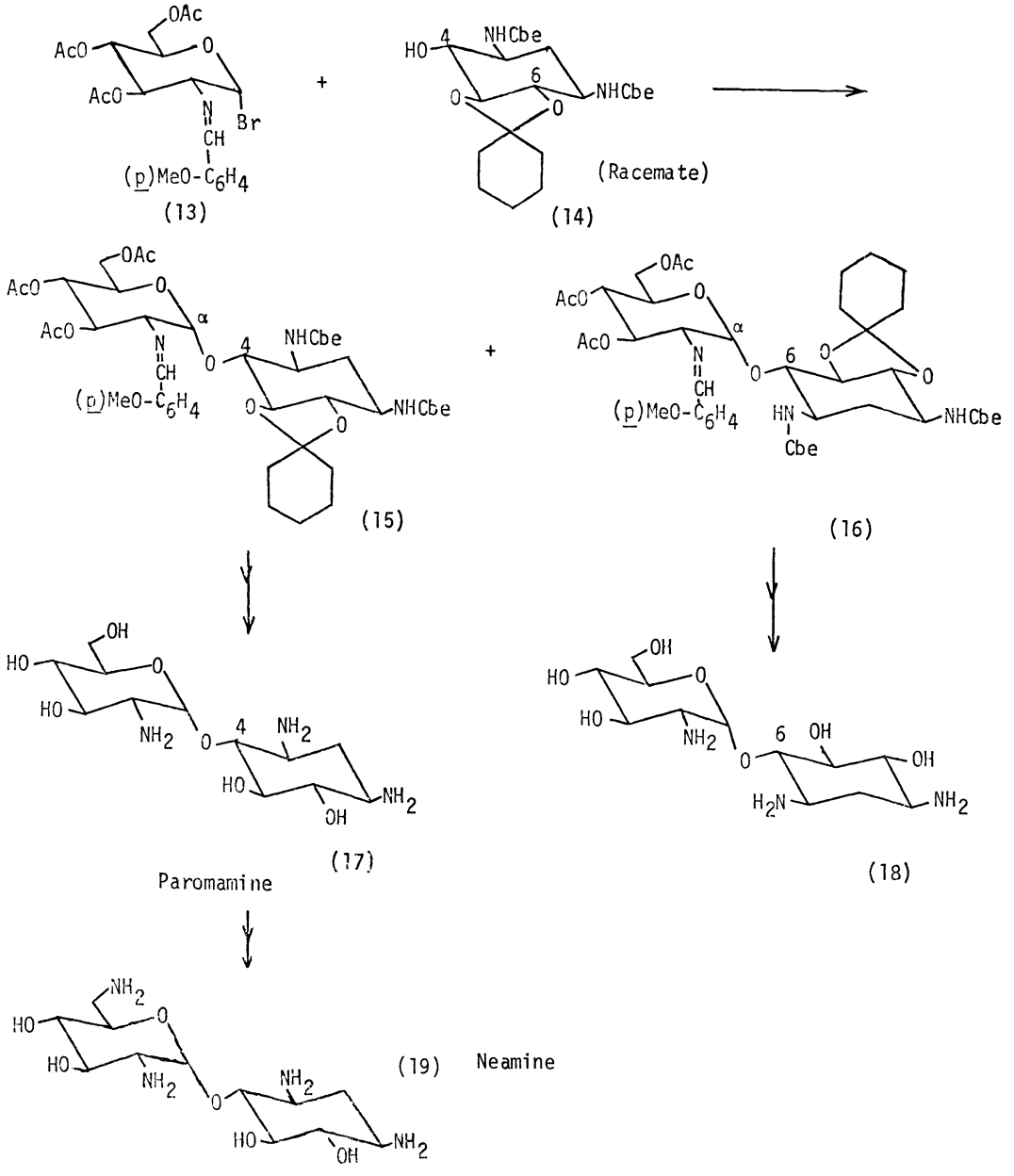
(11) β,β-glycoside : inactive



(12) α,α-glycoside : active

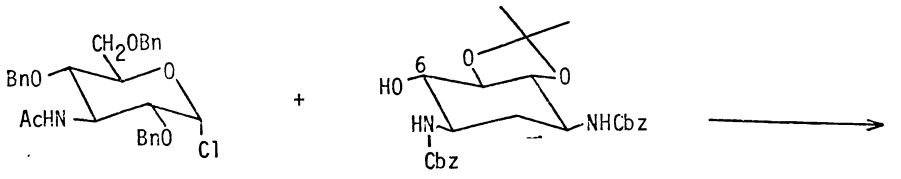
At that time, the preparation of α-D-glycopyranosides in high yields was an important problem in carbohydrate chemistry and the synthesis of aminoglycoside antibiotics involved the formation of such linkages and 1,2-cis-pyranosides. Recent progress in experimental techniques combined with an appreciation of the mechanisms of glycosidic bond formation has made available several useful procedures. Nevertheless, the Koenigs-Knorr condensation is still the most widely applicable to glycoside synthesis. The stereochemical control of the Koenigs-Knorr condensation is unreliable but it has become clear that the participation of the substituent at C-2 plays an important role. Thus, in the gluco series, the use of a non-participating C-2 substituent will favor the formation of α-glycopyranosides, although an appropriate catalyst and solvent must be used.

Our first targets were paromamine and neamine which are pseudodisaccharides. Paromamine (17) is a constituent of kanamycin C (10), paromomycins (5, 6), and many others. Neamine (19) is a constituent of neomycins (3, 4), kanamycin B (9), ribostamycin, butirosins, and many others. Both antibiotics were isolated from Streptomyces cultures.

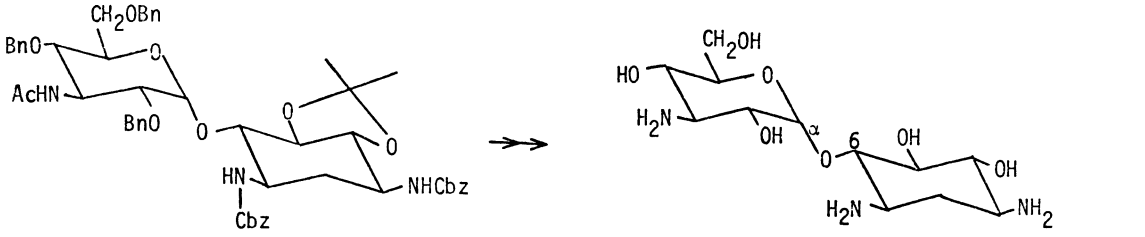


The synthesis of paromamine (Ref. 8) was carried out by the Koenigs-Knorr condensation of the 2-N-(2,4-dinitrophenyl) derivative of a glycosyl bromide with the bis-N-(2,4-dinitrophenyl) derivative of 2-deoxystreptamine in 1966. The use of dinitrophenyl group as a non-participating substituent at C-2 (Ref. 9) successfully gave α-glucopyranosides, and paromamine was obtained in low yield, because this reaction was not regioselective for the deoxystreptamine. However, this was the first synthesis of a naturally occurring aminoglycoside antibiotic. Several years later, we (Ref. 10) improved this synthesis by using a p-methoxy benzylidene derivative of 2-amino-2-deoxyglucose (13) (Ref. 11) and the 2-deoxystreptamine derivative (14); the total yield of α-glycosides was 85% and, since (14) was a racemate, the desired 4-O- and 6-O-α-glycosyl compounds (15, 16) were obtained in yields of 49 and 36%, respectively. Neamine (19) was derived from paromamine by selective tosylation of its primary hydroxyl group followed by azide displacement and catalytic reduction (Ref. 12). For amino sugars having a

hydroxyl group at C-2, the use of the benzyl group (Ref. 14) as a non-participating substituent at C-2 successfully gave α-glucopyranosides, as exemplified in the synthesis (Ref. 13) from (20) and (21) of 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (23) which is a pseudodisaccharide constituent of kanamycins. The foregoing modified Koenigs-Knorr reactions required strictly anhydrous conditions, therefore, we used carefully dried solvents and performed the condensations in a special dry room. Similar procedures were employed for the preparation of a number of related pseudodisaccharides.



(20) (21) Bn = CH_2Ph, Cbz = CO_2CH_2Ph

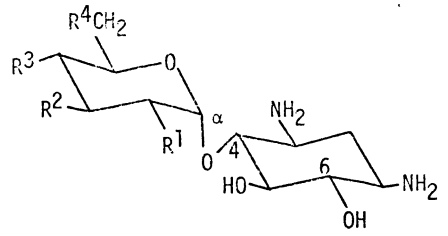


(22) (main product)

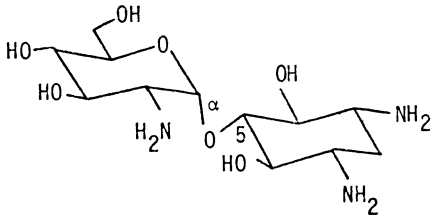
(23) 6-O-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine

The 2-amino-2-deoxy- and 6-amino-6-deoxyglucosyl compounds of 2-deoxystreptamine have antibacterial activity and 3-amino-3-deoxy and 4-amino-4-deoxy-glucosyl compounds (25 and 26) have no antibacterial activity. The α-D-glucosyl compound (27) was prepared by Lemieux and co-workers (Ref. 15) using the glycal-nitrosyl chloride procedure. We further prepared a number of 6-O-glycosyl compounds of deoxystreptamine and found that none of them had antibacterial activity.

5-O-Glycosyl compounds have also been synthesised . The 5-O positional isomer of paromamine (28) (Ref. 16) only had significant activity against Mycobacterium tuberculosis of the organisms tested.



	R^1	R^2	R^3	R^4	Activity
Paromamine:	NH_2	OH	OH	OH	+
Neamine:	NH_2	OH	OH	NH_2	+
(24)NK-1003	OH	OH	OH	NH_2	+
(25)*	OH	NH_2	OH	OH	-
(26)*	OH	OH	NH_2	OH	-
(27)*	OH	OH	OH	OH	-



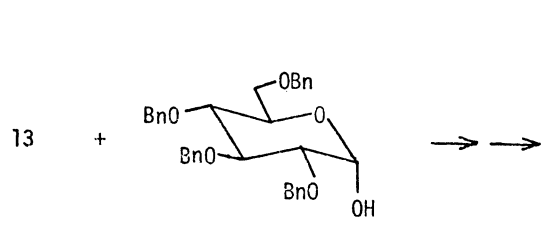
(28)* Isomer of paromamine: 5-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine. Activity + (TB)

\+ denotes that the aminoglycoside is active.

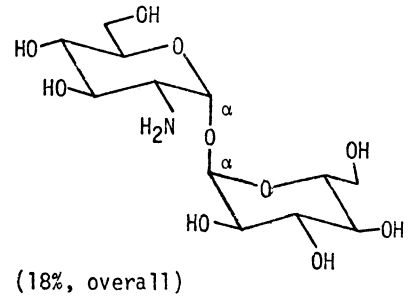
* unnatural compounds.

The above mentioned antibiotics are pseudodisaccharides; genuine disaccharide antibiotics have rarely been found. Trehalosamine (30) produced by a Streptomyces sp. is an α,α-disaccharide and is fairly active against Mycobacterium tuberculosis. We synthesised this antibiotic in 18% overall yield (Ref. 17) from (13) and (29).

The foregoing syntheses of pseudodisaccharides provided useful intermediates for further synthesis of more complex aminoglycosides, and we synthesised kanamycins A-C (8-10) from the kanosaminyldeoxystreptamine (23), neamine (19), and paromamine (17), respectively, by way of their suitably protected derivatives and protected glycosyl chlorides (Refs. 18-20).



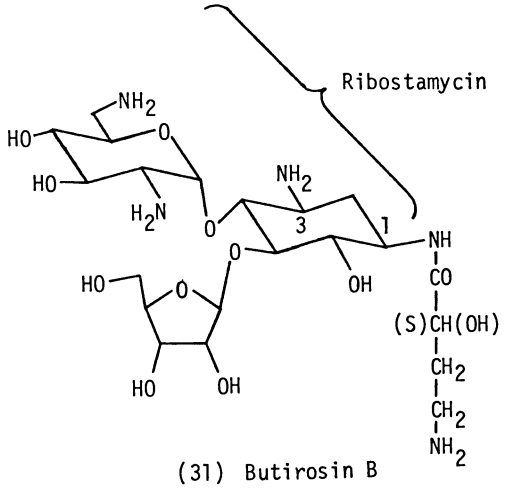
(29)



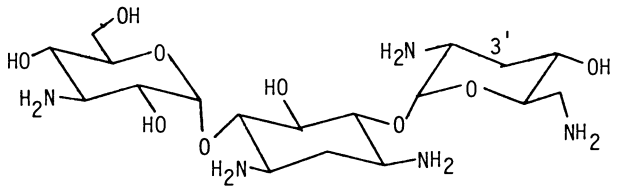
(18%, overall)

(30) α,α-Trehalosamine

We have further synthesised several representative aminoglycosides including butirosin B (31), tobramycin (32), dihydrostreptomycin (2), and streptomycin (1). Butirosins were discovered by Parke-Davis investigators (Ref. 21) and produced by a Bacillus sp. Tobramycin is also a broad-spectrum antibiotic reported by the researchers of Lilly Research Laboratories (Ref. 22). We have already reported the synthesis of butirosin B from ribostamycin (Ref. 23) and I present here its synthesis starting from monosaccharides.



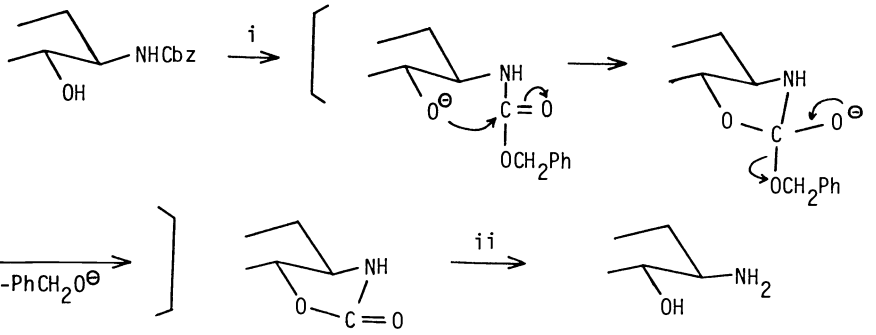
(31) Butirosin B



(32) Tobramycin
(3'-Deoxykanamycin B)

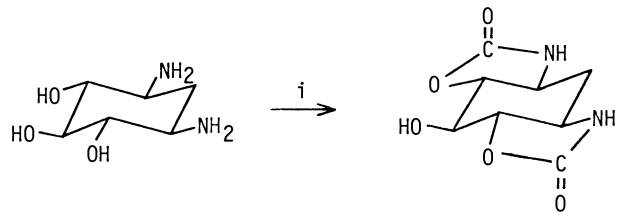
The synthesis involved new means of protecting vicinal trans-diequatorial amino and hydroxyl groups. Method A (Ref. 23) involves the treatment of a benzyloxycarbonylamino compound with sodium hydride in N,N-dimethylformamide and method B (Ref. 24) involves the treatment of an amino sugar with p-nitrophenoxycarbonyl chloride and Dowex 1x2 (HO^-) resin in aqueous media. The cyclic carbamate formed is readily removed by mild alkali, such as dilute barium hydroxide.

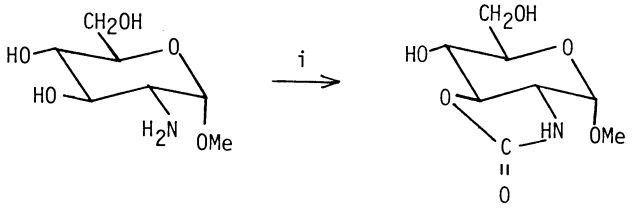
Method A :



i) $NaH/DMF/N_2$ ii) $Ba(OH)_2$/aq. dioxane

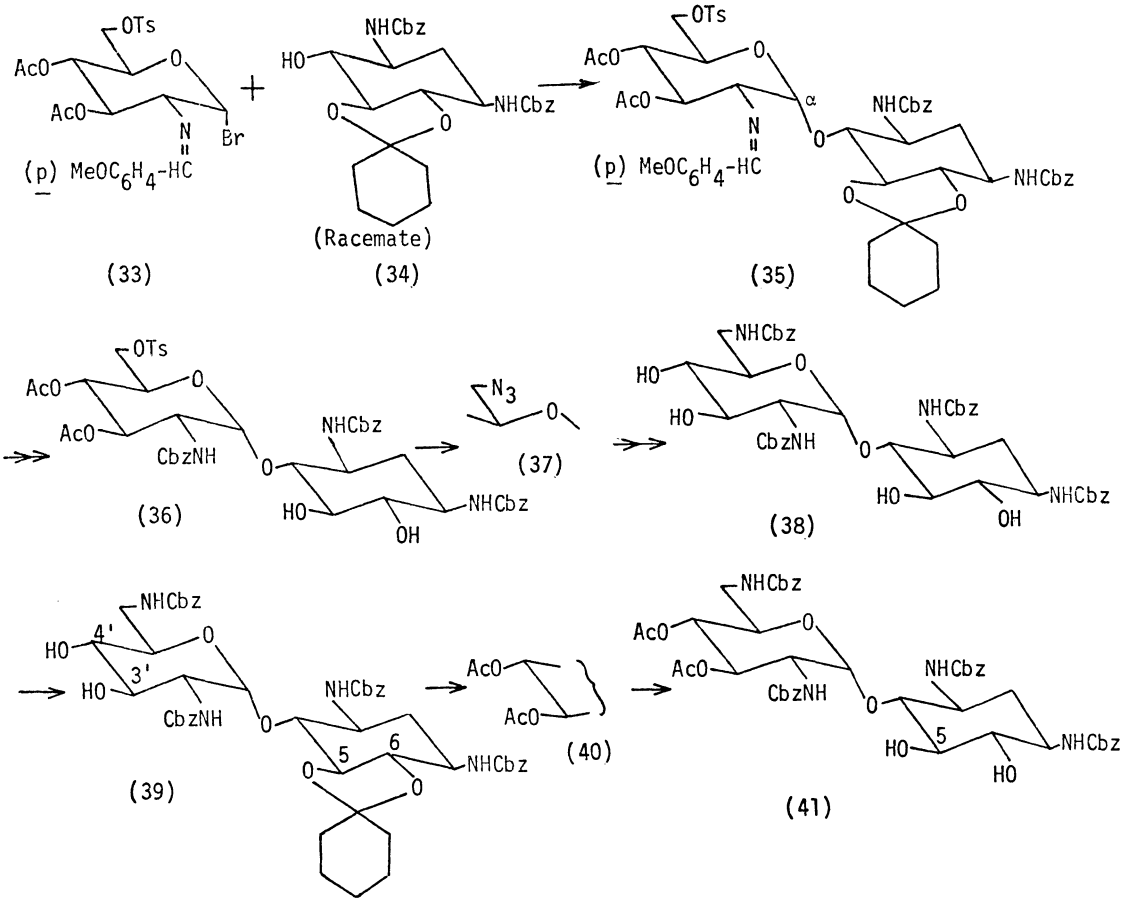
Method B :

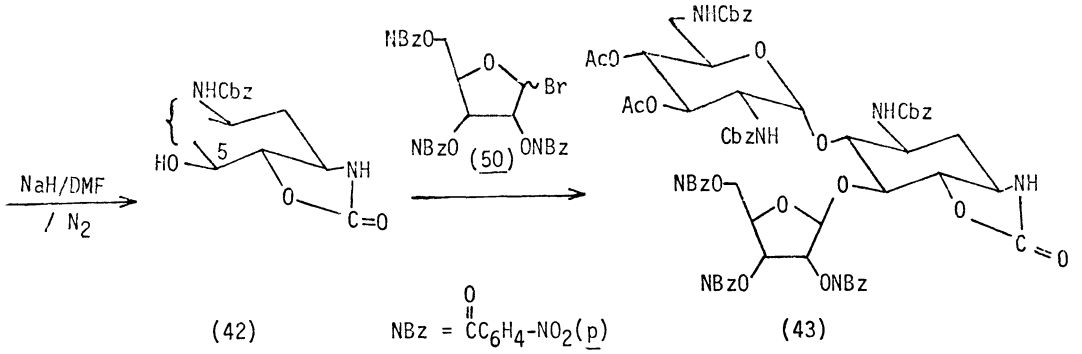




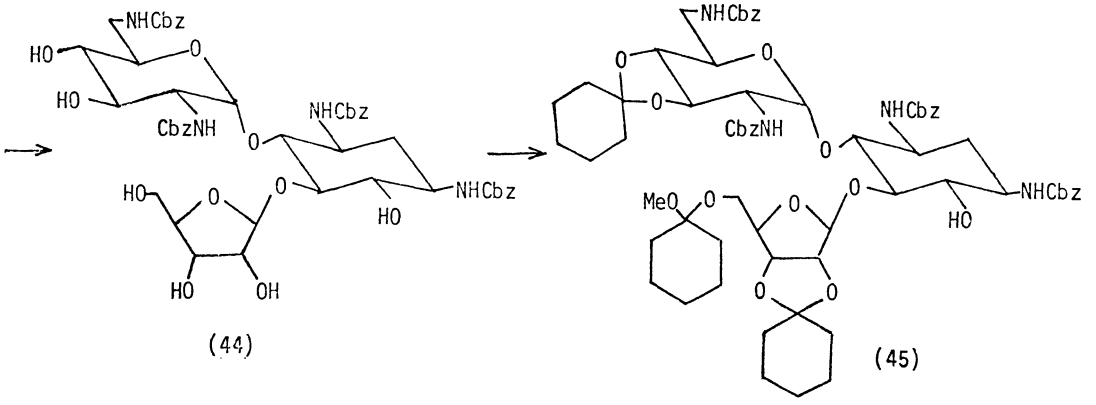
i) $\underline{p}$-$NO_2C_6H_4OCOCl$-Dowex 1x2(OH) or alkali/water; $\underline{p}$-$NO_2C_6H_4O^-$ and Cl^- are liberated.

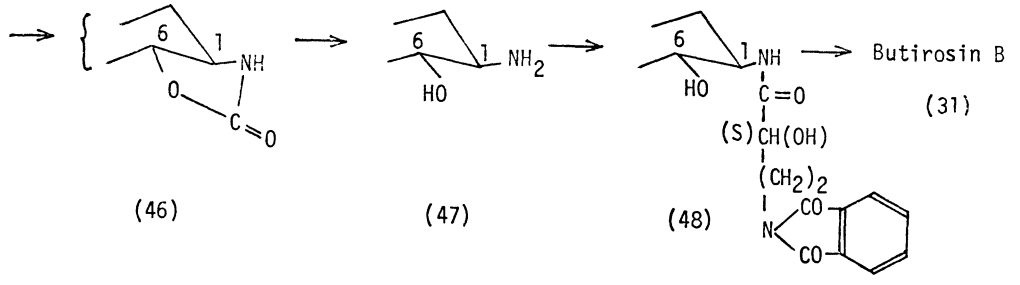
Of particular interest is the regioselective acylation of NH_2-1 of the 2-deoxystreptamine portion of aminoglycosides, and this was achieved by the above method. 3,4-Di-O-acetyl-2-deoxy-2-N-(p-methoxybenzylideneamino)-6-O-tosyl-D-glucosyl bromide (33) was condensed with a protected 2-deoxystreptamine (34) in dichloromethane containing mercuric cyanide. Since the latter aglycone is a racemate, the synthesis yields a mixture of two diastereomeric pseudodisaccharides, which could be separated by column chromatography to give the desired derivative (35). Removal of the cyclohexylidene group followed by replacement of TsO-6' with azide, hydrolysis of the acetyl groups, catalytic hydrogenation and benzyloxycarbonylation afforded tetra-N-(benzyloxycarbonyl)neamine (38) in good overall yield. Ketal exchange with 1,1-dimethoxycyclohexane in the presence of toluene-p-sulfonic acid in N,N-dimethylformamide gave a mixture of mono- and di-cyclohexylidene derivatives. However, we could differentiate the 3',4'-hydroxyl groups from the corresponding entity in the deoxystreptamine portion; the 3',4'-O-cyclohexylidene group on the glycosyl portion is less stable than the 5,6-acetal on the deoxystreptamine portion and the former can be easily removed by addition of methanol to the reaction mixture to give (39). Further acetylation gave a completely protected derivative (40). Decyclohexylidenation followed by treatment with sodium hydride in N,N-dimethylformamide afforded the expected cyclic carbamate (42). Thus, the desired regioselective glycosylation at C-5 of the 2-deoxystreptamine portion became possible. Condensation of (42) with 2,3,5-tri-O-(p-nitrobenzoyl)ribosyl bromide (50) in dichloromethane in the presence of mercuric cyanide gave the protected ribostamycin (43), which, by hydrolysis with barium hydroxide and benzyloxycarbonylation gave tetra-N-(benzyloxycarbonyl)ribostamycin (44). Acetalation then gave the tri-O-cyclohexylidene derivative (45), which has a methoxycyclohexyl form among the three cyclohexylidene groups. The ketal exchange reaction reported by Angyal et al. (Ref. 25) and Evans et al. (Ref. 26) was extremely useful in our syntheses. A high degree of acetalation is achieved by employing cyclohexanone dimethyl ketal in N,N-dimethylformamide with continuous removal of methanol; vicinal trans-hydroxyl groups on a pyranoid ring react as well as cis-hydroxyl groups. Treatment of (45) with sodium hydride in N,N-dimethylformamide formed a 1,6-cyclic carbamate (46) on the deoxystreptamine portion. Hydrolysis with 0.2M barium hydroxide followed by acylation with (S)-2-hydroxy-4-phthalimidobutyric acid gave (48), which, on removal of the protecting groups, afforded butirosin B (31).

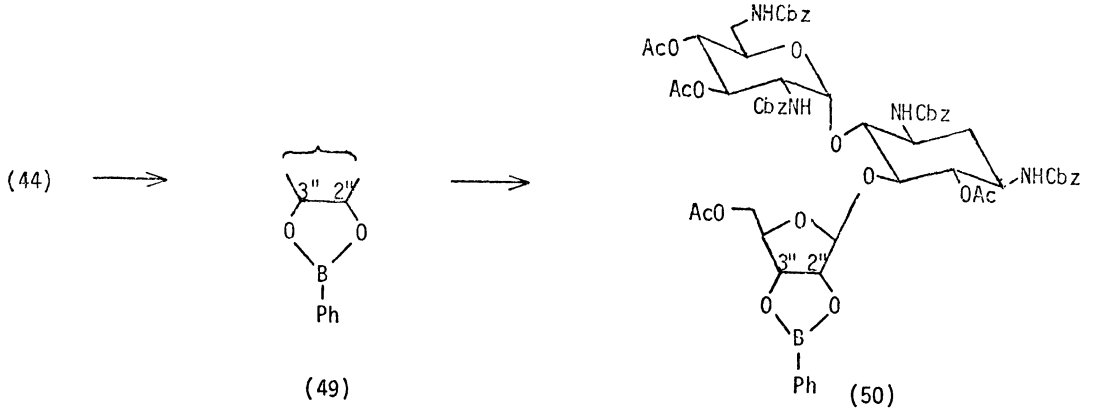




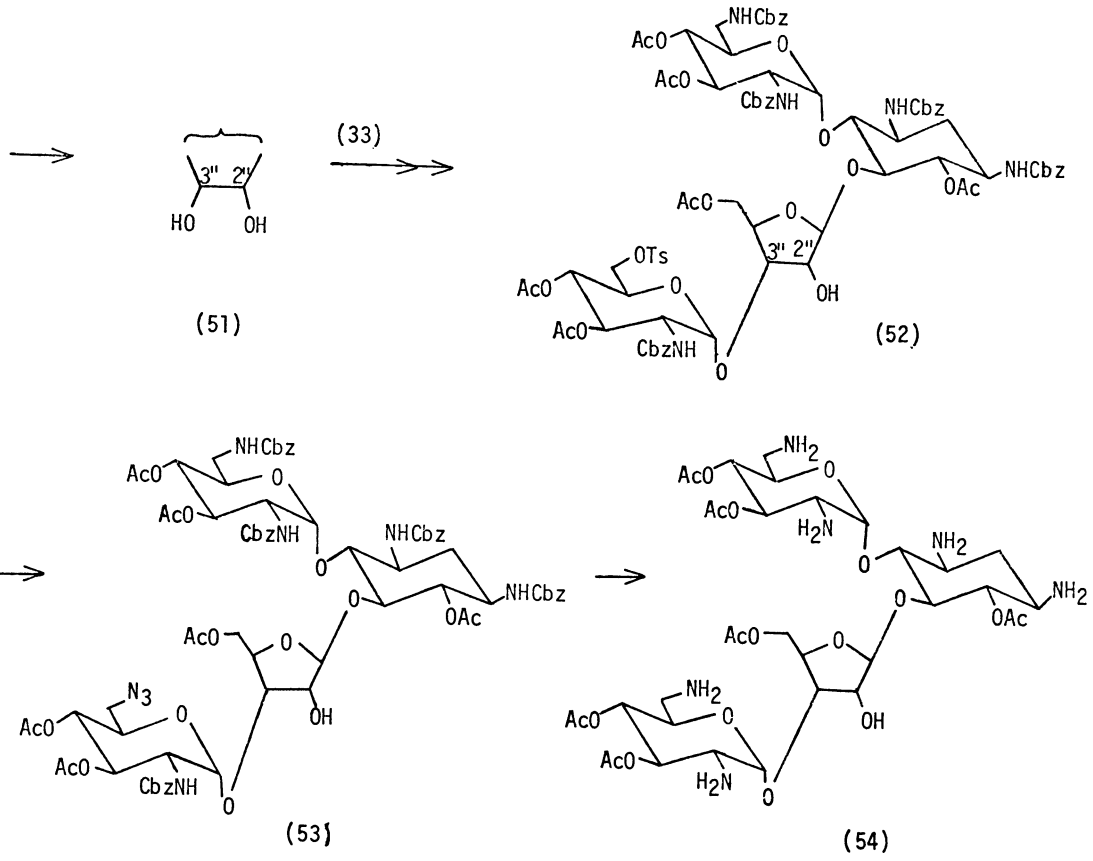
(42) NBz = $\overset{O}{\overset{\|}{C}}C_6H_4\text{-}NO_2(p)$ (43)



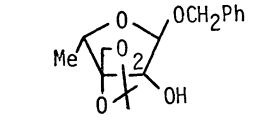




Last year (Ref. 27) we reported the total synthesis of neomycin C (4), that represented the first synthesis of an antibiotic of pseudotetrasaccharides. Neomycin was independently discovered by Umezawa's group in 1948 and Waksman's group in 1949. The neomycin complex is widely used for treating tropical infections. Elucidation of the complete structures of the neomycins was achieved by Rinehart and his co-workers (Ref. 28) in 1963. The starting compound for this synthesis was the tetra-N-(benzyloxy-carbonyl)ribostamycin (44). Benzeneboronic acid was useful for protection of HO-2",3" of the ribose portion. Treatment of (44) with benzeneboronic acid in pyridine gave the boronate (49). Acetylation then gave(50) and the boronate ester group was removed by treatment with propane-1,3-diol to give (51), as reported by Ferrier and Prasad (Ref. 29). The following α-glycosylation reaction is based on our prior findings that the 2-N-(p-methoxybenzylidene) Schiff base derivatives gave high yields of α-glycosides. Condensation of (51) with 3,4-di-O-acetyl-2-deoxy-2-(p-methoxybenzylideneamino)-6-O-tosyl-α-D-glucopyranosyl bromide (33) in anhydrous chloroform in the presence of silver carbonate, silver perchlorate, and Drierite gave a mixture of glycosides. Hydrolysis of the Schiff base moiety followed by benzyloxycarbonylation and chromatography on silica gel afforded the desired glycoside (52) in 34% overall yield from (51). Treatment of (52) with sodium azide then gave (53) and catalytic hydrogenation with palladium black followed by hydrolysis with M barium hydroxide completed the synthesis of neomycin C (4).



Streptomycin (1) and dihydrostreptomycin (2) were attractive targets for total syntheses, and we (Refs. 30, 31)achieved these syntheses in 1974. The structure of streptomycin was established by 1948except for the glycosidic linkage between streptose and streptidine which was revealed to be α-L by McGilveray and Rinehart (Ref. 32) in 1965. I should like to note several key stages of our synthesis. Of two possible routes for the synthesis of dihydrostreptomycin, one involved dihydrostreptobiosamine and subsequent coupling of a derivative with a protected streptidine, and the other involved the coupling of protected dihydrostreptose and streptidine followed by formation of the second glycosidic linkage with a protected 2-deoxy-2-methylamino-L-glucose. We chose the former route because the α-glycosidic linkage between dihydrostreptose and streptidine is less stable for hydrolytic treatment than that between 2-deoxy-2-methylamino-L-glucose and dihydrostreptose owing to the presence of MeNH-2 in the latter glycoside moiety. In the dihydrostreptobiosamine synthesis (Ref. 33), we used 2-amino-2-deoxy-L-glucose instead of the N-methyl derivative because we intended to use the Schiffs base for α-glycoside synthesis. Acetalation of the benzyl dihydrostreptoside by a ketal exchange reaction resulted in the formation of an isopropylidene group between the primary and tertiary hydroxyl groups rather than between the secondary and tertiary hydroxyl groups, giving (55). This is a rare structure which proved to be more stable to acids than the usual cyclic acetals. This acetalation enabled us to carry out the following glycosylation at C-2. Condensation of (55) with (56) followed by a sequence of reactions involving N-methylation and deprotection afforded the benzyl glycoside (57) of dihydrostreptobiosamine. On the other hand, the protected streptidine (59), which was soluble in organic solvents, was prepared by a sequence of reactions (Ref. 34). The protected glycosyl halide of dihydrostreptobiosamine used for glycosylation was (58) which has cyclic carbonate and carbamate groups. Coupling of(58 and 59)in anhydrous benzene in the presence of silver carbonate, silver perchlorate, and a molecular sieve gave a mixture of glycosides,



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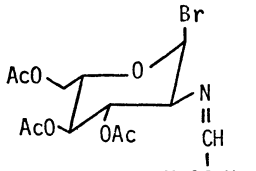

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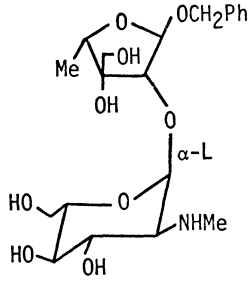

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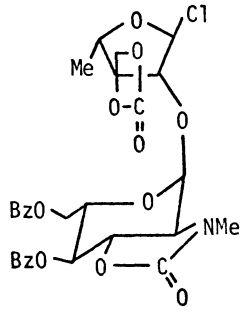

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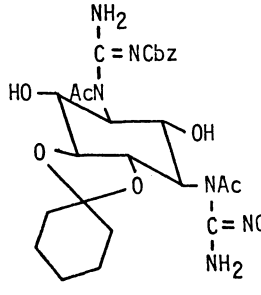
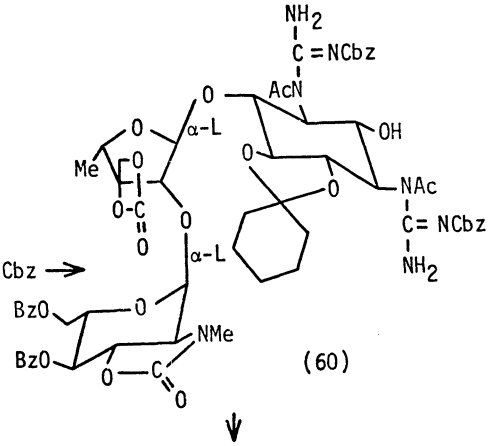


(Racemate)

(59)



(60)

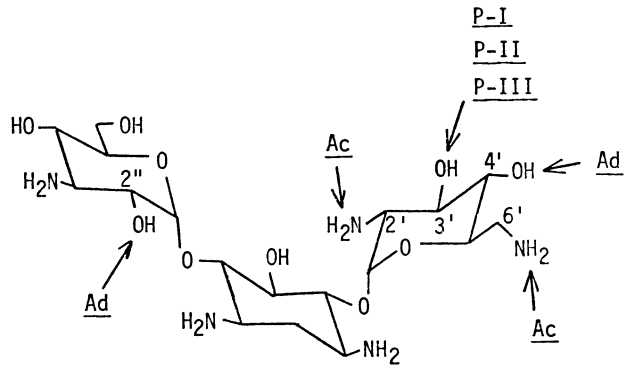
Dihydrostreptomycin (2)

Streptomycin (1)

which was separated by chromatography on silica gel to afford the protected dihydrostreptomycin (60). Treatment of (60) with dilute barium hydroxide simultaneously hydrolysed the acetyl, benzoyl, carbamate, and carbonate groups without transformation of the guanidine groups into ureido groups. Subsequent hydrolysis with aqueous acetic acid removed the cyclohexylidene group, and catalytic hydrogenolysis over palladium removed the benzyloxycarbonyl groups, yielding dihydrostreptomycin (2). Streptomycin (1) was synthesised from a protected dihydrostreptomycin by way of the Pfitzner-Moffatt oxidation with methyl sulfoxide, dicyclohexylcarbodi-imide, trifluoroacetic acid and pyridine (Ref. 35). Paulsen and his co-workers (Ref. 36) have recently synthesised streptobiosamine via the nitrosoglycal procedure using a new streptose derivative.

SYNTHESIS OF AMINOGLYCOSIDE ANTIBIOTICS ACTIVE AGAINST RESISTANT BACTERIA

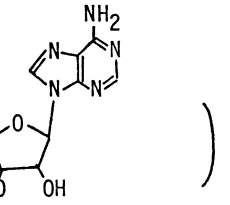
Drug resistance is a serious concern in present chemotherapy. Two kinds of resistance mechanism for aminoglycoside antibiotics have recently been revealed involving change of the 30S ribosomal subunit which is genetically controlled by the bacteria chromosome, and inactivating enzyme production which is genetically carried by plasmid. The former mechanism is found in most of laboratory-developed mutants and rarely in clinical isolates. Most of clinical isolates of drug-resistant bacteria show the latter form of resistance. The enzymatic inactivation mechanisms for kanamycin B so far reported are shown below.



P : Phosphorylation (OH → OPO_3H_2)

Ac: Acetylation (NH_2 → NHAc)

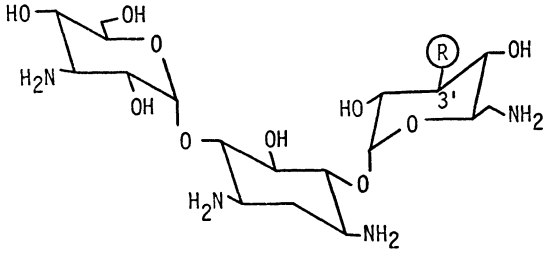
Ad: Adenylylation (OH → O-P(OH)-O ‖ O)



Inactivation of Kanamycin B

There are two modes of enzymatic acetylation, specific for NH_2-6' and NH_2-2', respectively. Resistant bacteria rarely produce adenylyl transferase enzymes to inactivate by adenylylation of HO-2". Moreover, another adenylyl transferase has recently been found to inactivate by adenylylation of HO-4'. However, the most commonly encountered mode of inactivation is 3'-phosphorylation and virtually all aminoglycoside antibiotics (e.g. neomycins, kanamycins and ribostamycin) having a hydroxyl group at position 3' are substrates for these enzymes. There are three different types (I-III) of phosphotransferase enzymes which differ in their substrate specificity (Ref. 37). A research group at our Institute elucidated the structure of kanamycin inactivated by enzymatic phosphorylation in 1967, and their further studies on the substrate specificity of the enzyme 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 HO-3' by this enzyme. The phosphorylation mechanism of resistance suggested that chemical modification of HO-3' might lead to a derivative that would be active against resistant bacteria. There are two approaches to new derivatives, namely, total synthesis or modification of the natural antibiotics. These syntheses entailed the regioselective removal or modification of amino groups on complex molecules.

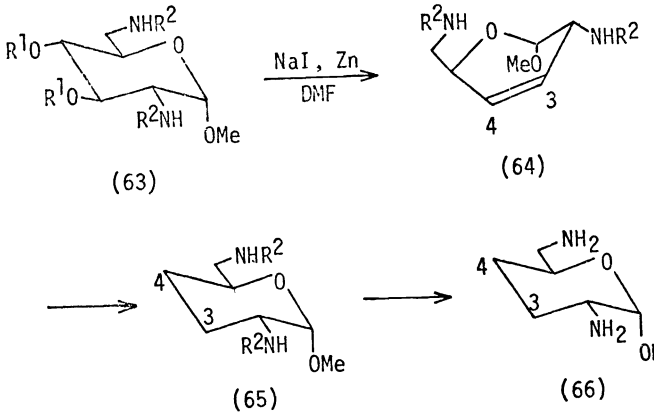
Our initial approach involved the total synthesis of 3'-O-methylkanamycin (62) and 3'-deoxykanamycin (61), and we found that deoxygenation at position 3' has a remarkable effect on the antibacterial activity (Table 1). The 3'-deoxykanamycin (61) (Ref. 38) is as active as the parent antibiotic, and, moreover, it is active against E. coli carrying R factor and resistant Pseudomonas, whereas the 3'-O-methyl derivative (62) (Ref. 39) is inactive except for slight activity against Bacillus subtilis, suggesting that, although HO-3' 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.



(61) 3'-Deoxykanamycin A : R = H

(62) 3'-O-Methylkanamycin A : R = OMe

Next, we contemplated the transformation of natural aminoglycosides into their 3'-deoxy derivatives. However, since deoxyaminosugars are a comparatively unknown class of compounds, we first studied their preparation. These studies will not be referred to in detail here, but a deoxy derivative was prepared (Ref. 40) from 2,6-diamino-2,6-dideoxy-D-glucose, a component of kanamycin B, neomycins, ribostamycin, butirosins and others. The two amino groups of the glycoside were protected with methoxycarbonyl, ethoxycarbonyl, or benzyloxycarbonyl groups and the two hydroxyl groups were mesylated to give the 3,4-dimesylate (63). Tosylation led to almost selective reaction of HO-3. Treatment of the dimesylate with sodium iodide and an excess of zinc dust in hot N,N-dimethylformamide afforded the 3,4-unsaturated sugar(64)in excellent yield. This procedure was first introduced by Tipson and Cohen (Ref. 41) in 1965, and, subsequently used by Horton and co-workers (Ref. 42) for the introduction of 2,3-unsaturation into D-glucose. Catalytic hydrogenation of(64) and deprotection then gave the 3,4-dideoxyaminoglycoside (66). This procedure opened the way to the transformation of neamine, kanamycin B, ribostamycin, and butirosin B into their 3',4'dideoxy derivatives.



(63) (64) (65) (66)

R^1: SO_2Me or SO_2CH_2Ph

R^2: CO_2Me, CO_2Et, CO_2CH_2Ph, or $SO_2C_6H_4Me(p)$

The deoxygenation of kanamycin B (9) was accomplished (Ref. 43) as shown below. The protected kanamycin (67) was regioselectively mesylated to give (68), which, on treatment with sodium iodide and zinc dust, gave the 3',4'-unsaturated derivative (69). Catalytic hydrogenation then gave the 3',4'-dideoxy derivative (70). Decyclohexylidenation followed by removal of the other protecting groups by hydrolysis with barium hydroxide yielded 3',4'dideoxykanamycin B (71, ~30% from kanamycin B).

Its antibacterial spectrum is shown in Table I compared with those of kanamycin and 3'-deoxykanamycin A (61). Resistant bacteria, including various strains of E.coli carrying R factors and Pseudomonas aeruginosa are remarkably sensitive to the 3',4'-dideoxy derivative. After clinical studies this semi-synthetic antibiotic assigned the generic name dibekacin has recently been commercialised as a drug for resistant infections.

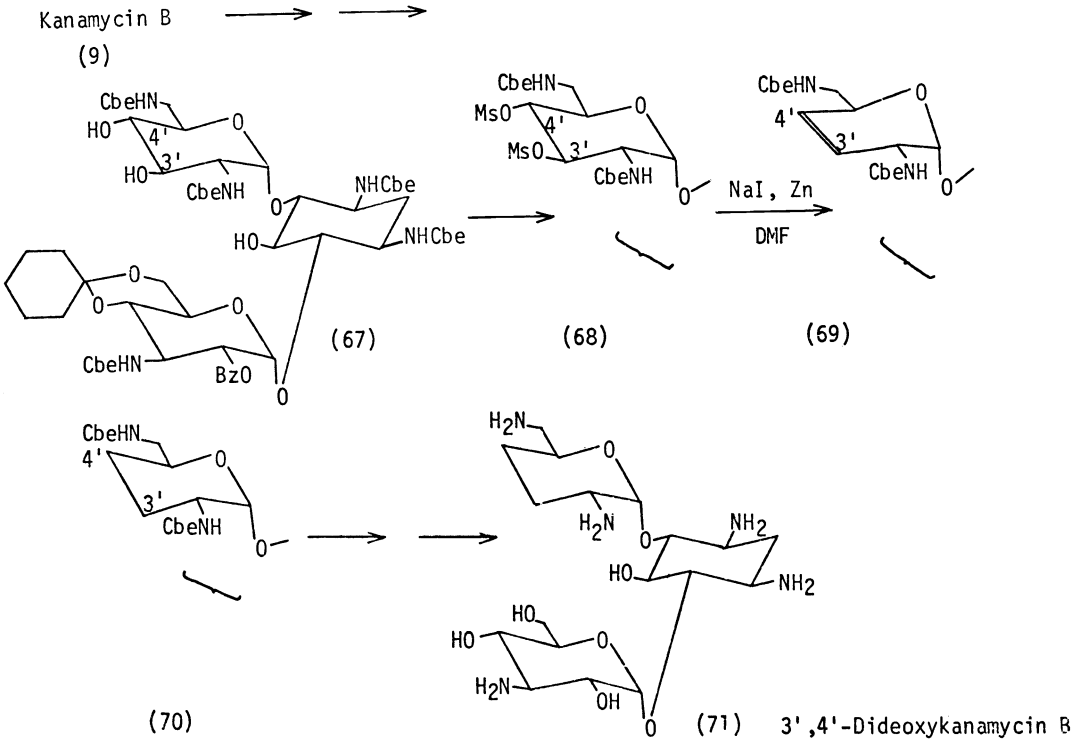


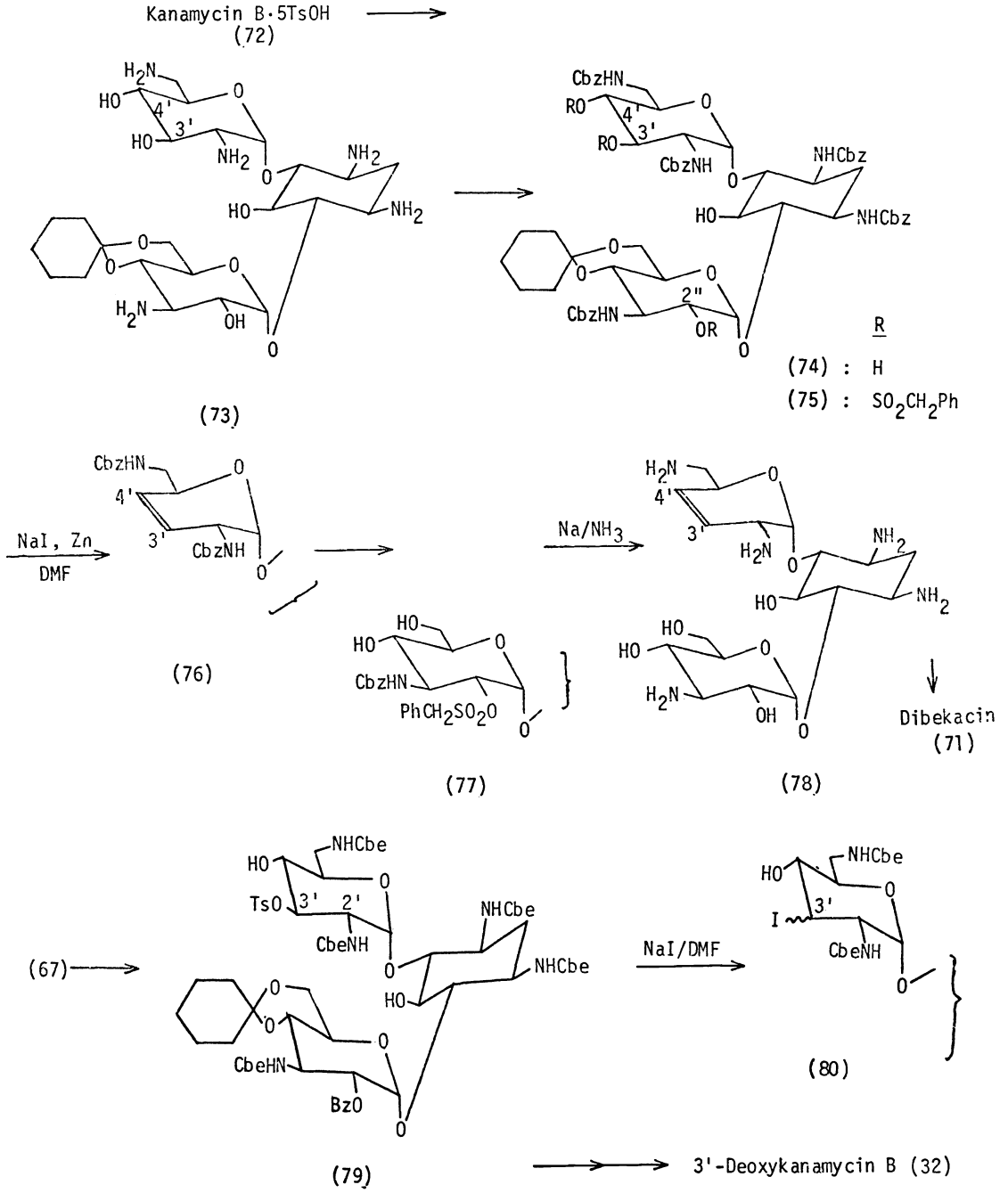
TABLE 1. Antibacterial spectra of 3',4'-dideoxykanamycin B, 3'-deoxykanamycin, and kanamycin

Test organisms*	Minimal inhibitory concentrations (μg/ml)		
	3',4'-Dideoxy-kanamycin B	3'-Deoxy-kanamycin	Kanamycin
Staphylococcus aureus FDA 209P	0.78	1.56	1.56
Escherichia coli K-12 CS-2	1.56	3.12	1.56
K-12 ML 1629	1.56	3.12	>50
K-12 ML 1630	3.12	3.12	>50
K-12 ML 1410	1.56	3.12	0.78
Pseudomonas aeruginosa A3	3.12	3.12	50
No. 11	3.12	12.5	>50
No. 45	0.78	1.56	50
Proteus rettgeri GN 311	12.5	12.5	6.25
GN 466	3.12	6.25	3.12

* Nutrient agar, 37°, 18 h.

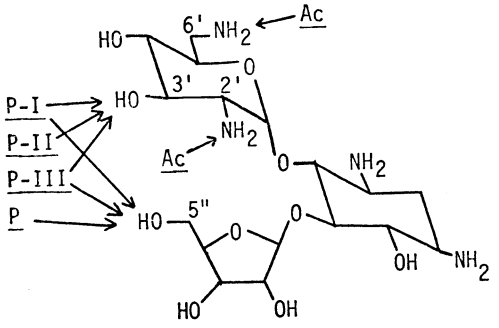
An improved synthesis of dibekacin is shown below. In general, protection of the amino groups is a prerequisite for transformation of the aminoglycoside molecule, but, in this synthesis, we protected HO-4",6" prior to the protection of amino groups. Thus, kanamycin was first converted into its pentatoluene-p-sulfonate salt (72) in order to raise the solubility in organic solvents. Controlled transketalization with cyclohexanone dimethyl ketal then gave 4",6"-O-cyclohexylidenekanamycin B (73) which retained

strong antibacterial activity. The amino groups of (73) were benzyloxycarbonylated to give (74). Benzyl sulfonylation was then used in an approach to 3',4'-unsaturation. Treatment of (74) with benzylsulfonyl chloride in pyridine at -20° for several hours gave the 3',4',2"-tri-O-benzylsulfonyl derivative (75, 98%) without benzylsulfonylation of HO-5 of the deoxystreptamine portion. Treatment of (75) with sodium iodide and zinc dust in N,N-dimethylformamide gave the 3',4'-unsaturated derivative (76, 86%). Decyclohexylidenation gave (77), and subsequent treatment with sodium in liquid ammonia readily removed the N-benzyloxycarbonyl and O-benzylsulfonyl groups to give (78), which was converted into dibekacin by catalytic hydrogenation. The overall yield of dibekacin from kanamycin B was >50% (Ref. 44).



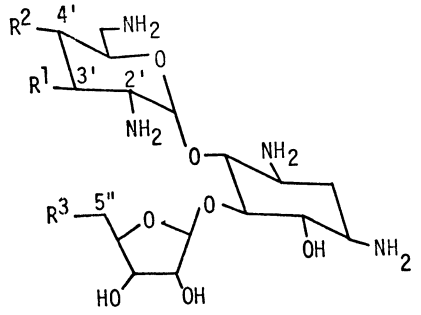
The intermediate (67) was also useful for the synthesis of 3'-deoxykanamycin B which was one of the desired derivatives and is identical with tobramycin. Tosylation of (67) was selective for HO-3' and iodination with sodium iodide in N,N-dimethylformamide afforded the iodo derivative (80) probably through an oxazoline intermediate formed by participation of the ethoxycarbonyl group at C-2'. Finally, catalytic hydrogenation with Raney nickel followed by deprotection gave 3'-deoxykanamycin B (32) identical with tobramycin (Ref. 45).

The inactivation mechanisms for ribostamycin (81) are shown below. There are four different types of phosphotransferase enzyme, which differ in their substrate specificities. Phosphotransferase-I phosphorylates HO-3' as well as HO-5" on the ribose portion, but does not inactivate butirosins, whereas phosphotransferase-II phosphorylates butirosins at position 3' and does not inactivate 3'-deoxyribostamycin and lividomycins (3'-deoxy compounds). Another phosphotransferase recently discovered preferentially phosphorylates HO-5" on the ribose portion. Phosphotransferase-III inactivates both butirosins and lividomycins.



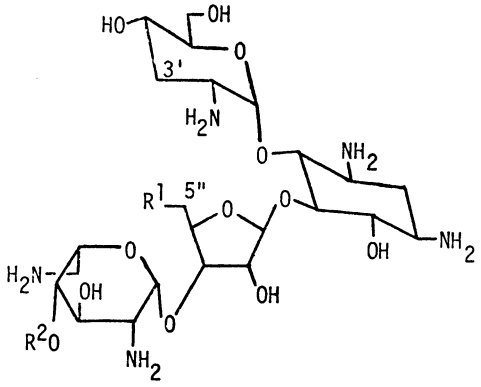
(81) Inactivation of Ribostamycin

Reaction sequences similar to those mentioned above allowed the synthesis of 3'-deoxy-, 3',4'-dideoxy-, and 3',4',5"-trideoxy derivatives of ribostamycin. 3'-Deoxyribostamycin (82) (Ref. 46) and 3',4'-dideoxyribostamycin (83) (Ref. 4) showed activity similar to that of ribostamycin against normal and resistant bacteria including Pseudomonas aeruginosa. On the other hand, 3',4',5"-trideoxyribostamycin (84) (Ref. 47) was less active than the parent antibiotic. From these experiments it appears that HO-5" of ribostamycin is important for antibacterial activity. The Dreiding model of ribostamycin suggests the presence of hydrogen bonding between HO-5" and NH_2-2' hindering the rotation of the ribose moiety.



	R^1	R^2	R^3
(81) Ribostamycin :	OH	OH	OH
(82) 3'-Deoxy- " :	H	OH	OH
(83) 3',4'-Dideoxy- " :	H	H	OH
(84) 3',4',5"-Trideoxy- " :	H	H	H

The important role of HO-5" was shown also with the lividomycins, aminoglycoside antibiotics reported by the Kowa Research Laboratories (Ref. 48). We synthesised the 5"-deoxy derivative (85) of lividomycin B, which is 3'-deoxyparomomycin I. Lividomycin B is active against normal and resistant bacteria including 3'-phosphorylating bacteria, however, 5"-deoxy derivatives (85, 87) showed markedly decreased activity (Refs. 49 and 50). Similarly, 5"-deoxy- and 5"-amino-5"-deoxy-lividomycin A (88) showed decreased activities (Ref. 50).

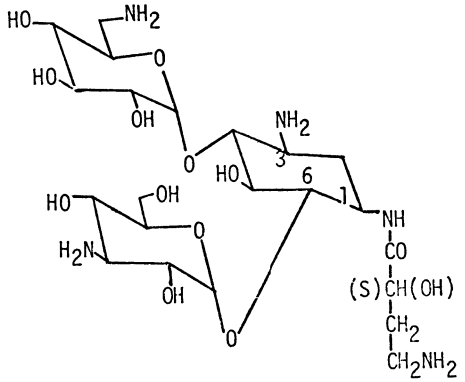


	R^1	R^2
(7) Lividomycin B :	OH	H
(85) 5"-Deoxylividomycin B :	H	H
(86) Lividomycin A :	OH	Mannosyl
(87) 5"-Deoxy- " :	H	Mannosyl
(88) 5"-Amino-5"-deoxy- " :	NH_2	Mannosyl

The deoxygenation of aminoglycosides is of current interest, and a research group at the Takeda Chemical Industries recently reported a new method involving a combination of enzymatic and chemical reactions; 3'-phosphates of aminoglycosides were prepared using the phosphotransferase enzymes and then converted into 3'-chloro derivatives by treatment with trimethylchlorosilane, which, on subsequent catalytic dechlorination, gave 3'-deoxy derivatives (Ref. 51).

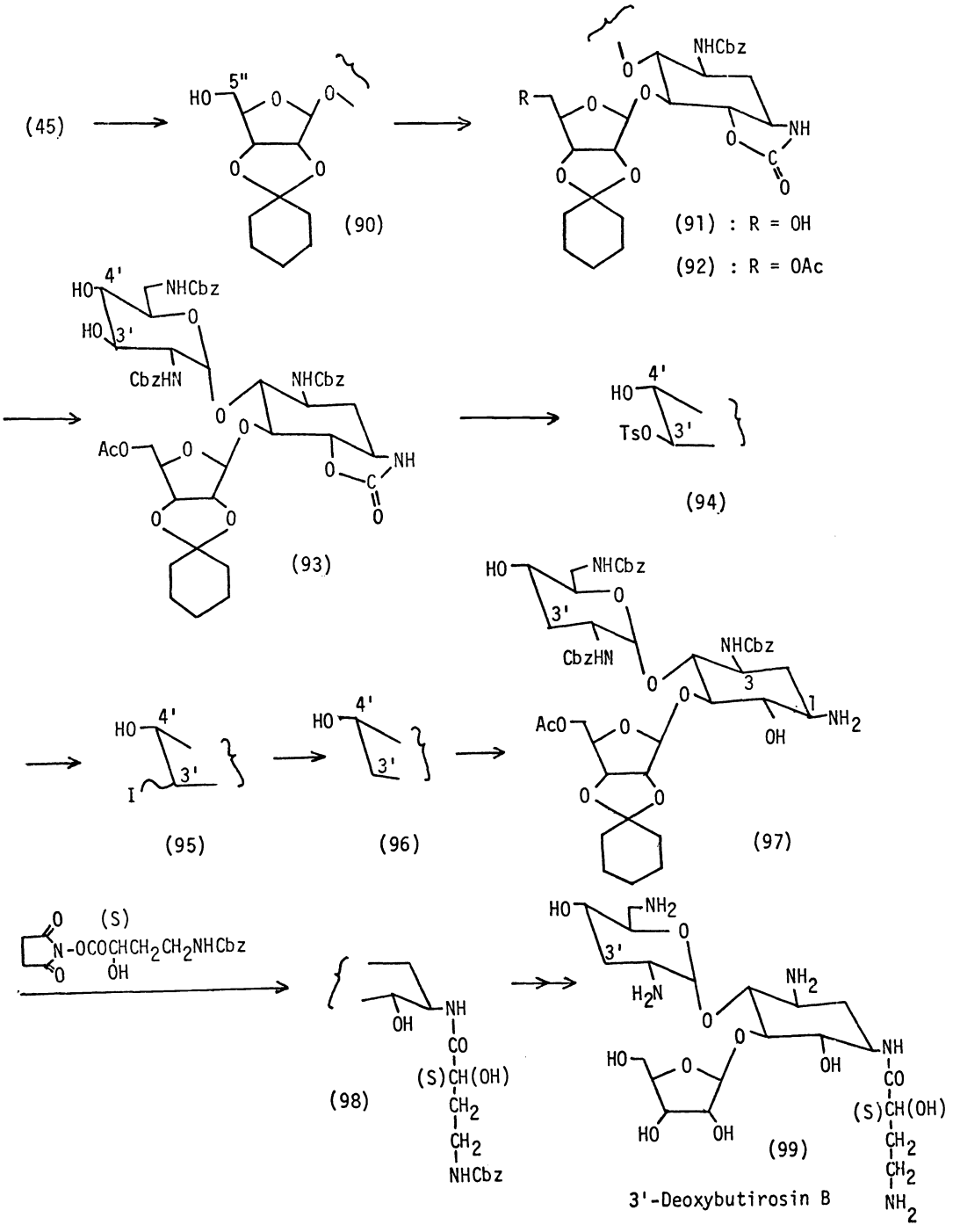
Another important modification of aminoglycoside antibiotics was suggested from the structures of the butirosins; butirosin B (31) is a derivative of ribostamycin acylated with the (S)-(-)-4-amino-2-hydroxybutyryl (AHBA) side chain attached to NH_2-1 of the deoxystreptamine moiety. A comparison of the antibacterial spectra of the butirosins and ribostamycin (81) showed that the former have a broader spectrum than ribostamycin, inhibiting some kanamycin-resistant bacteria including Pseudomonas strains. This relationship suggested that a favorable effect was obtained by acylation of NH_2-1 of the deoxystreptamine moiety in aminoglycoside antibiotics with this peculiar amino acid. Kanamycin modified in this way (89) was reported by Bristol-Banyu investigators in 1972 and was found (Ref. 52) to be equally or more active than kanamycin against both kanamycin-sensitive and resistant organisms. This derivative is assigned the generic name amikacin and was recently commercialised. We have synthesised 1-N-AHBA derivatives of dibekacin (71) (Ref. 53) and lividomycins (7, 86) (Refs. 54, 55), 3'-deoxybutirosins (Refs. 56, 58), 3',4'-dideoxybutirosin B (Ref. 59) and others.

The selective acylation of NH_2-1 of deoxystreptamine moiety is generally difficult. However, this acylation has been achieved in several cases through the above mentioned cyclic carbamate intermediate.

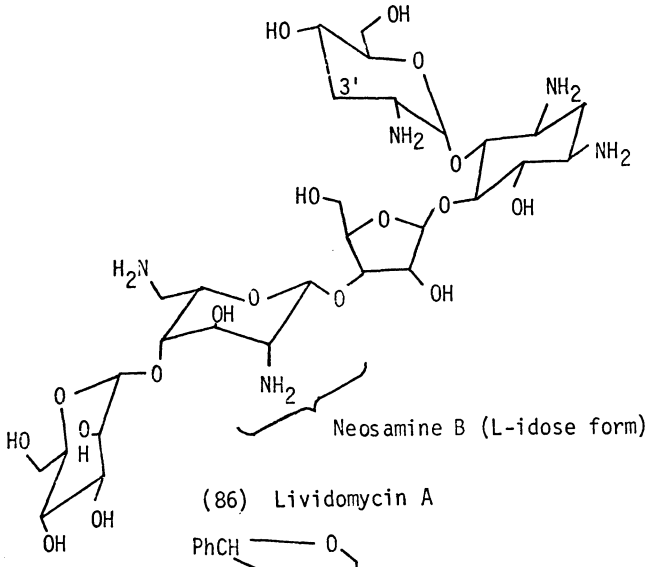


(89) Amikacin

The transformation of ribostamycin (81) into 3'-deoxybutirosin B (99) is shown below. The starting material (45) is an intermediate in the synthesis of butirosin B. Controlled hydrolysis of (45) with acetic acid removed the 5"-O-(methoxycyclohexyl) group to give(90),which, on treatment with sodium hydride in N,N-dimethylformamide, gave the cyclic carbamate(91). After acetylation of HO-5", the cyclohexylidene group was removed by controlled hydrolysis to give the 3',4'-diol (93). Selective tosylation of HO-3' followed by iodination and catalytic hydrogenation gave the 3'-deoxy derivative(96). Mild hydrolysis of the carbamate with dilute barium hydroxide gave(97),which, after 1-N-acylation and deprotection, provided 3'-deoxybutirosin B (99) (Ref. 56). This semisynthetic antibiotic shows an excellent antibacterial spectrum, including activities against resistant bacteria which produce phosphotransferase II and inactivate butirosins.

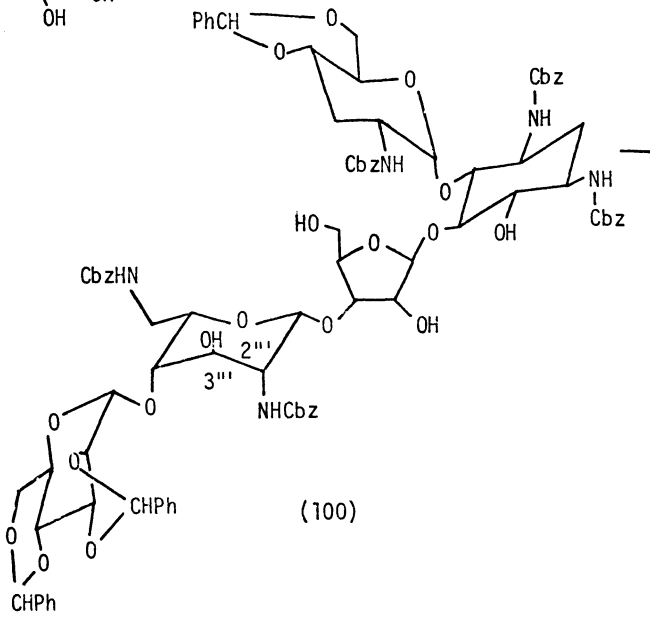


The 1-N-acylation of lividomycin A (86), which is a pseudopentasaccharide antibiotic, is shown below. Benzyloxycarbonylation of (86) followed by acetalation with benzaldehyde dimethyl acetal gave the penta-N-benzyloxycarbonyl-tri-O-benzylidene derivative (100), which, on treatment with sodium hydride in N,N-dimethylformamide, gave the cyclic carbamate (101). Cyclic carbamate formation between NH_2-2''' and HO-3''' on the neosamine B moiety (L-ido form) did not occur, suggesting that this moiety may be in 1C-like conformation which hinders the carbamate formation. Mild alkaline hydrolysis of the carbamate followed by 1-N-acylation with the amino acid and deprotection led to the 1-N-[(S)-4-amino-2-hydroxybutyryl]lividomycin A (103) (Ref. 55). The semisynthetic antibiotic showed a broader antibacterial spectrum than the parent antibiotic and inhibited the phosphotransferase I as well as II.



Neosamine B (L-idose form)

(86) Lividomycin A



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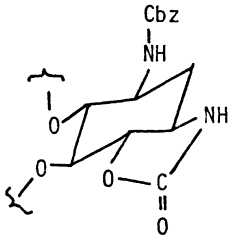

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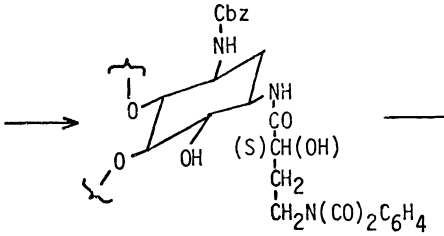

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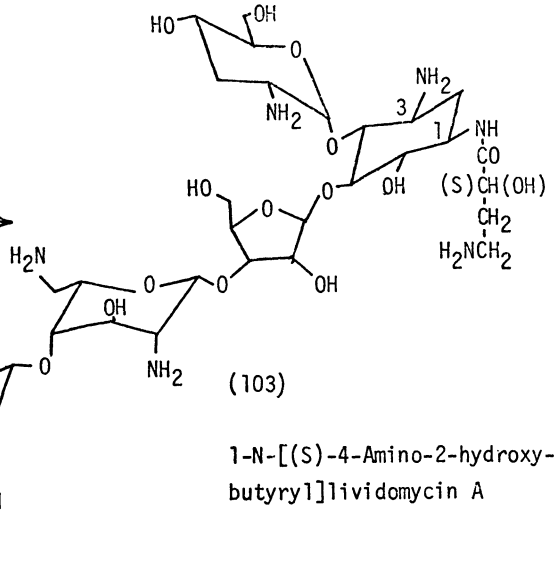
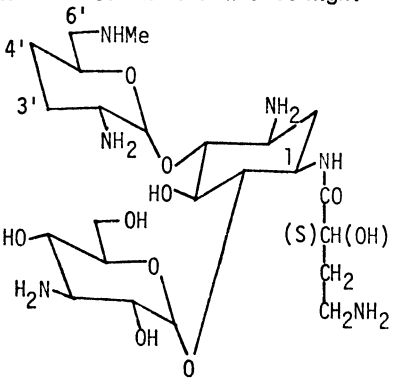


(103)

1-N-[(S)-4-Amino-2-hydroxybutyryl]lividomycin A

I shoula like to mention also an almost ideal form of kanamycin B which is active against various kinds of resistant bacteria. 1-N-(L-4-Amino-2-hydroxybutyryl)-3',4'-dideoxy-6'-N-methylkanamycin B(104) was synthesised from 3',4'-dideoxykanamycin B (71) by a research group of our Institute (Ref. 60). In this derivative, NH_2-6' is further methylated in order to prevent 6'-N-acetylation by the acetyltransferase enzyme. Table 2 shows the antibacterial spectra of this and another derivative, 6'-N-methyldibekacin, which we have also prepared. This derivative inhibits almost all inactivations by phosphotransferases, adenylyltransferases, and acetyltransferases. A combination of deoxygenation, N-acylation, and N-methylation provides significant activity against many resistant organisms. However, this compound was not commercialised because the cost was too high.



1-N-[(S)-4-Amino-2-hydroxybutyryl]-3',4'-dideoxy-6'-N-methylkanamycin B

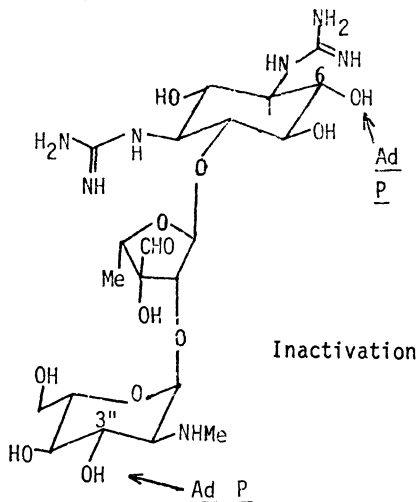
(104)

TABLE 2. The antibiotic spectra of 1-N-(L-4-amino-2-hydroxybutyryl)-3',4'-dideoxy-6'-N-methylkanamycin B (AHB-MDKB) and 3',4'-dideoxy-6'-N-methylkanamycin B (MDKB)

Test organisms	Minimum inhibitory concentrations (μg/ml)		Inactivation mechanisms
	AHB-MDKB	MDKB	
Staphylococcus aureus FDA 209P	0.78	≤0.20	
Sarcina lutea PC1 1001	3.13	12.5	
Bacillus anthracis	≤0.20	≤0.20	
Mycobacterium smegmatis ATCC 607	0.20	1.56	
Shigella dysenteriae JS 11910	3.13	6.25	
S. flexneri 4b JS 11811	3.13	3.13	
Salmonella typhi T-63	3.13	0.78	
Proteus vulgaris OX 19	1.56	1.56	
Klebsiella pneumoniae 22 # 3038	3.13	25	P-II(3'-O) 2"-O-Ad
Escherichia coli K-12	0.78	1.56	
E. coli K-12 R5	1.56	3.13	6'-N-Ac
E. coli K-12 ML 1629	1.56	3.13	P-1(3'-O)
E. coli K-12 ML 1630	0.78	6.25	"
E. coli K-12 ML 1410 R81	1.56	6.25	"
E. coli LA290 R55	0.78	12.5	2"-O-Ad
E. coli LA 290 R56	0.78	3.13	"
E. coli JR66/W677	3.13	25	P-II(3'-O) 2"-O-Ad
Pseudomonas aeruginosa A3	3.13	1.56	
P. aeruginosa TI-13	6.25	12.5	P-(I) (3'-O)
P. aeruginosa GN315	6.25	12.5	6'-N-Ac
P. aeruginosa 99	25	12.5	GM-3-N-Ac

In 1968 Umezawa and his co-workers reported that streptomycin (1) was inactivated by adenylylation, and in the same year, they and Davies and his co-workers proved that the inactivated product was the 3"-O-adenylyl derivative. Subsequently, phosphorylation of HO-3" by a phosphotransferase was reported, and, recently, other inactivations by phosphorylation and adenylylation of HO-6 of streptidine moiety have been described. Among these inactivating enzymes, the 3"-O-phosphotransferase is frequently found in clinical isolates.

We have recently synthesised the 3"-deoxydihydrostreptomycin (113) (Refs. 61, 62). The starting substance was benzyl dihydrostreptobiosaminide (57) obtained from dihydrostreptomycin by benzyl alcoholysis. N-Benzyloxycarbonylation of (57) followed by isopropylidenation and mesylation gave (105). Treatment of the 3'-O-mesyl derivative with sodium acetate in 2-methoxypropane gave the N,O-carbonyl-L-allo derivative (106). Mild hydrolysis of the cyclic carbamate followed by N-acetylation gave (107), which, on chlorination with sulfuryl chloride afforded the 3'-chloro derivative (108). Reduction with tributyltin hydride then gave the desired 3'-deoxy compound (109). Then, by a sequence of reactions, the isopropylidene group on the dihydrostreptose moiety was converted into a cyclic carbonate group to give (110), since the isopropylidene group is labile under the conditions for formation of the glycosyl chloride



Inactivation of Streptomycin

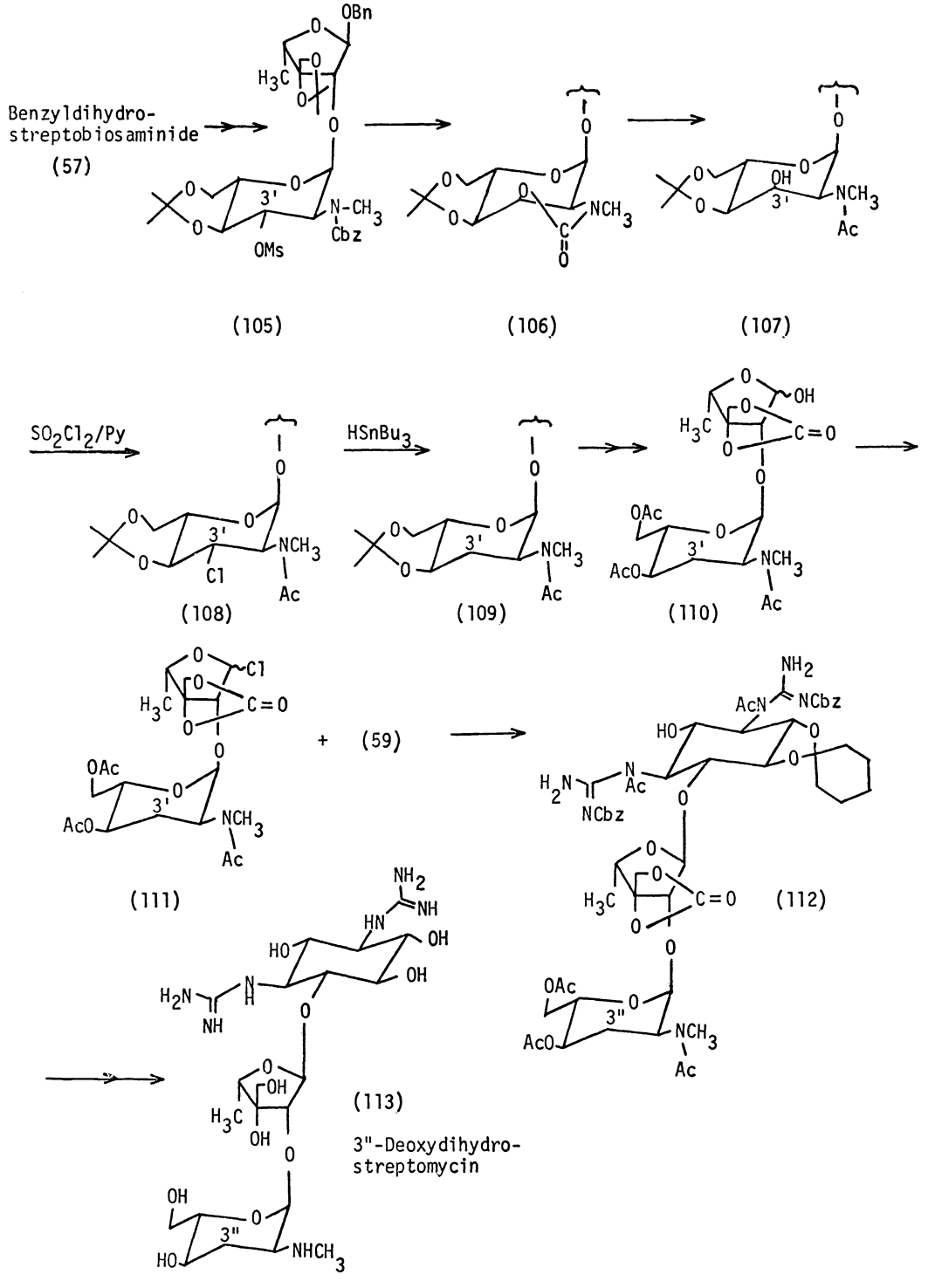
TABLE 3. Antibacterial spectrum of 3"-deoxydihydrostreptomycin (DODSM) compared with dihydrostreptomycin (DSM).

Test organisms*	Minimal inhibitory concentrations (μg/ml)		Resistance mechanisms
	DO-DSM	DSM	
Staphylococcus aureus FDA 209P	3.12	3.12	
Sarcina lutea PCI 1001	0.78	1.56	
Klebsiella pneumoniae PCI 602	3.12	3.12	
Escherichia coli NIHJ	1.56	3.12	
E. coli K-12	1.56	1.56	
E. coli K-12 ML 1629	1.56	>25.0	3"-O-Ad
E. coli K-12 JR66/W677	12.5	>25.0	3"-O-P
E. freundii GN 346	12.5	>25.0	
Salmonella typhi T-63	0.78	25.0	
Pseudomonas aeruginosa A3	12.5	12.5	
P. aeruginosa TI-13	>25.0	>25.0	
Proteus mirabilis IFM OM-9	12.5	25.0	
Mycobacterium smegmatis ATCC 607**	0.39	0.39	

* Agar dilution streak method (nutrient agar, 37°, 18 h).

** 48 h.

by treatment of(110)with thionyl chloride. Compound (111) was then condensed with the protected streptidine (59), an aglycone prepared previously in the total synthesis of dihydrostreptomycin, and since it is a racemate, the condensation product is a mixture of glycosides. Column chromatography gave(112) and deprotection gave the 3"-deoxydihydrostreptomycin (113), the antibacterial spectrum of which is shown in Table 3, compared to that of dihydrostreptomycin. 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.



In conclusion, the synthetic studies directed towards the aminoglycoside antibiotics now cover a wide area, and many references could not be included in this paper. For instance, there are extensive studies on gentamicins, a large group of aminoglycosides, by Daniels and associates of the Schering Corporation. Nevertheless, I hope that my approach indicated the present status of this field.

It has been shown that specific deoxygenation, N-acylation and N-alkylation are successful in increasing the activities of aminoglycoside antibiotics against resistant organisms. Moreover, combinations of these modifications are even more effective. There is a limit to the number of functional groups which can be removed or modified, but we have learned from Nature the structures of aminoglycosides such as spectinomycin, kasugamycin, sisomicin, butirosins, validamycins, apramycin, fortimicins, and others. In view of probable discoveries of new aminoglycosides, growing knowledge of mechanisms of antibiotic action and resistance, and developments in synthetic chemistry, further advances in useful aminoglycoside antibiotics may be expected.

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