STUDIES ON THE SYNTHESIS OF
AMINOGLYCOSIDE ANTIBIOTICS

by

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As members of the Final Examination Committee, we certify that we have read
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entitled Studies on the Synthesis of Aminoglycoside Antibiotics

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SIGNED: Mahendra N. Sharma
To My Parents
PREFACE

Aminoglycoside antibiotics are valuable, widely used chemotherapeutic agents for the treatment of bacterial infections, especially caused by gram-negative species. The effectiveness of earlier aminoglycosides, such as streptomycin and kanamycin, has become limited by the emergence of the resistant strains of bacteria that inactivate these antibiotics through enzymatic reactions. This problem led to the development of various analogs of streptomycin and kanamycin. Numerous analogs of streptomycin have been prepared by modifying its peripheral groups: formyl, guanidino and N-methylamino functions. However none of these analogs was superior to the parent antibiotic. Except for basic degradation of the guanidino groups, little modification of this moiety has been performed. Therefore, we synthesized a number of dihydrostreptomycin analogs through modification of its guanidino groups. However, none of them showed any significant antibacterial activity. In the kanamycin family, we successfully synthesized an amikacin analog, 1-N-[((S)-4-amino-2-hydroxybutyryl]-2'-deoxykanamycin B, from neamine. It differs from amikacin in the absence of a 2'-hydroxyl group and a 2'-amino group in place of a 2'-hydroxyl group. It is predicted that this compound will have antibacterial activity against most of the strains that are resistant to kanamycins. A sample has been submitted for antibacterial activity but the results are not yet available.
The author wishes to express his deepest gratitude to Dr. William A. Remers for his priceless contributions of guidance, patience and support as a dissertation director. In addition, special thanks go to Dr. T. A. Pursiano of Bristol Laboratories for antibacterial testing, to my colleagues Dr. Virendra Kumar, Dr. Dana L. Delaware, Dr. Bhashyam S. Iyengar for thoughtful discussion of chemistry and training in the lab and to my wife Madhuri for her patience and understanding. Finally, the author wishes to thank the National Institutes of Health for financial support.

Mahendra N. Sharma
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ABSTRACT

1-N-(Imidazolinyl)-1,3-dideamidinodihydrostreptomycin was synthesized by condensation of dideamidinodihydrostreptomycin with 2-methylthio-2-imidazoline hydroiodide. 1,3-Di(methylamidino)-dideamidinodihydrostreptomycin was prepared by reaction of N-methyl-S-methylisothiourea hydroiodide with dideamidinodihydrostreptomycin. Both of the compounds were found to have almost no antibacterial activity. In order to learn more clearly the role of guanidino groups on streptomycins for their antibacterial activity, 1,3-N,N-dimethyl-dihydrostreptomycin was synthesized starting from dihydrostreptomycin. The 2-aminomethyl group of dihydrostreptomycin was protected with a benzyl group followed by removal of the guanidino groups. The resulting product, 2'-N-benzyldideamidinodihydrostreptomycin, on treatment with benzyl chloroformate and reduction with lithium aluminum hydride, gave 1,3-N,N-dimethyl-2'-N-benzyldideamidinodihydrostreptomycin. Condensation of 1,3-N,N-dimethyl-2'-N-benzyldideamidinodihydrostreptomycin with S-methyl isothiocourea hydroiodide, followed by hydrogenation, gave the desired 1,3-N,N-dimethyldihydrostreptomycin. However, this analog was also found to be inactive. These findings support the existing opinion that alterations in the guanidino groups of dihydrostreptomycin will lead to inactive analogs.

In the kanamycin B family, a synthesis of 1-N-[(S)-4-amino-2-hydroxybutyryl]-2'-deoxykanamycin B was developed from neamine.
Since neamine has four amino and four hydroxyl groups, it was necessary to prepare an intermediate protected on all of these groups except the 1-amino and 6-hydroxyl group, then acylate the 1-amino group and condense the 6-hydroxyl group with the new protected sugar. This was done by way of the tetra-N-carbobenzyloxy derivative, which was converted into a 4';6':1,6-bis (cyclic carbamate). Following benzylolation of the remaining free hydroxyl groups, selective hydrolysis of the 5-membered cyclic carbamate gave the desired intermediate. The (S)-4-amino-2-hydroxybutytyl side chain was introduced at the 1-position and the hydroxyl group of the side chain was protected by forming another six membered ring carbamate. The resulting compound, having only one free hydroxyl group, was subjected to Koenigs-Knorr type coupling with 2,3-dideoxy-3-trifluoroacetamido-4,6-di-0-(p-nitrobenzoyl)-α-D-arabino-hexopyranosyl-1-bromide, followed by deprotection of all the blocking groups to give the desired 1-N-[(S)-4-amino-2-hydroxybutyryl]-2'-deoxy kanamycin B.
Waksman and coworkers\(^1\) coined the term "antibiotic" in 1942, although it had been known for many years that certain microorganisms were able to exert antagonist effects upon other organisms. The microorganisms in many cases had been found to produce active isolable substances (the antibiotics) which were responsible for biological activity. A large number of antibiotics (Table 1) composed essentially of carbohydrates were isolated from microorganisms. They are called aminoglycoside antibiotics because they contain several amino groups in their glycosidic moieties. Aminoglycoside antibiotics have assumed an important clinical role in the treatment of gram positive and especially gram negative infections for which other groups of antibiotics are ineffective. No other antibiotic group provides such efficient, comprehensive coverage of the pathogens most commonly found in the hospital environment as do aminoglycoside antibiotics. The remarkably successful clinical application of this group of antibiotics has aroused widespread interest and stimulated intensive, planned investigations of them by organic chemists and others, leading to marked advances in several areas of carbohydrate chemistry.

The first aminoglycoside antibiotic described, streptomycin\(^1\), was discovered by Waksman and coworkers\(^2\) in 1944. The development of streptomycin was the result of a well planned, scientific search for antibacterial agents. Waksman and coworkers examined a number of soil
<table>
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<th>Year</th>
<th>Antibiotic</th>
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<tr>
<td>1944</td>
<td>Streptomycin</td>
<td><em>Streptomyces griseus</em></td>
</tr>
<tr>
<td>1949</td>
<td>Neomycin</td>
<td><em>S. fradiae</em></td>
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<tr>
<td>1957</td>
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<td><em>S. kanamyceticus</em></td>
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<td>1959</td>
<td>Paromomycin</td>
<td><em>S. rimosus</em></td>
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<td>1961</td>
<td>Spectinomycin</td>
<td><em>S. spectabilis</em></td>
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<td>1963</td>
<td>Gentamicin C</td>
<td><em>Micromonospora purpurea</em></td>
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<td>1965</td>
<td>Kasugamycin</td>
<td><em>S. kasuganesis</em></td>
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<td>1968</td>
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<td><em>S. lividus</em></td>
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<td>1971</td>
<td>Butirosin</td>
<td><em>Bacillus circulans</em></td>
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<tr>
<td>1973</td>
<td>Apramycin</td>
<td><em>S. tenebrarius</em></td>
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<td>1974</td>
<td>Minosaminomycin</td>
<td><em>Actinomyces sp.</em></td>
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<td>1975-77</td>
<td>Seldomycin</td>
<td><em>S. hofuensis</em></td>
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<td>1976</td>
<td>Sorbistin</td>
<td><em>Pseudomonas sorbicinii</em></td>
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<tr>
<td>1977</td>
<td>Fortimicin</td>
<td><em>M. olivoasterospora</em></td>
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actinomycetes between 1939 and 1943 and demonstrated the elaboration by such organisms of a number of antibiotics, however none was clinically useful. In 1943 a strain of *Streptomyces griseus* was isolated that elaborated a potent antibacterial substance, streptomycin. The discovery of this new antibiotic was revealed in 1944 by Waksman, Schatz and Bugie.² It inhibited the growth of tubercle bacillus and a number of gram negative and gram positive microorganisms in vitro and in vivo. In less than two years, extensive bacteriological, chemical and pharmacological investigations of streptomycin had been carried out and its clinical usefulness was established. Streptomycin was found to have 2-methylamino-2-deoxy-L-glucose³ in a glycoside linkage and thereby became the first known "aminoglycoside antibiotic." The structure and numbering pattern of streptomycin are shown in Figure 1. The main disadvantages to streptomycin therapy were rapid occurrence of resistant strains of bacteria and toxicity. Today it is usually administered in combination with other antimicrobial agents only for the treatment of relatively unusual diseases, such as certain types of bacterial endocarditis, tularemia and plague; it is also used in tuberculosis in combination with isoniazide and p-aminosalicylic acid.

In continuing the search for better therapeutic agents, Waksman and Lechevalier⁴ isolated a soil organism, *Streptomyces fradie*, which produced a new antibiotic named neomycin. The isolated antibiotic consists of three components, neomycin A (neamine) ², neomycin B ³ and neomycin C ⁴. Neamine ² consists of 2'-deoxystreptamine and 2,6-diamino-2,6-dideoxy-D-glucose. Neomycin B ³ and neomycin C ⁴ differ
Figure 1. Streptomycin
only in the stereochemistry of the aminomethyl group attached to C-5\textsuperscript{''} in the ring D sugar residue. The structure was elucidated on the basis of chemical degradation,\textsuperscript{5} NMR studies\textsuperscript{6} and Reeve's copper complexing method.\textsuperscript{7} The component sugars, neosamine B\textsuperscript{8} (2,6-diamino-2,6-dideoxy-L-idose), neosamine C\textsuperscript{8-11} (2,6-diamino-2,6-dideoxy-D-glucose) and 2-deoxystreptamine\textsuperscript{12,13} had been synthesized. Synthesis of neomycin C was reported by Umezawa and coworkers.\textsuperscript{14} The structure and numbering system is shown in Figure 2.

In 1959 Haskel and coworkers\textsuperscript{15} isolated paromomycin from the culture broth of \textit{Streptomyces rimosus}. The structure was determined by chemical degradation procedure, NMR\textsuperscript{13} and mass spectral techniques.\textsuperscript{16}

In 1971 Mori and coworkers\textsuperscript{17} discovered lividomycins A and B and D-mannosyl paromomycin from \textit{Streptomyces lividus}.

The neomycins, paromomycins and lividomycins showed good antibacterial activity against a variety of gram negative and gram positive organisms. Unfortunately, relatively high renal toxicity and ototoxicity restricts their clinical use for the treatment of systemic infections. Neomycin has been widely used for topical application in burns, wounds, ulcers and infected dermatoses. Paromomycin is used for treatment of intestinal amebiasis and bacterial infections.

An entirely new structural class of aminoglycoside antibiotics, kanamycins were isolated in 1957 by Umezawa and coworkers\textsuperscript{18} from the culture broth of \textit{Streptomyces kanamyceticus}. Paper chromatography proved that kanamycin actually existed as a complex mixture of three closely related antibiotics which were given the trivial designations
Figure 2. Neomycins and Related Antibiotics

<table>
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<th>Neomycin</th>
<th>R₁</th>
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<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
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<tr>
<td>A (Neamine)</td>
<td>H₂N</td>
<td>O</td>
<td>H₂N</td>
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<tr>
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<td>OH</td>
<td>H</td>
<td>CH₂NH₂</td>
<td>H</td>
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<tr>
<td>A</td>
<td>OH</td>
<td>H</td>
<td>CH₂NH₂</td>
<td>H</td>
<td>D-Mannosyl</td>
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<tr>
<td>B</td>
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<td>H</td>
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<td>H</td>
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kanamycin A, kanamycin B and kanamycin C. All were found to be active in varying degrees against *Mycobacterium tuberculosis* and a number of gram negative bacteria. The structures and numbering pattern are shown in Figure 3.

Gentamicin C was isolated by Weinstein and coworkers at the Schering Corporation in 1963. Two gentamicin producing cultures, *Micromonospora purpurea* and *Micromonospora echinospora*, have been described. The gentamicin C is a mixture of gentamicin C₁, gentamicin C₁₃ and gentamicin C₂. The structural studies were done by Cooper and coworkers and Schaffner and colleagues. The structure and numbering pattern are shown in Figure 4.

Gentamicins C₁, C₂ and C₁₃ are composed of 2-deoxystreptamine, a branched-chain aminopentose, garosamine and a diaminohexose purpurosamine. Commercial preparations of gentamicin C are comprised of 23% of C₁, 34.6% of C₁₃ and 37.1% of C₂. Gentamicin C, like other aminoglycoside antibiotics, has weak activity against streptococci, but it is highly active against gram positive bacteria and *M. tuberculosis*. It is more active than the previously described aminoglycosides against gram-negative bacteria. It also demonstrated a marked inhibitory effect against most strains of *Pseudomonas aeruginosa*.

Sisomicin, reported by Weinstein and coworkers in 1971, was isolated from the fermentation broth of *Micromonospora inyoensis*. The sisomicin structure is composed of 2-deoxystreptamine, garosamine and 2,6-diamino-2,3,4,6-tetrahydroxy-D-glycero-hex-4-enose which corresponds to a dehydropurpurosamine. Its structure was assigned
Figure 3. Kanamycins and Tobramycin

<table>
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<th>R₂</th>
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<tr>
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<tr>
<td>11</td>
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<td>OH</td>
</tr>
<tr>
<td>16</td>
<td>Tobramycin</td>
<td>NH₂</td>
<td>H</td>
</tr>
</tbody>
</table>
Figure 4. Gentamicins
by biogenetic analogy to the gentamicins. The structure of sisomicin is shown in Figure 5. Sisomicin is a very potent broad spectrum antibiotic active against various strains of bacteria and effective against Rickettsia akari infection in mice.

Stark and colleagues reported the isolation of tobramycin, which was separated from an antibiotic complex nebramycin, produced by Streptomyces tenebrarius. Tobramycin has a broad spectrum of activity against a variety of bacteria including Pseudomonas and Proteus. The structure of tobramycin, reported by Koch and Rhoades, is shown in Figure 3. Tobramycin is 3'-deoxykanamycin B. The partial synthesis of tobramycin was accomplished by Umezawa and coworkers from kanamycin B.

Ribostamycin was isolated by Shomura and coworkers from the culture filtrate of Streptomyces ribosidicus in 1970 in Japan. Its structure was elucidated by Akita and colleagues. On methanolysis, ribostamycin gives neamine and a methyl D-riboside. The furanoid nature of the D-ribose moiety was shown by the results of periodate oxidation of tetra-N-acetylribostamycin. It is structurally related to neomycin B and C but it is without the ring D of those compounds. It is a relatively non-toxic compound active against many gram-negative bacteria, but not Pseudomonas. It is used clinically in Japan and in a limited number of other countries. The structure of ribostamycin is shown in Figure 6.

Butirosins A and B were isolated by Woo and coworkers of Parke-Davis from the culture broth of Bacillus circulans and
Figure 5. Sisomicin
Figure 6. Ribostamycin and Related Antibiotics
characterized as aminoglycosides structurally similar to ribostamycin. This was the first report of an aminoglycoside being produced by a member of the order Eubacteriales, as all other antibiotics of this type were isolated from streptomycetacea, organisms assigned to the order Actinomycetales. Acid hydrolysis studies of butirosin A (the major component) confirmed the liberation of D-xylose, neosamine C (2,6-diamino-2,6-dideoxy-D-glucose), 2-deoxystreptamine, neamine and a novel amino acid \((S)(-)^{4-}\text{amino-2-hydroxybutyric acid.}\) Mass spectral studies showed that three of the structural units, \((S)(-)^{4-}\text{amino-2-hydroxybutyric acid, neosamine C and a pentose are individually attached, as amide and O-glycoside, to the fourth unit, 2-deoxystreptamine.}\) The unusual amino acid, \((S)(-)^{4-}\text{amino-2-hydroxybutyric acid, in amide linkage with the C-1-amino group in 2-deoxystreptamine was the first of its kind reported in the case of 2-deoxystreptamine containing aminoglycoside antibiotics. The structure of butirosins A and B are shown in Figure 6. A synthesis of butirosin B from ribostamycin has recently been reported by Umezawa and coworkers. Butirosins are broad spectrum antibiotics including significant activity against Pseudomonas. They have a toxic potential less than one-half that of ribostamycin and one-eighth that of gentamicin C.

H. Umezawa and coworkers isolated kasugamycin and reported its unique structure (Figure 7). Kasugamycin is produced by Streptomyces kasugaeensis. Unlike the other aminoglycoside antibiotics, kasugamycin does not have an aminocyclitol moiety. It showed useful
Figure 7. Kasugamycin and Spectinomycin.
antifungal activity and has found commercial application as an inhibitor of *Percularia oryzae* in rice plants.

Spectinomycin \(^{21}\) was isolated from *Streptomyces spectabilis* at the Upjohn Company \(^{44}\) and from *S. flavopesiscus* by workers from the Abbott Laboratories. \(^{45}\) It is moderately active against a wide variety of bacteria and it is used clinically for the treatment of uncomplicated gonorrhea. The structure was determined by Wiley and coworkers \(^{46}\) in 1963 and the stereochemistry was confirmed by X-ray crystallographic analysis. \(^{47}\)

Nara and coworkers \(^{48}\) recently isolated the seldomycins, a new class of aminoglycoside antibiotics from the fermentation broth of *Streptomyces hofuensis*. The antibiotic mixture consists of four factors designated seldomycins 1, 2, 3 and 5. These compounds exhibit a broad spectrum of antibacterial activity, with factor 5 being the most active. The structures of these compounds are shown in Figure 8.

Fortimicins are a new group of potent aminoglycoside antibiotics discovered by T. Nara and coworkers \(^{49,50}\) in 1977. They are produced by fermentation of a novel micromonospora species, *M. olivoasterospora*. The structures of fortimicins have been elucidated by Egan and coworkers \(^{51}\) and Shirhata and coworkers. \(^{52}\) They consist of \(\psi\)-disaccharides of purpurosamine C or 6-epipurpurosamine B and a novel 1,4-diaminocyclitol (Figure 9).

H. Umezawa and coworkers \(^{53}\) in 1979 isolated spraracin A and B. These antibiotics are similar to fortimicins and have a diaminocyclitol moiety.
Figure 8. Seldomycins
Figure 9. Fortimicins and Sporamycins
Mechanism of Action ⁵⁴, ⁵⁵

The aminoglycoside antibiotics are taken up by bacteria in an active transport process. They bind to bacterial ribosomes and inhibit protein synthesis. They are bactericidal in action. The principal observed effects are misreading of the genetic code in translation on polysomes and inhibition of protein synthesis mediated by effects on ribosomal subunits.

The primary intracellular site of action ⁵⁶ of the aminoglycoside antibiotics is the 30 S ribosomal subunit which consists of 21 proteins and a single 16 S molecule of RNA. They also disrupt the normal cycle of ribosomal function by interfering, at least in part, with the first step of protein synthesis that occurs at the ribosome (initiation). However, further studies showed that the antibiotic does not prevent the formation of initiation complexes. Instead, it interacts with free ribosomes (or their initiating subunits) in a way that allows initiation complexes to form, but with distorted information. They not only fail to continue into chain elongation, but they spontaneously dissociate with a half life at 37° of 3-5 minutes. ⁵⁷

Another effect of aminoglycoside antibiotics is their capacity to induce misreading of the genetic code of m-RNA templates. This effect causes incorrect amino acid incorporation into the growing polypeptide chain. ⁵⁸ The aminoglycoside antibiotics vary in their ability to cause misreading and this property presumably depends on differences in their affinities for specific ribosomal proteins.
Antibacterial Activity of the Aminoglycoside Antibiotics

Aminoglycoside antibiotics are broad spectrum compounds capable of inhibiting both gram negative and gram positive organisms. Their principal use is against infections caused by aerobic gram negative bacilli such as *E. coli*, *Klebsiella*, *Proteus* and *Enterobacter*. Few aminoglycosides are capable of inhibiting *Pseudomonas aeruginosa*. Many aminoglycoside antibiotics are also active against *Mycobacterium tuberculosis*.

Aminoglycoside antibiotics are highly polar cations at physiological pH; they are thus poorly absorbed from the intestinal tract. This lack of absorption is utilized in certain clinical situations: for example, to achieve sterilization of the gut prior to bowel surgery in order to minimize the risk of post operative wound infection. Intramuscular injection is the normal mode of administration in cases of systemic infections. Aminoglycoside antibiotics are often drugs of choice for septicemia and other serious infections of the central nervous system, urinary tract, respiratory tract, gastrointestinal tract, skin, bone and soft tissues (burn) caused by sensitive gram negative bacilli. In case of infections caused by *Pseudomonas aeruginosa*, carbenicillin is often administered concommitantly with a suitable aminoglycoside antibiotic, since the action of the two antibiotics is synergistic.
Limitations of Aminoglycoside Antibiotics

The limitations of aminoglycoside antibiotics are the common ones among therapeutic agents. They are:

1. Toxicity

Toxicity

All aminoglycoside antibiotics are potentially toxic. They can produce nephrotoxicity, which is generally reversible when the drug is withdrawn, and ototoxicity, which can affect both the balance and hearing functions and which is often irreversible. Toxicity can be minimized by careful control of antibiotic concentrations in plasma.

Nephrotoxicity

Very high concentrations of aminoglycoside antibiotic accumulate in the renal cortex and urine and thus cause nephrotoxicity. Neomycin is the most nephrotoxic aminoglycoside antibiotic and it is no longer administered systemically for this reason. Gentamicin appears to be the most nephrotoxic of the commonly used drugs. Elderly patients are more susceptible to nephrotoxic effects of these drugs, as are patients with shock, dehydration, pre-existing renal disease or oliguria.

Nephrotoxicity from aminoglycosides is essentially a form of acute tubular necrosis and is essentially manifested by the inability to concentrate the urine. This damage does not usually occur until after at least five to seven days of therapy, it progresses as administration of the drug is continued. The urine then characteristically
contains protein and tubular cell casts, followed by reduction in glomerular filtration rate, with elevation of aminoglycoside, creatinine and urea in plasma. The histological features are those of acute tubular damage with secondary interstitial damage. These changes are usually reversible and regeneration of renal cells occurs if the drug is discontinued.  

Ototoxicity  

Both auditory and vestibular dysfunction can follow the administration of any of the aminoglycosides. Drug accumulates in the perilymph of the inner ear especially when the concentration in plasma is very high and diffusion back into the blood stream is slow. The half lives of aminoglycosides are five to six times longer in the otic fluids than in plasma. The degree of permanent dysfunction correlates with the number of destroyed or altered sensory hair cells and this is directly related to sustained exposure to the drug. Repeated courses of aminoglycosides, each resulting in the loss of more cells, can lead to deafness. Older patients are more susceptible to ototoxicity than young patients. It is recommended that patients receiving aminoglycosides be carefully monitored for ototoxicity, because the initial symptoms may occur several weeks after the therapy is discontinued.

Bacterial Resistance  

The second important problem with aminoglycoside antibiotics involves the development of resistance in previously sensitive bacteria. Professor H. Umezawa of Japan and Professor Davies of the U.S.A. have investigated this type of resistance.
Resistance to aminoglycosides can arise in three ways:  

1. By alteration of the ribosomal binding site such that it can no longer bind the drug. Resistance of this type occurs by natural ribosomal mutation, but leads to high levels of resistance only to compounds of the streptomycin group. The reason for this seems to be that streptomycin has only one effective binding site on the ribosome whereas other aminoglycoside antibiotics have several effective binding sites.

2. Impermeability of the organism to the antibiotic. This is a common mode of bacterial resistance for antibiotics of several classes. Aminoglycoside resistance due to this mechanism is found only in strains of *Pseudomonas aeruginosa*.

3. The most important mechanism of resistance to aminoglycoside antibiotics involves inactivation of the antibiotic by bacterial enzymes. The clinically isolated strains of bacteria which are resistant to one or more of the aminoglycosides have been shown to possess enzymes that chemically modify the aminoglycoside via acetylation, phosphorylation or adenylylation to produce inactive derivatives. Such resistance can be transferred between organisms of the same species or between organisms of different species by means of extranuclear genetic materials called R-factors or plasmids. To date, some 12 different enzymatic modifications have been characterized in clinical isolates of gram negative and gram positive bacteria (Table 2).
Table 2. Enzymes Modifying Aminoglycoside Antibiotics Found in Resistant Gram Negative and Gram-Positive Isolates

<table>
<thead>
<tr>
<th>Modification</th>
<th>Enzyme</th>
<th>Typical Substrates</th>
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<tbody>
<tr>
<td>Acetylation</td>
<td>AAC(2')</td>
<td>Gentamicin, Tobramycin</td>
</tr>
<tr>
<td></td>
<td>AAC(6')</td>
<td>Tobramycin, kanamycin, amikacin, neomycin, gentamicin C&lt;sub&gt;1α&lt;/sub&gt;</td>
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<tr>
<td></td>
<td>AAC(3)</td>
<td>Gentamicin, tobramycin, kanamycin</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>APH(3')</td>
<td>Kanamycin, neomycin</td>
</tr>
<tr>
<td></td>
<td>APH(3&quot;)</td>
<td>Streptomycin</td>
</tr>
<tr>
<td></td>
<td>APH(2&quot;)</td>
<td>Gentamicin</td>
</tr>
<tr>
<td></td>
<td>APH(5&quot;)</td>
<td>Ribostamycin</td>
</tr>
<tr>
<td>Adenylylation</td>
<td>AAD(4')</td>
<td>Amikacin, tobramycin, kanamycin</td>
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<tr>
<td></td>
<td>AAD(2&quot;)</td>
<td>Gentamicin, tobramycin, kanamycin</td>
</tr>
<tr>
<td></td>
<td>AAD(3&quot;)</td>
<td>Streptomycin, spectinomycin</td>
</tr>
<tr>
<td></td>
<td>AAD(6)</td>
<td>Streptomycin</td>
</tr>
</tbody>
</table>
**Acetylation:** The enzyme aminoglycoside antibiotic acetyltransferase (AAC) of different species utilizes acetyl-coenzyme A to transfer an acetyl group to amino groups of antibiotics. They are found in strains of *E. coli*, *P. aeruginosa*, *E. cloacae*, *E. aerogens* and *P. stuartii*.

**Phosphorylation:** The enzyme aminoglycoside antibiotic phosphotransferase of different species utilizes an ATP molecule to transfer a phosphate group to a hydroxyl group of an antibiotic. These enzymes are found in strains of *E. coli*, *P. aeruginosa*, *E. cloacae*, *S. aureus* and *K. pneumoniae*.

**Adenylation:** Besides the above two major inactivating enzymes, a lesser modifying enzyme, aminoglycoside antibiotic adenylyltransferase, inactivates antibiotics by adenylylation of a hydroxyl group. It is found in *E. coli*, *K. pneumonia*, *E. cloacae* and *P. aeruginosa*.

Inactivation of kanamycin A is shown in Figure 10.

Aminoglycoside antibiotics have established an important position in the antibiotic armamentarium of the clinician. The realization that the spectrum of microorganisms susceptible to the aminoglycoside could be significantly broadened if derivatives refractory to the action of such enzymes were obtained has led to an increased effort to find new enzyme resistant antibiotics from natural sources, i.e., through soil screening programs. In addition, knowledge of inactivation products has encouraged the chemist to develop semisynthetic
Figure 10. Enzymatic Inactivation of Kanamycin A.
aminoglycosides which are less susceptible to inactivation. The first examples of new antibiotics specifically designed in this way are dibekacin \(^{72}\) (3',4'-dideoxykanamycin B) and amikacin. \(^{73}\) Dibekacin does not have 3' and 4'-hydroxyl groups which were susceptible to enzymatic inactivation. Amikacin, a derivative of kanamycin A which has a 1-N-[(S)-4-amino-2-hydroxybutyryl] side chain, is resistant to aminoglycoside acetyl transferase (3) and aminoglycoside adenyl transferase (2''). It is believed that this (S)-AHBA side chain protects amikacin from inactivation. This concept will be discussed in the kanamycin analog part.
INTRODUCTION TO STREPTOMYCINS

Streptomycin was discovered by Waksman and coworkers in 1944 from *Streptomyces griseus*. Carter and colleagues obtained relatively pure streptomycin by charcoal adsorption, followed by elution with methanolic hydrochloride and finally by chromatography using alumina. It was shown to be effective against tubercle bacilli and a number of gram negative and gram positive microorganisms *in vitro* and *in vivo*. By late 1945, the medical world was reporting miraculous results in treating infections caused by *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas tularensis* and many other microorganisms. However, it has no significant activity against viruses, fungi or the protozoan *Entamoeba histolytica*. Garrod and O'Grady studied the spectrum of activity of streptomycin and showed that it has a remarkable range of effectiveness against gram negative bacteria other than *P. aeruginosa* and certain strains of *Proteus sp.* and that it also possesses some activity against some gram positive bacteria. Thus, Waksman was eminently successful in his calculated effort to find an antibiotic which was inhibitory for many of those organisms which are refractory to the action of naturally produced penicillins. The structure of streptomycin was first proposed by Kuehl and coworkers, who showed that 2-methylamino-2-deoxy-L-glucose was a
component of streptomycin. In the streptomycin family were placed
streptomycin, dihydrostreptomycin, mannosidostreptomycin, hydroxy-
streptomycin, N-demethylstreptomycin, bluensomycin (glebomycin) and
their derivatives (Figure 11).

By classical degradation methods, streptomycin and other members
of this group were shown to consist of three moieties. Streptidine,
streptose or its reduced or oxidized form and 2-methylamino-2-deoxy-
L-glucose. Streptidine, which was considered to be the aglycone of
the antibiotic, was identified as 1,3-diguanyl-1,3-dideoxyinositol,
In the case of bluensomycin the aglycone is bluensidine (glebidine).
The streptose unit was identified as 3-formyl-5-deoxy-L-xylene.
Streptose and the third component, 2-methylamino-2-deoxy-L-glucose
composed the disaccharide streptobiosamine.

The glycosidic linkage between streptose and 2-deoxy-2-
(methylamino)-L-glucose was identified as α-L on the basis of nuclear
magnetic resonance studies done by Rinehart and coworkers. 82

The absolute configuration of the streptidine moiety was
determined by Dyer and Todd 83 by applying Reeve’s copper complexing
method to N,N’-diacetyl-4-deoxy-streptamine derived from streptomycin
by degradation. Moreover, the same conclusion was revealed by
Tatsuok and coworkers 84 on applying Reeve’s method to 2,6-di-0-
methylstreptamine derived from dihydrostreptomycin; this observation
revealed that the streptobiosamine moiety is attached to C-4 in the
R configuration. The absolute structure of streptomycin was confirmed
by an X-ray analysis 85 of streptomycinxime selenate.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
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<th>R3</th>
<th>R4</th>
<th>R5</th>
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<tr>
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<td>Me</td>
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<td>H</td>
</tr>
<tr>
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<td>Me</td>
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</tr>
<tr>
<td>23 Mannosidostreptomycin</td>
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<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>D-Manno pyranosyl NH-C-NH₂</td>
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<tr>
<td>24 Hydroxystreptomycin</td>
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<td>CH₂OH</td>
<td>Me</td>
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<td>25 Dihydrodeoxystreptomycin</td>
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<td>28 Bluensomycin</td>
<td>CH₂OH</td>
<td>OH</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
</tr>
</tbody>
</table>

Figure 11. Streptomycins
Bleunsomycin had been shown to be an analog of dihydrostreptomycin in which the streptidine moiety of the latter is replaced by bluensidine; however, its absolute configuration was not established. Barlow and Anderson made a polarimetric study of the interaction of the tetraamine copper reagent with bleunsomycin, its deamidinodecarbamoyl derivative and several reference compounds of the streptomycin series and the results supported the absolute structure as shown in Figure 11.

**Biosynthesis**

The biosynthesis of streptomycin has been extensively studied. The knowledge of carbohydrate entities of streptomycin implied that glucose was the ultimate precursor of the antibiotic. Possible biosynthetic pathways have been established through fermentation experiments using labeled precursors. 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 12), notably those of Horner, Rieder, Baddily and Weiner. This was confirmed by a $^{13}$C labeling experiment by Rinehart and coworkers.

The position of incorporation of specifically labeled glucose has also been determined for streptose. $^{1-14}$C-glucose labeled streptose at its C-1 position. The formyl group at the C-3' of streptose was derived from C-3 of glucose. Labeling studies by Briton demonstrated that when glucose was fed into *S. griseus* fermentation broths it was
Figure 12. Carbons of streptomycin labeled by specifically labeled D-glucose
incorporated into myo-inositol as well as into the streptidine moiety. This suggested that myo-inositol was a precursor of streptidine.

The comprehensive studies by Walker at Rice University indicate why streptamine is not incorporated. As shown in Figure 13, 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 carbamidinylated to give streptidine phosphate, which is incorporated into streptomycin. Thus added streptidine can be converted into streptomycin via streptidine phosphate, but streptamine is found nowhere on the biosynthetic pathway.

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 presence of added streptidine.

Some of the intermediates between glucose and dihydrostreptose also are known as a result of the work by Grisebach, who demonstrated that [14C] glucose-d-TDP was converted to 4-keto-4,6-dideoxyglucose-dTDP, which in turn was converted to dihydrostreptose-dTDP as shown in Figure 14. Partial purification of the enzymes involved in these bioconversions has been carried out and the stereochemistry of the glucose-dTDP → 4-keto-4,6-dideoxyglucose-dTDP conversion has been investigated recently by Floss who showed that C-4 to C-6 hydride transfer occurs with inversion at C-6.
Figure 13. Biosynthetic pathway from D-glucose to streptidine
Figure 14. D-Glucose conversion
There are a few links still missing in the biosynthetic pathway to streptomycin. Nothing is known about intermediates between glucose and N-methyl-L-glucosamine, in which every asymmetric center of glucose has been inverted. However, recent studies with streptidine-t-phosphate and dihydrostreptose d-TDP have provided evidence that it is the glycosidic bond between streptose and streptidine which is formed first in streptomycin.

**Mode of Action**

The mode of inhibitory action of the streptomycin group of antibiotics has been exhaustively investigated. Streptomycin is bactericidal in nature. Protein biosynthesis is rapidly inhibited by streptomycin with subsequent changes in polysome patterns: the disappearance of polysomes and accumulation of monosomes carrying mRNA and f-met-tRNA but incapable of protein synthesis. The streptomycin binds to the 30S ribosomal unit and is critical for protein synthesis. Traub and his colleagues and Strachelin and Meselson pinpointed the site of action in the 23 core particle of the 30S ribosomal unit. Ozaki and coworkers showed that a particular ribosomal protein (S12) had a great effect on the capacity of ribosomes to bind 3H-dihydrostreptomycin. The S12 protein also seemed to determine the course of streptomycin activity in sensitive cells as well as the occurrence of misreading which caused the incorporation of a wrong amino acid in the peptide chain.
Bacterial Resistance

Resistance to streptomycin can arise in three ways:

1. By alteration of the ribosomal binding site such that it can no longer bind the drug. Single step mutations in *E. coli* or *Pseud. aeruginosa* that result in the substitution of a single amino acid in a crucial ribosomal protein may prevent binding of the drug. This type of resistance occurs only for the antibiotics of streptomycin family.

2. Impermeability\(^{105}\) of the organism to the antibiotic. This type of resistance generally is found only in the strains of *Pseudomonas aeruginosa*.

3. The most important mode of streptomycin resistance involves inactivation of the antibiotic by R-factor mediated enzymes. In 1961 H. Umezawa\(^{106}\) and coworkers reported that a particular *E. coli* (K12 ML 1629) carried an R-factor that coded for inactivation of streptomycin through an enzymatic process which requires ATP. Umezawa further identified the enzyme as adenylyltransferase, and the inactivated product as adenylylstreptomycin, as shown in Figure 15. Davies and coworkers\(^{107}\) and Umezawa and colleagues\(^{108}\) determined the structure of this product and proposed that the adenylylation occurred at the C-3 hydroxyl group of 2-methylamino-2-deoxy-L-glucose. Further biochemical studies by Smith and coworkers\(^{109}\)
Figure 15. Inactivation sites of the streptomycin family
showed that dihydrostreptomycin and bluensomycin inactivation was also catalyzed by the same enzyme.

Another R-factor containing strain *E. coli* (JR35), was found to be resistant to streptomycin. It was later identified to contain a phosphorylating enzyme which inactivated streptomycin at the same 3'-OH group as the streptomycin adenylyltransferase. The streptomycin phosphotransferase was also isolated in other strains, *Pseudomonas aeruginosa* (TL-13, TL-11, Cape 18, etc.) by S. Mitsuhashi and others. As expected, inactivated products of both enzymes were shown to be incapable of binding to streptomycin sensitive ribosomes.

**Synthesis of Dihydrostreptomycin and Streptomycin**

The synthesis of two of streptomycin's components, streptidine and 2-methylamino-2-deoxy-L-glucosamine, had been reported by Polglase and coworkers and by Kuehl and colleagues. The unique branched sugar "streptose" was synthesised by Dyer in 1965 and by Paulson and coworkers in 1972.

The first total synthesis in the streptomycin series was achieved in 1974 by S. Umezawa and coworkers. There are apparently two basic approaches to dihydrostreptomycin; one involves the synthesis of dihydrostreptobiosamine and subsequent coupling of its derivatives with protected streptidine, and the other involves the coupling of protected derivatives of dihydrostreptose and streptidine followed by formation of the second glycosidic linkage with a protected
derivative of 2-deoxy-2-methylamino-L-glucose or 2-amino-2-deoxy-L-glucose. Umezawa chose the former route because of the α-linkage between dihydrostreptose and streptidine is less stable for hydrolytic treatment than that between 2-deoxy-2-methylamino-L-glucose and dihydrostreptose, because of the presence of the methylamino group at C-2 of the latter glycosidic moiety. The isopropylidine derivative of benzyl-L-dihydrostreptoside and 3,4,6-tri-O-acetyl-2-deoxy-2-(p-methoxybenzylidene)-amino-L-glucopyranosyl bromide were condensed through a modified Koenig's-Knorr reaction. Deblocking of the N-protecting group and reacting with methyl chloroformate gave the N-(methoxycarbonyl) derivative. Then the necessary deblocking of all the protecting groups except the benzyl group gave benzyl α-L-dihydrostreptobiosaminide (Figure 16).

The benzylstreptobiosaminide was diisopropylidinated, the isopropylidine group of the 2-amino-2-deoxy-L-glucose was selectively removed, and the free hydroxyl groups were reprotected with benzoyl chloride. The N-methyl group and the adjacent hydroxyl group were blocked by the formation of a cyclic carbamate and the remaining isopropylidino group was replaced with a cyclic carbonate. The desired glycosyl chloride was obtained by hydrolysis of the benzyl glycosidic linkage and treatment of the resulting intermediate with thionyl chloride.

Also, the aglycone was suitably protected. The C-4 and C-5 hydroxyl groups of streptidine were transformed into the corresponding cyclohexylidinoketal with 1,1 dimethoxycyclohexane. The guanidino
Figure 16. Synthesis of dihydrostreptomycin and streptomycin
functions were acetylated and treated with benzyl chloroformate to afford di-N-acetyl-di-N-benzyloxycarbonyl-4,5-O-cyclohexyldiene-streptidine. Condensation of the protected disaccharide and the blocked aglycone resulted in dihydrostreptomycin after the removal of all of the protecting groups. Using mild oxidation conditions, dimethylsulfoxide and N,N'-dicyclohexylcarbodiimide, dihydrostreptomycin was converted into streptomycin.

**Synthetic Modifications (Structure Activity Relationship)**

The peripheral functionalities of streptomycin: the formyl, guanidino and N-methyl groups provided opportunities for chemical transformations. However, the synthesis of suitable derivatives was beset with numerous difficulties. The streptose-streptidine linkage in streptomycin and the dihydrostreptose-streptidine linkage in dihydrostreptomycin were cleaved in acid. Alkaline conditions were also found unsuitable due to the instability of streptomycin. (It readily degrades to maltol in the presence of base. The guanidino groups were unstable to strongbase, whereby strepturea-streptobiosaminide, and finally streptamine-streptobiosaminide were formed.

With the knowledge of these problems, Bartz and coworkers and Peck and his colleagues independently reduced the C-3 formyl group of the streptose moiety of streptomycin using Adams catalyst to get dihydrostreptomycin. The new analog showed activity equal to that of streptomycin.
Consequently, further investigations at the carbonyl functionality were performed. Oxidation of the formyl group with bromine/water resulted in streptomycinic acid, a biologically inactive analog \(^ {127}\) (Figure 17). Fried \(^ {128}\) reacted streptomycin with carbonyl reagents to get the corresponding streptomycin oxime, phenylhydrazone, semicarbazone and thiosemicarbazone, but none was active. The only promising compound, streptomycin isonicotinylhydrazone \(^ {129}\), was shown to be hydrolyzed into its components in aqueous solution. \(^ {130}\) Its activity was equivalent to the combined germicidal activity of streptomycin and isonicotinylhydrazine. \(^ {131}\)

Winsten and coworkers \(^ {132}\) reacted streptomycin with propyl, butyl, octyl, decyl, octadecyl, cyclohexyl, D,L-phenethyl and furfuryl amines. The resulting Schiff's bases were hydrogenated over platinum to yield the corresponding streptomycylamines. These streptomycylamines showed a wide range of antibacterial activity but still lower than the parent compound. \(^ {133}, {134}\)

Winsten \(^ {135}\) also prepared an aminohydrin derivative by the condensation of streptomycin with nitromethane followed by catalytic reduction. His research group later synthesized an amino analog by reduction of the streptomycin oxime. \(^ {136}\) Recently, Heding converted the formyl moiety of streptomycin into a methyl group by formation of the diethylthio ketal and reduction with Raney nickel. \(^ {137}\)

Waksman and colleagues \(^ {138}\) showed that as the pH was increased from 5 to 8, streptomycin's antibacterial action increased several
Figure 17

PtO₂/H₂
Adams catalyst

\[ R = \text{NHC-NH₂} \quad R₁ = \text{CH₂OH} \]

22 Dihydrostreptomycin

\[ R₁ = \text{CO₂H} \]

39 Streptomycinic acid
fold. Eagle and coworkers also reported that the highest activities of the aminoglycoside antibiotics occurred when they were in the nonionized form. The roles of the amino group of the glucosamine moiety and the guanidino groups of the streptidine were evaluated. Dideamidinodihydrostreptomycin and dideguanyldihydrostreptomycin were obtained by alkaline degradation of dihydrostreptomycin. The later was 4000 times less active than dihydrostreptomycin and the former 250 to 500 times less active. The reaction of acetylacetone with the guanidino groups afforded N,N′ bis-(4,6-dimethyl-2-pyrimidinyl) streptamine dihydrostreptobiosamine, which was inactive.

The addition of formaldehyde to streptomycin gave the N-hydroxymethyl derivative which was claimed to have biological activity comparable to the parent compound. The N-acetates of both streptomycin and dihydrostreptomycin were prepared by Comie and coworkers and they were one-fourth as active as the parent compounds. Ikada and Mitsukiko reported the synthesis of N-acetyl, N-caproyl and N-succinyl-bis-(dihydrostreptomycin).

Heding prepared a number of derivatives with variations at the formyl and the N-methylamino groups. Condensation of nitromethane and sequential reduction of the nitro function yielded an aminohydrin. Oxidation with nitrous acid converted this derivative into the corresponding glycol and nitrosation occurred at the N-methylamino group. The compounds were shown to be less active than dihydrostreptomycin. Nitrosation of dihydrostreptomycin introduced the nitroso functionality at the N-methylamino substituent to afford a
derivative which also was inactive. N,N-Dimethyldihydrostreptomycin has been synthesized and its activity was only one-fourtieth that of the parent compound. S. Umezawa and coworkers synthesized 3''-deoxydihydrostreptomycin in 1976. In their route, 2-O-(2-acetamido-4,6-di-O-acetyl-2,3-dideoxy-N-α-L-glucopyranosyl)-3,3'-O-carbonyl dihydrostrepbose was prepared from dihydrostreptobiosamine and transformed into its glycosyl chloride with thionyl chloride. This chloride was then coupled with di-N-acetyl-di-N-benzyloxycarbonyl-4,5-O-cyclohexyldine streptidine. After the separation and removal of protecting groups, the desired compound was obtained. Since the 3''-hydroxyl group involved in the enzymatic inactivation by resistant strains of bacteria had been removed, the analog was active against these organisms and its activity was comparable to a dihydrostreptomycin on sensitive strain. Some of the analogs of streptomycin and their activities are shown in Table 3.

Rationale for New Guanidino Group Analogs

Modification of the guanidino groups has been quite limited. After Polglase and others showed that the degradation of dihydrostreptomycin to dideamidinodihydrostreptomycin and dideguanyldihydrostreptomycin resulted in severe loss of activity, the general consensus was that alteration at these positions was not allowed. All of these degradation products had seriously decreased pKa value and the size requirement at the former guanidino position. The lack of activity of N,N-bis (4,6-dimethylpyrimidinyl)-streptamine-dihydrostreptobiosamine seemed to confirm this. The
Table 3. Antibiotic activity of common analogs of streptomycin

<table>
<thead>
<tr>
<th>Derivative</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Streptomycin</td>
<td>0.4</td>
</tr>
<tr>
<td>2. Dihydrostreptomycin</td>
<td>0.4</td>
</tr>
<tr>
<td>3. Dihydroseryxystreptomycin</td>
<td>0.4</td>
</tr>
<tr>
<td>4. N-Demethylstreptomycin</td>
<td>3.2</td>
</tr>
<tr>
<td>5. Methylstreptomycin</td>
<td>6.3</td>
</tr>
<tr>
<td>6. Streptomycinic acid</td>
<td>100.0</td>
</tr>
<tr>
<td>7. Dihydro-N-demethylstreptomycin</td>
<td>3.2</td>
</tr>
<tr>
<td>8. Mannosidostreptomycin</td>
<td>3.2</td>
</tr>
<tr>
<td>9. N,N-Dimethylidihydrostreptomycin</td>
<td>25.0</td>
</tr>
<tr>
<td>10. Dideamidinodihydrostreptomycin</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Tested against sensitive E. coli strain Novo 01, estimated by the conventional serial dilution method.
pyrimidyl derivative had altered the guanidino's electronic properties by modifying its resonance contributors. It also added a new bulky aromatic constituent.

More recently, Delaware\textsuperscript{147} made a number of dihydrostreptomycins starting from dideamidinodihydrostreptomycin. Condensation of various aldehydes with the primary amino groups of dideamidinodihydrostreptomycin afforded Schiff's base analogs and subsequent reduction with sodium borohydride gave N-aralkyl derivatives. The biological testing of these compounds showed no activity. Delaware also prepared N,N'-di-(methylimidate)-dideamidino-dihydrostreptomycin\textsuperscript{45} by condensation of ethyl acetimidate hydrochloride with dideamidino dihydrostreptomycin, but this analog was also inactive (Figure 18).

Recently Umezawa and colleagues\textsuperscript{148} prepared 1-deamidino, 3-deamidino- and 1,3-dideamidino-dihydrostreptomycin. He found 3-guanidino group of dihydrostreptomycin to be more important than 1-guanidino group for the antibacterial activity because 1-deamidino dihydrostreptomycin showed weak activity in a level similar to bluensomycin whereas the other two showed almost no antibacterial activity. He also prepared 1-N-[(S)-4-amino-2-hydroxybutyryl] and 1-N-[(S)-4-guanidino-2-hydroxybutyryl] derivatives of 1-deamidinodihydrostreptomycin and found no improvement in antibacterial activity over 1-deamidinodihydrostreptomycin. All these findings indicate that a 3-guanidino group is crucial for activity of dihydrostreptomycin and streptomycin. We thought it worthwhile to investigate further the
Figure 18. Dihydrostreptomycin analogs
role of the guanidino group of dihydrostreptomycin, based on the concept that modifications which only slightly alter this function may be allowed. The replacement of a guanidino group with a cyclic guanidino group might be helpful. Also, other minimally modified derivatives should be investigated to extend the scope of the previously reported data. For these reasons, some new compounds in which the role of guanidino groups of dihydrostreptomycin might be interpreted more precisely have been devised. They are shown in Figure 19.

**Synthesis of Analogs**

Polglase\textsuperscript{149} in 1962 reported a two step synthesis of dideamidinodihydrostreptomycin. Dihydrostreptomycin sulfate was treated with a saturated solution of barium hydroxide. After filtering off the precipitated barium sulfate, the filtrate was refluxed for 28 hours or so until evolution of ammonia ceased. Excessive barium hydroxide was neutralized by introduction of carbon dioxide. After filtration and evaporation, 1,3-dideamidinodihydrostreptomycin was isolated as a bicarbonate salt. The free base was obtained by heating at 110° for two hours in vacuum. Utilizing the Polglase method, Delaware\textsuperscript{147} prepared a white compound whose melting point and elemental analysis was not consistent with the reported data. He purified this material by chromatography on an Amberlite\textsuperscript{®} cation exchange GC-50 resin column. Elution of the column with aqueous ammonia resulted in a white crystalline dideamidinodihydrostreptomycin sesquihydrate\textsuperscript{49}. Dideamidinodihydrostreptomycin sesquihydrate was used for the
Figure 19. Proposed analogs of dihydrostreptomycin.
preparation of new analogs. The synthesis of alkyl substituted guanidino analogs of dihydrostreptomycin was attempted through the reformation of the group on dideamidinodihydrostreptomycin 49.

It has been reported in the literature that clonidine 50, a drug used in hypertension, has a cyclic guanidino group. Clonidine was prepared in good yield by the condensation of 2,5-dichloroaniline and 2-methylthio-2-imidazoline hydroiodide. Other workers also employed the 2-methylthio-2-imidazoline hydroiodide to synthesize cyclic guanidino analogs of various amino acids and 2-hydroxyamines 90,91. Utilizing this approach, the following synthetic schemes were proposed (Figure 20).

Since 2-methylthio-2-imidazoline hydroiodide was commercially available through Aldrich Chemical Company, the synthesis of N,N-bis-(2-imidazolinyl)-dideamidino dihydrostreptomycin 46 was attempted by condensing the reactants in DMF. The product was obtained after work-up and purification. The elemental analysis and other data show that only one of the amino groups of 1,3-dideamidino dihydrostreptomycin reacted with 2-methylthio-2-imidazoline. This probably is 1-NH$_2$ position because the 3-NH$_2$ group seems hindered for this bulky group. The structure is shown in Figure 21. The Structure 51 was established by the Δ[M]TACu method. 148 In this method, TACu can only form a complex with a pair of vicinal amino and hydroxyl groups having the relative spatial orientation of a 60° dihedral angle. This method was used by Umezawa 148 to distinguish between dihydrostreptomycin derivatives in which a guanidino group was present either at N-1 and N-3. When
Figure 20. Proposed Synthetic Scheme
Figure 21.
the method was applied to dihydrostreptomycin, TACu formed a complex only at $C''(2)\text{NH}-\text{CH}_3 - C''(3)\text{OH}$ and $\Delta[M]_{\text{TACu}} + 1020^\circ$ was observed. In the case of 3-deamidino dihydrostreptomycin, Umezawa found $\Delta[M]_{\text{TACu}} + 1570^\circ$, which indicates that two copper complexes are formed, one at $C(2')\text{NH}-\text{CH}_3 - C(3)\text{OH}$ and one at $C(2)\text{OH} - C(3)\text{NH}_2$. In the case of 1-deamidinodi-hydrostreptomycin he observed the $\Delta[M]_{\text{TACu}} + 830^\circ$ which indicates the presence of a $C(1)\text{NH}_2$ group because, in this case, complexes can be formed between the amino group at C-1 and $C(6)\text{OH}$ and between the amino group at C-1 and $C(2)\text{OH}$ competitively, thus resulting in cancellation of the $\Delta[M]_{\text{TACu}}$ values for these sites. In our case of 51, which is a similar situation to 3-deamidino dihydrostreptomycin, we found $\Delta[M]_{\text{TACu}} + 1980.6^\circ$. This indicates that copper complexes are formed at two places, one at $C(2)\text{OH} - C(3)\text{NH}$ of the substituted streptamine moiety and another at $C(2')\text{NH}-\text{CH}_3 - C(3')\text{OH}$. The possibility of the alternative structure, 3-(imidazoliny1)-1,3-dideamidinodi-hydrostreptomycin, has been ruled out on the basis of the high observed $\Delta[M]_{\text{TACu}}$ value. In this case the copper complex would have been formed between the amino group at C-1 and $C(2)\text{OH}$ and between the amino group at C-1 and $C(2)\text{OH}$ competitively, thus resulting in cancellation of the $\Delta[M]_{\text{TACu}}$ values in these sites. The structure of 51, therefore was decided to be 1-N-(imidazoliny1)-1,3-dideamidinodi-hydrostreptomycin. N-Methyl-S-methyl isothiourea hydroiodide was prepared by heating methylthiourea with methyl iodide in ethanol. Dideamidinohydrostreptomycin 49 was condensed with N-methyl-S-methyl isothiourea hydroiodide in N,N-dimethylformamide (DMF) to get the desired product 47.
In order to learn more clearly the role of guanidino groups in streptomycins for their antibacterial activity we decided to prepare 1,3-N,N-dimethylidihydrostreptomycin 48. The basic strategy for the synthesis of 48 is shown in Figure 22. In this synthetic scheme, the 2''-amino group of 22 was protected in the very beginning of the synthesis. This was nicely done by first reacting the dihydrostreptomycin free base with benzaldehyde and then reducing with sodium cyanoborohydride which gave the 2''-N-benzyl derivative. This derivative was directly treated with a saturated aqueous solution of barium hydroxide and the resulting 2''-N-benzyl derivative of dideamidinodihydrostreptomycin 52 was obtained in good yield. In the preparation of the carbobenzyloxy derivative 53, we noticed that methanol is not a useful solvent in this reaction. Therefore we used toluene for this purpose. A suspension of the carbobenzyloxy derivative 53 was further treated with lithium aluminum hydride using tetrahydrofuran as solvent. The product 54 was purified using Amberlite IRC-50 (cation exchange resin). S-Methylisothiourea hydroiodide was reacted with 1,3-N,N-dimethyl-2''-benzyl-dideamidinodihydrostreptomycin 54 in DMF. The reaction was smooth but product purification was quite difficult. Excess S-methylisothiourea hydroiodide was removed by ion exchange resin chromatography. The hydrogenation of 55 gave 48 which was purified as described in the Experimental.

Antibacterial Activity

Compounds 47, 48 and 51 were submitted to Bristol Laboratories, Syracuse, New York, for biological evaluation as antibacterial agents. The minimal inhibitory concentration (MIC) of the three
Figure 22. Synthesis of 1,3-N,N-Dimethyldihydrostreptomycin
semi-synthetic dihydrostreptomycin analogs were determined by a standard broth dilution assay with various microorganisms. For all of the assays the medium was Mueller-Hinton Broth.

A broth dilution test involves the incorporation of serial dilutions of an antimicrobial agent into a nutrient broth and then the inoculation with a standard suspension of the microorganisms being tested. Mueller-Hinton medium is considered to be the medium of choice since it shows rather good batch to batch reproducibility for susceptibility, is low in sulfonamide and tetracycline inhibitors, and gives good growth of rapidly growing pathogens. Usually the serial dilutions of the antimicrobial agent are prepared using the standard twofold carryover dilution technique with the broth as the solvent. The serial dilutions are inoculated with the test microorganisms whose final concentration is approximately $5 \times 10^5$ cells/ml of broth. The culture tubes are gently mixed and allowed to incubate for sixteen to eighteen hours at 37° C. The lowest concentration of the compound being tested that produces inhibition of growth after the incubation period becomes the minimal inhibitory concentration (MIC).

For the three dihydrostreptomycin analogs, a broad spectrum of microorganisms was employed as test strains. Compounds 47, 48, and 51 were found inactive as compared to dihydrostreptomycin against the resistant and sensitive strains of bacteria at the dosages tested (Table 4).

The lack of antibacterial activity of compounds 47, 48, and 51 supports Polglase and others' views on the activity of the streptomycin family of antibiotics. Basic degradation of dihydrostreptomycin to
Table 4. Antibacterial Activity of Dihydrostreptomycin Analogs in Mueller-Hinton Broth

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (mg/ml)</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>S. aureus</td>
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<tr>
<td>A-9537</td>
<td>&gt;63</td>
</tr>
<tr>
<td>A22210</td>
<td>&gt;63</td>
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<td>K. pneumoniae</td>
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<tr>
<td>P. aeruginosa</td>
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</table>
dideamidinodihydrostreptomycin eliminates the guanidino groups and antibacterial activity. Alternation of the guanidino group of dihydrostreptomycin does not restore any antibacterial activity. Replacement of the amidino group with an imidazoline group gave (51) which showed almost no activity. The substitution of the methyl groups (47) for one of the hydrogens of amidino group of dihydrostreptomycin destroys any antibacterial activity. The antibacterial activity of 48 implies that an unsubstituted guanidino group is essential for the antibacterial activity of dihydrostreptomycin and its analogs.

**Experimental**

Dihydrostreptomycin sulfate was bought from Sigma Chemical Company, St. Louis, Missouri 63178.

Melting points were determined using a Laboratory Devices Mel-Temp® apparatus with open capillary tubes and are reported uncorrected.

Infrared spectra were obtained on a Beckman IR-33 infrared spectrophotometer.

Nuclear magnetic resonance spectra were determined on a Varian Associates EM-360 spectrometer. Tetramethylsilane and sodium 2,2-dimethyl-2-silapentane-5-sulfonate were used as the internal standard.

Elemental analyses were determined by the Chemalytics Inc., Tempe, Arizona 85282, Galbraith Laboratories Inc., Knoxville, Tennessee 37921 and University Analytical Center, Department of Chemistry, University of Arizona, Tucson, Arizona 85721.
Dideamidinodihydrostreptomycin sesquihydrate (49)

Dihydrostreptomycin sulfate (2.923 g 2.0 mmol) was dissolved in 10 ml of water and 125 ml of saturated solution of barium hydroxide was added. The precipitated barium sulfate was filtered off and the filtrate was refluxed for 30 h (until the evolution of ammonia ceased). The straw-colored reaction mixture was cooled and filtered. The excess of barium hydroxide was neutralized with carbon dioxide. The solution was filtered again and concentrated to a thick gummy syrup. The syrup was dissolved in hot methanol, cooled and filtered. Concentration of the methanol solution to 20% of its original volume, and cooling yielded (1.08 g, 98.5%) of 49 as a white solid, mp 167-171°C (d).

The crude 49 (0.2g) was further purified by charging it on an Amberlite IRC-50 (NH₄⁺) C.P. ion exchange resin column. The column was eluted with 0.1 N ammonium hydroxide solution and all of the fractions which gave a positive ninhydrin test were combined and concentrated to dryness to afford 110mg of 49 as a white crystalline powder; mp 170-171°C (d) (lit. 149 mp 170-171°C); I.R. (KBr) 3350 (Strong, OH, NH), 1580, 1460, 1360 cm⁻¹.

1-N-(2-Imidazolinyl)-1,3-dideamidinodihydrostreptomycin (51)

Dideamidinodihydrostreptomycin 49 (100 mg, 0.19 mmol) was dissolved in dry N,N-dimethylformamide (DMF, 10ml) and 2-methylthio-2-imidazoline (266mg, 1.09mmol) was added and the reaction mixture was heated for 40 h at 100-110°C. The DMF was evaporated under vacuum and the resulting brown solid was dissolved in water and chromatographed on
Amberlite IRC-50 column (cation exchange resin). The resin was first treated with ammonium hydroxide solution and then washed several times with water in order to remove excess ammonium hydroxide (to pH 7.0). The aqueous solution of the brown reaction mixture was charged onto the column and then the column was washed with 30ml of water. On elution with ammonium hydroxide solution (0.1 N to 0.5 N), the fractions from which a sample charred on 5% alcoholic sulfuric acid treatment were combined. On concentration and recrystallization from a water-acetone mixture 51 (68mg, 60%) was obtained as a light yellow solid: mp 200°C (d); IR(KBr), cm\(^{-1}\), 3300 (Strong, OH, NH), 1660 (Strong, C=N), 1360; NMR(CDCl\(_3\)), \(\delta\) 1.2 (d, 3H, 4'-CH\(_3\)), 2.4 (s, 3H, 2''-N-CH\(_3\)), 3.6 (bs, 4H, imidazoliny1). \(^{13}\)C NMR \(\delta\) 164 (C=N, imidazoliny1), 109 (C-1''), 101 (C-1'''), 88.5 (C-4''), 83.0 (C-3''), 45 (CH\(_2\), imidazoliny1), 36 (N-CH\(_3\)), 15 (4'-CH\(_3\)); [M]+\(_{436}\) - 1287°(c 0.6, H\(_2\)O), \(\Delta[M]_{TACu} = 1980.6°\). Anal. calcd. for C\(_{22}\)H\(_{44}\)N\(_4\)O\(_{12}\)·3H\(_2\)O: C, 42.51; H, 7.56; N, 11.27; found: C, 42.23; H, 7.3; N, 11.16.

1,3-Di(methylamidino)-dideamidinodihydrostreptomycin (47) Dideamidinohydrostreptomycin 49 (100mg, 0.19mmol) was dissolved in 5ml of dry DMF and N-methyl-S-methylisothiourea hydroiodide (0.22g, 1.0mmol) was added to it. The reaction mixture was heated in an oil bath at 100-110°C for two days. The DMF was evaporated under vacuum and the brown residue was purified using Amberlite IRC-50 resin. The resin was first treated with 1.0 N ammonium hydroxide and then washed several times with water until
pH 7.0. The neutral resin was packed in a short column and the brown residue was dissolved in water and charged onto the column. The column was first eluted with 30ml of water and then with ammonium hydroxide solution (0.1 N to 0.5 N), the fractions showing a single spot on TLC (n-butanol-acetic acid-water, 4:1:2) were combined. On concentration and precipitation by acetone 47 was obtained as a light yellow solid. It was dried in vacuum at 50°C for 4 h. (67mg, 50%): mp 270°C (dec.; it charred and did not melt up to 300°C); IR (KBr), 3450 - 3360 (OH, NH, broad), 1640 (C = N), 1540 cm⁻¹; NMR (D₂O), δ1.1 (d, 3H, 4'-CH₃), 2.1 (s, 3H, 2''-NCH₃), 2.7 (bs, 6H, amidinomethyl) Anal. calcd. for C₂₃H₄₅N₇O₁₂·H₂CO·2H₂O: C, 40.62; H, 7.10; N, 13.82; found: C, 40.28; H, 6.63; N, 13.87.

Dihydrostreptomycin free base (22)

Dihydrostreptomycin sulfate (2.923g, 2.0mmol) was dissolved in 20ml of water. An aqueous solution of barium hydroxide was added in small portions until pH 12.0 was observed. The white precipitate of barium sulfate was filtered off. The filtrate was filtered again through a diatomaceous earth pad. It was concentrated to a small volume and filtered again. The free base 22 was precipitated from this solution by addition of acetone. The white crystalline base 22 (1.0g, 85%) did not melt up to 300°C; it charred at 240°C, turning black up to 300°C (lit. mp > 300°C); IR (KBr) 3300 (OH, NH, strong), 1660 (guanidino groups, strong), 1640 (C=N, strong); NMR (D₂O) δ1.2 (d, 3H, 4'-CH₃), 2.35 (s, 3H, 2''-N-CH₃).
Dihydrostreptomycin free base 22 (1.0g, 1.7 mmol) was suspended in water and acetonitrile (20ml, 1:1) and benzaldehyde (1.06g, 10mmol) was added. The mixture was stirred for 6 h at room temperature. Sodium cyanoborohydride (1.26g, 20mmol) was added and the stirring was continued overnight with a few drops of acetic acid to keep mixture nearly neutral. The acetonitrile was removed under vacuum and 150ml of saturated barium hydroxide solution was added. The white precipitate was filtered off and the filtrate was refluxed for 30 h (until the smell of ammonia ceased). The reaction mixture was cooled and filtered through a diatomaceous earth pad. The excess of barium hydroxide was neutralized by introduction of carbon dioxide. The precipitated barium carbonate was removed again by filtration and the filtrate was concentrated to a small volume which was chromatographed using Amberlite IRC-50 ion exchange resin. This resin was first treated with 1.0 N ammonium hydroxide solution and then it was washed several times with water until pH 7.0 was obtained and then packed in a short column. The above mentioned solution of the reaction product was charged onto the column. The column was first washed with water and then eluted with ammonium hydroxide with a gradual increase in concentration (0.1 N to 0.5 N). The fractions with a single spot on TLC and charring on spray of 5% sulfuric acid solution were combined and concentrated. The procedure was repeated to remove remaining inorganic salt (barium carbonate). The product 52 was obtained as a carbonate (0.6g, 60%); mp 235-237°C (dec.);
IR (KBr) 3340 (OH, NH broad), 1660, 1550 cm\(^{-1}\); NMR (D\(_2\)O) \(\delta\) 1.2 (d, 3H, 4'-CH\(_3\)), 2.5 (s, 3H, 2''-N-CH\(_3\)), 7.4 (s, 5H, aromatic); Anal. calcd. for \(C_{26}H_{43}N_3O_{12} \cdot 2H_2CO_3 \cdot 4H_2O\): C, 42.80; H, 7.0; N, 5.30; found: C, 42.90; H, 7.42; N, 4.82.

1,3-N,N-Dimethyldeamidino-2''-N-benzylidihydrostreptomycin (54)

Sodium carbonate (1.0g) was added to a suspension of 52 in water and toluene (15ml, 2:1) and the reaction mixture was cooled in an ice-salt bath with constant stirring. A solution of benzyl chloroformate (1.0ml, 95%) in toluene (5ml) was added gradually to the reaction mixture. The stirring was continued further for 2 h at 0° and then reaction mixture was refrigerated overnight. The white solid that formed was collected by filtration and washed with ether and water (1:1). The product 53 was dried overnight under vacuum at 50°C (0.45g, 61%): mp 200°C (d); IR (KBr) 3400 (OH, NH, broad), 1700, 1680 (C=O), 1540 cm\(^{-1}\); NMR (DMSO-d\(_6\)) \(\delta\) 1.1 (d, 3H, 4'-CH\(_3\)), 1.7 (s, 3H, 2''-N-CH\(_3\)), 7.3 (15H, aromatic).

The carbobenzyloxy derivative 53 (0.34g, 0.4mmol) was suspended in dry and freshly distilled tetrahydrofuran (60ml) and cooled in an ice-salt bath. Lithium aluminum hydride (1.0g, Aldrich) was added gradually to the reaction mixture with constant stirring under a nitrogen atmosphere. After half an hour, the ice-bath was replaced with a heating mantle and the reaction mixture was refluxed for three days. The excess of lithium aluminum hydride was destroyed by addition of dilute sodium hydroxide solution at 0°C. The resulting aluminum
salt was filtered off and washed with tetrahydrofuran (2 x 20ml). The filtrate and washings were combined and concentrated. A short column packed with Amberlite IRC-50 ion exchange resin \((\text{NH}_4^+\)) was used for purification. The reaction product was dissolved in a small amount of water and charged onto the column. The column was washed first with 100ml of water followed by ammonium hydroxide solution with gradual increase in the concentration (0.1 N to 0.5 N). The fractions showing a single spot on TLC (n-butanol-acetic acid-water, 4:1:2) were combined and concentrated. The product 54 was further purified by dissolving it in methanol/water and precipitating with acetone to give 0.17g (60%): mp 156-160°C; IR 330-3400 (OH, NH, broad), 1650, 1550 cm\(^{-1}\); NMR \((\text{DMSO-}\text{d}_6\)) \(\delta1.1\) (d, 3H, 4'-CH\(_3\)), 2.5 (s, 9H, 3CH\(_3\)), 7.3 (s, 5H, aromatic). Anal. calcd. for \(\text{C}_{28}\text{H}_{47}\text{N}_3\text{O}_{12}\cdot\text{H}_2\text{CO}_3\cdot2\text{H}_2\text{O}\): C, 47.4; H, 7.6; N, 5.7; found: C 47.4; H, 7.3; N, 5.9.

1,3-N,N-Dimethyl dihydrostreptomycin (48)

A mixture of 54 (0.20g, 0.32mmol) in 5.0ml of DMF and S-methylisothiourea hydroiodide (0.438g, 1mmol) was heated in an oil bath at 100-110°C for 48 h. The reaction mixture was concentrated to dryness at low pressure and the excess of S-methylisothiourea hydroiodide was removed by column chromatography. Amberlite IRC-50 ion exchange resin was treated firstly with ammonium hydroxide and secondly washed with water (to pH 7.0). The ion exchange resin was packed in a short column and the reaction mixture dissolved in the minimum amount of water and charged onto the column. The column was
washed with water (100ml) and then eluted with ammonium hydroxide with a gradual increase in concentration (0.1 N to 0.5 N). The fractions with positive ninhydrin test were combined and concentrated to a small volume. Addition of acetone gave 55, which was precipitated and dried in vacuum. (0.13g, 50%): mp 220°C (dec) blackens: IR (KBr) 3420 (OH, NH, broad), 1700, 1665 (C=N), 1570, 1420 cm⁻¹; NMR (D₂O) δ 1.2 (d, 3H, 4'-CH₃), 2.8 (9H, 3CH₃), 7.4 (5H, aromatic). A mixture of 55 (0.05g) dissolved in 15ml of water and dioxane (10:5), palladium on activated carbon (10%, 0.2g) and acetic acid (0.5ml) was hydrogenated (Parr hydrogenation apparatus) at 50psi for 24 h. at room temperature. The reaction mixture was filtered and concentrated under vacuum. On co-evaporation with toluene (5ml) a white solid was obtained, which was purified by chromatography on Amberlite IRC-50 (NH₄⁺). The resin was treated with ammonium hydroxide solution and washed with water until the pH was 7.0. The reaction product was dissolved in the minimum amount of water and charged onto the column. The column was washed first with water followed by ammonium hydroxide solution with gradual increase in the concentration (0.1 N to 0.5 N). The fractions with a positive ninhydrin test were combined and concentrated. The 1,3-N,N-dimethyldihydrostreptomycin 48 was recrystallized from a water/methanol/acetone mixture and dried in vacuum to give 0.03g (70%): mp 240°C (dec., blackens): IR (KBr) 3360 (OH, NH, broad), 1665, 1650 (guanidino C=N), 1560 cm⁻¹; NMR (D₂O) δ 1.3 (d, 3H, 4'-CH₃), 2.0 (s, 9H, 3CH₃) Anal. calcd. C₂₃ H₄₅ N₀.4 H₂ CO₃·2H₂O: C, 40.62; H, 7.19; N, 13.82; found: C, 40.48; H, 7.04; N, 14.05.
SYNTHESIS OF 1-N-[(S)-4-AMINO-2-HYDROXYBUTYRYL]-
2"-DEOXYKANAMYCIN B

Introduction to Kanamycins

Kanamycin was discovered by Professor H. Umezawa of Japan in 1957. The strain from which the substance was isolated was assigned the name Streptomyces kanamyceticus. Kanamycin showed excellent antibacterial activity against resistant staphlococci and tubercle bacilli and it was much less toxic than either streptomycin or neomycin. Paper chromatographic studies proved that kanamycin actually existed as a mixture of three closely related aminoglycoside antibiotics which were given the trivial name kanamycin A, kanamycin B and kanamycin C. All were found to be active against a number of gram negative and gram positive bacteria and mycobacterium tuberculosis. Their structures and numbering patterns are shown in Figure 23.

The structure proof of kanamycin A was independently provided by Cron and coworkers and Maeda and colleagues. Acid hydrolysis of kanamycin A gave 2-deoxystreptamine (2-DOS) and two aminosugars, namely 3-amino-3-deoxy-D-glucose (kanosamino) and 6-amino-6-deoxy-D-glucose. The glycosidic linkage between kanosamine and 2-DOS in kanamycin A is more resistant to acid hydrolysis than that between the latter and 6-amino-6-deoxy-D-glucose, because of the proximity of the amino group in kanosamine to the glycosidic linkage. Hydrolysis of
Figure 23. Structure of kanamycins and related sugars.
0-permethylated, N-acetylated kanamycin A gave 1,3-di-N-acetyl-5-0-methyl-2-deoxystreptamine which was optically inactive. Periodic oxidation of kanamycin A, followed by hydrolysis, afforded 2-DOS only. These results indicated the C-4,6 substitution pattern in the 2-DOS. The α-D-anomeric linkages indicated by optical rotation and IR spectral studies. The 100 MHz NMR spectrum of kanamycin A was studied by Lemieux and coworkers, who assigned the chemical shift of the anomeric proton at higher field to the 6-0-glycosyl group and the one at lower field to the 4-0-glycosyl group.

Kanamycin B and C differ from kanamycin A only in that the 6-amino-6-deoxy-D-glucose residue of kanamycin A is replaced by 2,6-diamino-2,6-dideoxy-D-glucose in kanamycin B and by 2-amino-2-deoxy-D-glucose in kanamycin C. The diamino sugar of kanamycin B is also present in neomycin and the aminosugar of kanamycin C is also present in the paromomycins.

Kanamycin B was isolated and characterized by Schmitz and coworkers and Wakazawa and colleagues. Both reported that kanamycin B was less active than kanamycin A against mycobacteria but was more biologically active, in general, against both gram positive and gram negative bacteria. Various researchers have reported kanamycin B being at least two-fold more active than kanamycin A against Staphylococcus aureus and Proteus sp. and four times more efficient as a broad spectrum antibiotic. Recently Kuze reported that kanamycin B is very effective against atypical strains of mycobacteria. It has been demonstrated to be superior to kanamycin A as an
ophthalmic antibacterial agent. Its most effective spectrum of activity is against penicillin-resistant Staphylococci, coliform organisms, Salmonella and Shigella.

Kanamycin C, which has a hydroxyl group at C-6' rather than amino group of kanamycins A and B, is a much more weaker antibiotic than the other two. Its activity against S. aureus and E. coli is about one fourth that of kanamycin A and one-eighth of kanamycin B.

Kanamycins, like other aminoglycoside antibiotics, are important chemotherapeutic agents; however, their use is somewhat limited because of toxicity. Possible major side effects include action upon the eighth cranial nerve leading to irreversible high frequency deafness (ototoxicity), and severe nephrotoxicity due to accumulation of the drug in high concentration in the urinary excretory system. Kanamycin B has been reported to be more ototoxic than kanamycin A, with the relative toxicities of the kanamycins expressed as neomycin >> kanamycin B > kanamycin C > kanamycin A, in order of decreasing toxicity.

The structure of kanamycin B was studied by workers at Meiji Seika in Japan. By means of hydrolytic degradation of kanamycin B, they were able to isolate neamine. This information coupled with the findings of Schmitz and coworkers, who established that kanosamine and 2-DOS were components of the kanamycin B molecule, allowed them to write the empirical formula $C_{18}H_{37}N_{5}O_{10}$. Data obtained through periodic acid oxidation of kanamycin B derivatives and spectral studies of the oxidation products enabled them to write the structure as
shown in Figure 23. X-ray data supporting this structural assignment has been provided by Umezawa. 

**Synthesis**

Kanamycin A

Total synthesis of kanamycin A has been reported by two groups of workers. Umezawa and coworkers synthesized it by coupling 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine with 6-amino-6-deoxy-D-glucose by way of their suitable protected derivatives and respectively.

Nakajima and coworkers synthesized kanamycin A by an alternative route. In this route, condensation of 4-O-(6-acetamido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3-di-N-(benzyloxy carbonyl)-2-deoxystreptamine with glycosyl chloride gave protected kanamycin A which led to Kanamycin A (Figure 24).

Kanamycin B

The total synthesis of kanamycin B was achieved by Umezawa and coworkers. The neamine derivative 4-O-(3,4-di-O-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-α-D-glucopyranosyl)-N,N'-dicarbobenzoxy-2-deoxystreptamine was condensed with 3-acetamido-2,4,6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl chloride in presence of Drierite and mercuric cyanide. Subsequent removal of the protecting groups, followed by ion exchange chromatography gave the pure kanamycin B (Figure 24).
Figure 24. Synthesis of Kanamycins
Kanamycin C

Umezawa and coworkers synthesized kanamycin C by coupling a suitably blocked derivative of kanosamine with a suitable blocked derivative of paromamine (Figure 24).

Mechanism of Action

Kanamycins apparently elicit their antibacterial properties in sensitive bacteria via inhibition of protein synthesis in a manner similar to that of streptomycin. Studies by Davies and co-workers indicated that site of interaction of kanamycins was the 30S ribosomal subunit. Inhibition could occur by (a) prevention of attachment of mRNA to the ribosome (b) interference with the movement of mRNA relative to the 30S subunit (translocation) (c) blockage of the aminoacyl (acceptor) site. Kanamycin B produces much higher levels of misreading or inhibition of peptide bond formation than streptomycin, which has been attributed to multiple binding of the drug with 30S ribosomal subunit.

Bacterial Resistance

The biochemical mechanism of aminoglycoside antibiotics resistance have been revealed by Umezawa and others. Most of the clinically isolated strains of bacteria which are resistant to kanamycins as well as other aminoglycosides have been shown to possess enzymes that modify the structures of antibiotics by phosphorylation, acetylation or adenylylation to produce inactive derivatives. The inactivation of kanamycin B by various enzymes is shown in Figure 25. The N-acetylation
APH = Aminoglycoside antibiotic phosphotransferase
AAD = Aminoglycoside antibiotic adenylyltransferase
AAC = Aminoglycoside antibiotic acetyltransferase

Figure 25. Enzymatic Inactivation of Kanamycin B.
or O-adenylylation mechanisms are not very common; however, the 3'-O-phosphorylation is most commonly encountered and all aminoglycoside antibiotics possessing a hydroxyl group at 3' are substrates for the phosphorylating enzyme.

An understanding of the biochemical nature of resistance has allowed the rationale for modifications of many aminoglycoside antibiotics to improve antibacterial activity against resistant microorganisms. Modification of existing aminoglycoside antibiotics can be categorized in three major types of analogs.

(1) Deoxy Analogs. The chemical removal of the hydroxyl group that is inactivated resulted in deoxyaminoglycosides which are effective against phosphorylating enzymes. The naturally obtained antibiotics tobramycin 16 (3'-deoxy), gentamicins 12, 13, 14 (3',4'-dideoxy) and recently discovered sisomicin 15 (3',4'-dideoxy,4',5'dehydro) and verdamicin (6'-C-methyl sisomicin) are highly active against phosphorylating enzymes. The semisynthetic 3'-deoxy-kanamycin B (tobramycin) 16 and 3',4'-dideoxykanamycin B (dibekacin) 65,33,187 are potent antibacterial agents possessing good activity against strains elaborating phosphorylating enzymes. 33,187-190 However, these antibiotics are still labile to those strains which produce aminoglycoside antibiotic adenylyltransferase 191-196 (Figure 25). The 2'-deoxy-gentamicin C2 and 2'-deoxy-gentamicin C1a showed activity against gentamicin-resistant adenylylating strains. However the epimerization of the 2''-position of gentamicin C1 produced epigentamicin C1 which was inactive. 214
Figure 26. Dibekacin and Amikacin.
(2) 1-N-Acyl Analogs. Acylation of the 1-amino group of aminoglycoside antibiotics with (S)-4-amino-2-hydroxybutyryl [(S)-AHBA] chain gave improved antibiotics with a broad spectrum of activity compared with that of the parent antibiotic. The novelty of this chain was learned from the isolation of butirosins. The acylation of ribostamycin with (S)-AHBA gave butirosin B which was identical with natural butirosin B and showed improved activity over the parent ribostamycin. The addition of this side chain not only inhibits the 3'-phosphotransferase, but also 2''-adenylylation and 3-acetylation as well. The most remarkable and probably most active aminoglycoside antibiotic is amikacin, a semisynthetic compound from acylation of kanamycin A with (S)-AHBA (Figure 26). Netilimicin, a 1-N-ethyl derivative of sisomicin, is a potent broad spectrum antibiotic and is refractory to 2''-O-modifying and certain 3-N-acetylating enzymes. More recently Nagabhushan and coworkers synthesized 1-N-[(S)-3-amino-2-hydroxypropionyl]-gentamicin B (Figure 27). It is a broad spectrum antibiotic not susceptible to inactivation by the most important aminoglycoside modifying enzymes. More recently Richardson and coworkers synthesized 1-N-(1,3-dihydroxy-2-propyl) kanamycin B and its activity is about half to that of kanamycin B.

(3) 6'-N-Alkyl Analogs. Each of aminoglycoside antibiotics having a 6'-amino group is vulnerable to acetylation. The alkylation (methylation or ethylation) of this amino group gave an antibiotic that is resistant to acetylation. Gentamicin C₁ and sagamicin (6'-N-methyl) are resistant to acetylation enzymes. Semisynthetic
Figure 27. Semisynthetic Gentamicins
6'-N-methyl-tobramycin and 6'-N-methyl-3,4'-dideoxykanamycin showed respectable antibacterial activity.

**Synthetic Modifications in Kanamycins**

(1) Modifications in the 2-Deoxystreptamine (2-DOS) Moiety

(a) N-Acylation of Kanamycin A. Kawaguchi and coworkers acylated kanamycin A's C-1-NH₂ group with (S)-AHBA and a series of related acyl groups to determine whether such derivatives would acquire any improvement in activity. Among them amikacin [with (S)-AHBA sidechain] was as active as gentamicin but markedly more resistant to enzymatic inactivation. Umezawa and coworkers acylated kanamycin A with β-amino-α-hydroxypropionic acid and the resulting antibiotic was almost as active as amikacin. However, increasing the aliphatic chain to more than five carbon proved to be detrimental to activity. The amino acid used for acylation must contain no less than three and no more than five carbons; it must have an α-hydroxyl group in the (S)-configuration; and it must have an ω-amino group.

Acylation of C-3-NH₂ group of 2-DOS results in a compound devoid of antibiotic activity.

(b) N-Acylation of Kanamycin B. 1-N-[(S)-4-Amino-2-hydroxybutyryl]-kanamycin B and 1-N-[(S)-3-amino-2-hydroxypropyl]-kanamycin B have been prepared. These compounds were found to have superior anti-pseudomonanl activity to the kanamycin B. However their activities against *E. coli* and *S. aureus* were comparable to parent antibiotic.
(c) **N-Acylation of Kanamycin C.** 1-N-(S)-AHBA-kanamycin C\textsuperscript{204} has been made and but its activity was similar to the parent compound.

(2) **Modifications in the 4-Substituent of 2-DOS**

(i) **N-Alkylation.** 6'-N-Methylkanamycin A and 6'-N-methyl kanamycin B have been prepared.\textsuperscript{205} Their activities are very similar to those of the parent compounds. As expected, these alkylated derivatives were no longer susceptible to inactivation by the enzyme AAC(6').

(ii) **Deoxygenation.** 3'-Deoxykanamycin A\textsuperscript{204} is as active as the parent compound against *S. aureus* and *E. coli* and 25 fold more active against *P. aeruginosa*. 3',4'-Dideoxykanamycin B was synthesized by Umezawa and coworkers\textsuperscript{188} and it was about one-half as active as gentamicin against all test organisms. Another compound, 6'-N-methyl-3',4'-dideoxykanamycin B has activity comparable to that of the 3',4'-dideoxykanamycin B and was resistant to inactivation by AAC.

(3) **Modifications in the 6-Substituent of 2-DOS**

(i) **C-3"-Modification.** C-3"-N-Acetyl and C-3"-N-(S)-AHBA derivatives have been prepared.\textsuperscript{204} The former was less active than the parent compound and the latter was inactive.

(ii) **C-6" Modifications.**\textsuperscript{206-208} Deoxygenation at C-6" yielded a methyl derivative of kanamycin A with activity comparable to the parent compound. The corresponding kanamycin B analog has been prepared and found to have activity identical to that of kanamycin B. Kanamycin A derivatives in which C-6" has been modified to give a
carboxyl group, an ethyl ester of this acid or the corresponding amide were prepared by Kobayashi and colleagues and all have considerably less antibacterial activity than the parent compound.

(4) Modification of 2-DOS and the 4-Substituent

3',4'-Dideoxykanamycin B was acylated at the C-1-amine with (S)-AHBA. It has essentially the same potency as amikacin. Acylation of the C-3-NH$_2$ group of 3',4'-dideoxykanamycin B with (S)-AHBA eliminates activity. However when 3',4'-dideoxykanamycin B was acylated at both the C-1 and C-2' amines it showed good activity against S. aureus and E. coli, but not P. aeruginosa.

(5) Modifications in 2-DOS and the 6-Substituent

(i) N-Alkylation. Compounds in which the C-1 and C-3 amino group of 2-DOS and the C-3''-amine of its 6-substituent were N-alkylated with various substituents were prepared, but none was as active as the parent antibiotic.

(ii) Amikacin modified at C-6''. A derivative of amikacin having a C-6''-NH$_2$ group was prepared. It was 1/8 as active as parent compound.

(6) Modifications in the 4- and 6-Substituents

Umezawa and coworkers and Lemieux and colleagues prepared Kanamycin A and B analogs by modification in the 4- and 6-substituents of 2-DOS but none of them possesses significant antibacterial activity.
(7) Modifications in 2-DOS and the 4- and 6-Substituents

Fujii and his coworkers prepared a series of 16 tetra-N-aralkyl derivatives of kanamycin A. Although all compounds had some antimicrobial activity, five of them were significantly active, but none was superior to the parent antibiotic.

Rationale for 1-N-[(S)-4-Amino-2-hydroxybutyryl]-2''-deoxykanamycin B

Although the isolation of new aminoglycoside antibiotics from natural sources continues to be important and certain products of controlled biosynthesis such as the hybrimycins might offer some therapeutic advantages over related natural compounds, it appears that semisynthetic aminoglycosides will achieve an increasingly important position in future years. New semisynthetic aminoglycosides such as amikacin and 3',4'-dideoxykanamycin B have clearly established this trend and a large number of other semisynthetic compounds show interesting activities in preliminary assays.

One of the main problems in preparing semisynthetic aminoglycosides is that of regiospecificity. For example, the kanamycin B molecule contains five amino groups and six hydroxyl groups, which means that complex schemes of blocking and deblocking are required for reaction at a particular functional group. An alternative approach to the development of new aminoglycosides is to begin with a portion of the structure, for example neamine, and couple another sugar by glycosidic linkage. This approach has the advantage of affording new combinations of the constituent parts of aminoglycosides not accessible by
modification of the intact molecule. Its main disadvantage lies in problems of regiospecificity and anomeric specificity in forming the glycosidic bond with the new sugar.

In order to prepare a new derivative of kanamycin B active against kanamycin-resistant bacteria, we decided to synthesize 1-N-[ (S)-4-amino-2-hydroxybutyryl]-2'-deoxykanamycin B 70 (Figure 28). This compound has a neamine unit. This unit is important because it occurs in the structure of a number of antibiotics including kanamycin B, neomycin, ribostamycin, and the butirosins. The compound 70 also has the N-1-[ (S)-4-amino-2-hydroxybutyryl] side chain. It has been demonstrated that the N-1-[ (S)-4-amino-2-hydroxybutyryl] side chain containing antibiotics butirosins and amikacin are resistant to inactivation by certain enzymes. This (S)-AHBA side chain protects the 3-NH₂, 3'-OH and 2''-OH groups of amikacin from the inactivation by AAC, APH and AAD enzymes. The other key unit of the compound is the 2-deoxykanosamine attached to the 6-O-position of the neamine. Since 2''-OH group is susceptible to inactivation by AAD, APH a 2''-deoxy compound should be active against these enzymes producing resistant bacteria.

Synthesis

The basic strategy for the synthesis of 70 was to prepare a neamine derivative that has all groups protected except the 1-amino and 6-hydroxyl groups (Figure 29). This neamine derivative was coupled with a protected (S)-AHBA side chain at N-1-position. The (S)-AHBA
Figure 28. Proposed Analog of Kanamycin B
Figure 29. Retrosynthetic Analysis of Target Compound
coupled neamine derivative then would be subjected to Köenigs-Knorr type coupling with an appropriate bromosugar. Finally, deprotection of all the protecting groups would give the target compound 70.

Our starting material for the synthesis of 70 was commercially available neomycin sulfate 3 (Figure 30). After hydrolysis of neomycin sulfate with methanolic hydrogen chloride, the resulting neamine hydrochloride 2 was precipitated from the solution by the addition of dry ether. Since neamine has four hydroxyl and four amino-groups, we used the Kumar and Remers 212, 213 method for the conversion of neamine into a derivative 74 that has all groups protected except the 1-amino and 6-hydroxyl groups. Thus on treatment with benzyl chloroformate, neamine hydrochloride was converted into tetra-N-benzylloxycarbonylneamine 71 in good yield. Treatment of this derivative with sodium hydride in N,N-dimethylformamide (DMF) led to the formation of a bis (cyclic carbamate) derivative 72. The remaining hydroxyl groups were protected by treatment with benzyl bromide to give 73. Since we wanted to do coupling at the 1- and 6-positions of the 2-deoxystreptamine part of the molecule, we had to hydrolyze preferentially the 5-membered cyclic carbamate. We were successful in doing so by using 0.05M barium hydroxide solution.

The resulting product 74 was treated with an activated (S)-AHBA derivative 76 in dry tetrahydrofuran to give 77. The activated (S)-AHBA derivative 76 was prepared 73 by treatment of N-hydroxysuccinimide with (S)-4-benzylloxycarbonylamino-2-hydroxybutyric acid 75 in presence of N,N'-dicyclohexylcarbodiimide.
Figure 30. Synthesis of 1-N-[S)-4-aminobutyryl]-2'-deoxykanamycin B.

\[ \text{RHN} \text{Z1 COCH2Ph} \]

\[ \text{R} = -\text{C-OCH} \]

\[ \text{Z3 CO2CH2Ph} \]

\[ \text{CH2Ph} \]

\[ \text{R} \]

\[ \text{Z2 CO2CH2Ph} \]

\[ \text{CH2Ph} \]

\[ \text{H} \]

\[ \text{R} \]

\[ \text{Z1 COCH2Ph} \]

\[ \text{Z} \]

\[ \text{NEOMYCIN SULFATE} \]

\[ \text{H2N} \]

\[ \text{4Cl} \]

\[ \text{NM} \]
Figure 30.—Continued

73

\[
\begin{align*}
\text{O} & \text{N} \\
\text{O} & \text{O} \\
\text{R-N-R}_1 & \text{O}
\end{align*}
\]

74

\[
\begin{align*}
\text{R-N-R}_1 & \text{O} \\
\text{R-N-R}_1 & \text{OH} \\
\text{NH}_2 & \text{H}
\end{align*}
\]

75

\[
\begin{align*}
\text{O} & \text{N} \\
\text{O} & \text{O} \\
\text{R-N-R}_1 & \text{O}
\end{align*}
\]

76

\[
\begin{align*}
\text{R-N-R}_1 & \text{O} \\
\text{R-N-R}_1 & \text{OH} \\
\text{NHCOCH}_{\text{O}} & \text{H}
\end{align*}
\]

77

\[
\begin{align*}
\text{O} & \text{N} \\
\text{O} & \text{O} \\
\text{R-N-R}_1 & \text{O}
\end{align*}
\]

78

\[
\begin{align*}
\text{R} & \text{CO}_2\text{CH}_2\text{Ph} \\
\text{R}_1 & \text{CH}_2\text{Ph}
\end{align*}
\]

-\text{(CH}_2\text{)}_2\text{NH}\text{R}
Figure 30.—Continued
(DCC). The (S)-AHBA side chain of \textbf{77} has a free hydroxyl group at its 2-position. This hydroxyl group was protected by making a six-membered cyclic carbamate \textbf{78} by treatment of \textbf{77} with sodium hydride in N,N-dimethylformamide. The appropriate bromosugar \textbf{82} was subjected to Koenigs-Knorr type coupling with \textbf{78} in the presence of mercuric cyanide and Drierite\textsuperscript{®} in dichloromethane to give the coupled product \textbf{83}. This product was treated with barium hydroxide solution, followed by hydrogenation using 10% palladium charcoal as catalyst, to give the desired product \textbf{70}.

The bromosugar \textbf{82} was synthesized as shown in Figure 31. The starting compound methyl 3-trifluoroacetamido-4,6-0-benzylidene-2,3-dideoxy-\textalpha-D-arabinohexopyranoside\textsuperscript{214, 215} \textbf{79} was hydrolyzed by 50% acetic acid to give \textbf{80}. The hydroxyl groups of \textbf{80} were protected as p-nitrobenzoyl esters to give \textbf{81}. Finally, the product \textbf{81} was converted to the bromosugar \textbf{82} by reaction with hydrogen bromide in methylene chloride.

**Antibacterial Activity and Discussion**

Based on the structure-activity relationships and the various modes of bacterial resistance to kanamycins that were described in the introduction, we designed and synthesized a new amikacin analogs, 1-N-[(S)-4-amino-2-hydroxybutyryl]-2''-deoxykanamycin B \textbf{70}. It differs from amikacin in the absence of a 2''-hydroxy group and a 2'-amino group in place of a 2'-hydroxy group. It is predicted that this compound will have antibacterial activity against most of the strains that are resistant to the kanamycins. A sample has been submitted to
Figure 31. Synthesis of (S)-AHBA Ester and Bromosugar
Bristol Laboratories for antibacterial screening, but the results are not yet available.

The novelty and importance of our synthesis of J 70 lies in the sequential regiospecific substitution onto the neamine molecule of two key units that form an amikacin analog: the N-1-(S)-4-amino-2-hydroxybutyryl side chain and the 6-0-glycoside with 2-deoxykanosamine. The synthesis was accomplished from the known bis (cyclic) carbamate 72 by the procedure described above. It should be a general synthesis that will be useful for the preparation of other amikacin analogs with different N-1-acyl groups and 0-6-glycosides. Such analogs can help to define further the aminoglycoside structure-activity relationships.

**Experimental**

Neomycin sulfate was bought from Sigma Chemical Company, St. Louis, Missouri 63178.

Melting points were determined using a Laboratory Devices Mel-Temp® apparatus with open capillary tubes and they are reported uncorrected.

Thin layer chromatography was performed on Analtech precoated gass plates (Silica gel GF, thickness 250 microns). For column chromatography silicagel 60 (70-270 mesh ASTM, supplied by Brinkman Instrument, Inc.) was used. Ion exchange resin chromatography was done on Amberlite® IRC-50 C.P. (20-50 mesh, cation exchange resin) and Amberlite® CG-50 (100-200 mesh, cation exchange resin) supplied by Mallinckrodt, Inc.
Infrared spectra were obtained on a Beckman IR-33 Infrared Spectrophotometer.

Nuclear magnetic resonance spectra were determined on a Varian Associates EM-360 spectrometer. Tetramethyilsilane and sodium 2,2-dimethyl-2-silapentane-5-sulfonate were used as the internal standards.

Elemental analyses were determined by Chemalytics Inc., Tempe, Arizona 85282 or Galbraith Laboratories Inc., Knoxville, Tennessee 37921 or University Analytical Center, Department of Chemistry, University of Arizona, Tucson, Arizona 85721.

Neamine hydrochloride (2)

A mixture of neomycin sulfate 3 (5.0 g) and 600 ml of anhydrous methanol saturated with 8.3 g of hydrogen chloride was refluxed for 2.5 h. Complete solution occurred after 1 h. The colorless solution was chilled in an ice bath and diluted with 200 ml of anhydrous ether. The flocculant white precipitate of neamine hydrochloride which formed was removed by filtration on a tared sintered glass funnel, washed with 25 ml of anhydrous ether and dried in a vacuum desiccator over phosphorous pentoxide. This procedure yielded 2.4 g of 2 as a white solid, mp 250°C (dec) (lit.216 mp 250°C); IR (KBr) cm⁻¹ 3380 (broad OH, NH), 1590, 1490.

Tetra-N-benzyloxycarbonylneamine (71)

A mixture of neamine hydrochloride 2 (0.5 g, 1.06 mmol) and sodium bicarbonate (1.7 g) in 70% aqueous methanol (20 ml) was cooled with stirring in an ice bath and benzyl chloroformate (2.0 g, 11.72
mmol) was added dropwise with the aid of 5 ml of methanol. The reaction mixture was stirred for 2 h at 0°C and then evaporated to dryness under reduced pressure. The residue was extracted three times with 25 ml of dry p-dioxane. The dioxane extract was concentrated to a small volume (5 ml) and treated with ether (50 ml). The resulting white solid was filtered and washed several times with ether to give a pure product 71, 0.7 g (78%), mp 259°C (dec) [Lit. mp 259°C (dec)]. IR. (KBr) cm⁻¹ 3580-3220 (OH and NH), 1705, 1695, 1690 (NHCO I), 1530 (NHCO II).

3,2'-Di-N-benzyloxy carbonyl-1,6: 4',6'-N,O-carbonyl neamine (72)

A solution of tetra-N-benzyloxy carbonyl neamine 71 (1.0 g, 1.1 mmol) in 15 ml of dry N,N-dimethylformamide under nitrogen was cooled to 0°C in an ice bath. Sodium hydride (0.15 g, 50% oil dispersion, 3.125 mmol) was added and the reaction mixture was stirred 30 minutes at 0°C and 2 h at room temperature (24°C). After 1 h of stirring the reaction mixture solidified and 15 ml of dry N,N-dimethylformamide was again added. The reaction mixture was neutralized (pH 7.0) with glacial acetic acid (3.0 ml), concentrated under reduced pressure, and treated with ice-cold water (50 ml). The resulting white solid was filtered and washed thoroughly with cold water and finally with ether to yield 72, 0.575 g (57%), mp 230°C (dec). [Lit. mp 230°C (dec)] IR (KBr), cm⁻¹ 3600-3200 (OH and NH), 1760 (5 membered carbamate) 1720 (6 membered carbamate), 1690 (NHCO I) and 1540...
(NHCO II). NMR (DMSO-d$_6$) $\delta$ 5.1 (bs, anomeric proton) 7.3 (s, aromatic protons) and 8.1 (bs, carbamate protons).

3,2'-Di-N-benzyloxycarbonyl-5,3'-di-O-benzyl-1,6:4',6'-N,O-carbonylneamine (73)

3,2'-Di-N-benzyloxycarbonyl-1,6:4',6'-N,O-carbonylneamine 72 (0.624 g, 1.0 mmol) was dissolved in dry dimethylformamide (15 ml) under a nitrogen atmosphere, sodium hydride (0.384 g, 8.0 mmol, 50% mineral oil dispersion) was added, and the mixture was stirred at 25° for 45 minutes. Benzyl bromide (2.064 g, 12 mmol) was added dropwise to the reaction mixture and the stirring was continued for 24 h. The excess of benzyl bromide was destroyed by the addition of 50 ml of methanol. The reaction mixture was evaporated to dryness and the residue was extracted with chloroform (100 ml). The chloroform layer was washed with water (3 x 20 ml), dried over anhydrous sodium sulfate and concentrated to a small volume (5 ml). On addition of n-hexane (20 ml) a white solid was precipitated. It was filtered and washed with n-hexane to give a white powder 73 0.68 g (70%). The product (small amount) was purified on a silica gel column by eluting with benzene to which an increasing amount of ethyl acetate was added. The desired product was eluted from benzene-ethyl acetate (1:1) to give a white powder, mp 124°C (lit. $^{213}$ mp 124°C); IR, (KBr), $\mathrm{cm}^{-1}$ 3500 (weak), 3060, 3020, 2920(s) 1770, 1720 (carbamates) 1480.; NMR (CDCl$_3$), $\delta$ 7.0 (30 H, aromatic), 5.3 (NH).
3,2'-Di-N-benzyloxy carbonyl-5,3'-O-dibenzyl-1,6-N,O-carbonyl neamine (74)

To a solution of 73 (1.01 g, 1 mmol) in dioxane (50 ml) was added 0.05 M barium hydroxide solution (10 ml). The mixture was stirred at 60°C for 1 h and another aliquot (10 ml) of the barium hydroxide solution was added. The solution was stirred for a further 2 h. The excess of barium hydroxide was neutralized by introduction of carbon dioxide gas and the reaction mixture was filtered and washed with dioxane. After evaporation of the filtrate and wash, the residue was extracted with 100 ml of chloroform, washed with water (2 x 50 ml) and dried over anhydrous sodium sulfate. Concentration of the above solution (5 ml) and addition of n-hexane (20 ml) gave a white solid 74 which showed no peak near 1760 cm⁻¹ in the infrared spectrum. This solid was used for the next step without further purification.

Yield = 0.88 g (88%), mp 93-96°C.

N-Hydroxysuccinimide Ester of (S)-4-Benzyl oxycarbonylamino-2-hydroxybutyric acid (75) (76)

A solution of (S)-4-benzyloxy carbonylamino-2-hydroxybutyric acid 75 (4.24 g, 16.8 mmol) and N-hydroxysuccinimide (1.92 g, 16.8 mmol) in 80 ml of dry ethyl acetate was cooled to 0°C and then dicyclohexyl carbodiimide (3.44 g, 16.8 mmol) was added. The mixture was stirred for 15 minutes and then kept overnight in a refrigerator. The precipitated dicyclohexylurea was filtered off and the filtrate was concentrated to 20 ml under reduced pressure to give colorless crystals of 76 which were collected by filtration. The filtrate was
evaporated to dryness in vacuum and the crystalline residue was washed with 10 ml of a benzene-n-hexane mixture (1:1) to give an additional amount of 76. The total yield was 5.22 g (90%), mp 121-122°C (lit.73 mp 121-122.5°C); IR (KBr) cm⁻¹ 3500, 3340 (NH, OH), 1805, 1775, 1740, 1680 (C = O).

3,2'-Di-N-benzyloxycarbonyl-1-N-[(S)-4-benzyloxycarbonylamino-2-hydroxybutryl]-5,3'-di-O-benzyl-1,6-N,O-carbonylneamine (77)

A solution of 74 (1.8497 g, 1.92 mmol) and the N-hydroxysuccinimide ester of (S)-4-benzyloxycarbonyl amino-2-hydroxybutyric acid 76 (0.7996 g, 2.36 mmol) in 14 ml of tetrahydrofuran and a small amount of triethylamine (2 drops) was stirred overnight at room temperature, the reaction mixture was evaporated under reduced pressure. The residue was extracted with chloroform (100 ml) and washed with water (50 ml). The chloroform layer was dried over anhydrous sodium sulfate. The solution was concentrated to a small volume (5 ml) and on addition of petroleum ether (20 ml) a white 77 solid was obtained. The while solid was recrystallized from chloroform-n-hexane. (1:2) to give 77, 1.61 g (70%), mp 79-81°C; IR (KBr) cm⁻¹ 3350 (NH, OH), 1750, 1680 (C = O); NMR. (CDCl₃) δ 7.2 (35H, aromatic), 5.1(2H, CH₂); Anal. calcd. for C₁₁%H₂₇N₂O₁₅; C, 68.37; H, 6.02; N, 5.78; found: C, 68.57; H, 5.96; N, 5.63.

1-N-[(S)-4-Amino-2-hydroxybutyryl]-3,2'-di-N-benzyloxycarbonyl-5,3'-di-O-benzyl-1,6: 4",2"-N,O-carbonylneamine (78)

An ice cooled solution of 77 (5.0 g, 4.13 mmol) in 50 ml of dry N,N-dimethylformamide under an atmosphere of nitrogen was treated
with sodium hydride (0.297 g, 12.3 mmol, of 50% dispersion in oil) and the mixture was stirred under nitrogen for 0.5 h at 0°C and then two hours at room temperature. The reaction mixture was neutralized (pH 7.0) with glacial acetic acid and concentrated to a small volume (5 ml). On addition of cold water (25 ml) a white solid was obtained. It was filtered and washed thoroughly with water (50 ml) and dried. The product was recrystallized from chloroform-petroleum ether (1:2) to give 78, 3.2 g (71%) mp 110°C; IR (KBr), cm⁻¹ 3440, 3120 (NH, OH), 1750, 1680 (C = O); NMR (CDCl₃) δ 7.3 (3OH, aromatic), 1.3 (d, 2H carbamate), 2.9 (d, 2H, carbamate); Anal. calcd. for C₆₂H₆₅N₅O₁₄: C, 67.45; H, 5.89; N, 6.34; found: C, 67.19; H, 5.85; N, 6.36.


Dry hydrogen bromide was passed for 10 minutes into cold methylene chloride (10 ml) and treated with a solution of 81 (1.0 g) in 50 ml of dry methylene chloride. After stirring half an hour at 0°C and 1 h at room temperature the solution was evaporated to dryness and extracted with methylene chloride (50 ml). The methylene chloride extract was washed first with water (50 ml) and then with aqueous sodium bicarbonate (20 ml, 10%) again with water (2 x 50 ml). Finally, upon drying and evaporating a bromosugar 82 was obtained which was used for further reaction.

A mixture of 78 (1.0 g, 0.9 mmol), mercuric cyanide (1.01 g), Drierite® (2.0 g) and dry methylene chloride (30 ml) was stirred at
25°C for 4 h under nitrogen. A solution of 82 (1.0 g) in 20 ml of dry methylene chloride was added and the reaction mixture was further stirred at reflux for 24 h. The sugar 82 (0.5 g) was again added and stirring was continued for three more days. The reaction mixture was diluted with methylene chloride and filtered through a pad of diatomaceous earth. On concentration of the filtrate to a small volume (5 ml) and dilution with petroleum ether (40 ml) a slightly yellowish solid was obtained which showed one major and several minor spots on TLC (9:1 chloroform-methanol). Purification on a silica gel column using benzene ethyl acetate as solvent gave 83 as a slightly yellowish solid. It was recrystallized from chloroform and petroleum ether (0.445 g, 30%) mp 120-123°C; IR (KBr) cm⁻¹ 3400, 3300 (NH, 1745, 1725, 1710 (C = O) 1520, 1350 (NO₂); NMR (CDCl₃) δ 8.3 (8H, aromatic), 7.3 (30H, aromatic) 5.4-4.1 (benzyl and carbamate NH protons) Anal. calcd. for C₈₄H₆₈O₂₆F: C, 61.38; H, 4.93; N, 6.82; found: C, 61.21, H, 4.60; N, 6.93.

1-N-[{(S)-4-Amino-2-hydroxybutyryl}]-2"-deoxykanamycin B. (70)

A solution of 83 (0.2 g, 0.12 mmol) in 10 ml of dioxane was heated at 50°C. Barium hydroxide (10 ml, 0.2M) solution was added in portions. After 1 h intervals two additional 10 ml portions of 0.2M barium hydroxide solution were added. Following a total reaction time of 5 h, the reaction mixture was neutralized with carbon dioxide gas and filtered through a pad of diatomaceous earth. The pad was washed with dioxane (20 ml). The filtrate and wash were combined and
concentrated to a small volume (3 ml) and on addition of acetone (20 ml) gave a light yellow solid which showed no absorption characteristic of cyclic carbamate group in its infrared spectrum.

A solution of the above light yellow solid in 20 ml of dioxane and 5 ml of water was treated with 0.2 g of 10% palladium on carbon and 1.5 ml of acetic acid. The mixture was shaken with hydrogen at 50 psi and 25°C for 24 h on a Parr hydrogenation apparatus. The mixture was filtered and concentrated under reduced pressure. On coevaporation with toluene (5 ml) an off-white solid was obtained. This solid was purified by successive column chromatographic separations on Amberlite® IRC-50 (NH₄⁺ cation exchange resin) with ammonium hydroxide solutions (0.1N - 0.5N) and Amberlite GC-50 (NH₄⁺) with 0.1N - 0.5N ammonium hydroxide solutions. Concentration of the final eluate gave an off-white solid 70 that was recrystallized from water-acetone to give 0.024 g (35%); mp 201°C (dec; blackens); [α]₂⁰⁵°⁰⁰ +94° (c 0.5, H₂O);

IR (KBr) cm⁻¹ 3340 (broad, NH, OH), 2960, 1650 (NH-G⁻) 1580, 1430, 1370; NMR (250 MHz, D₂O) δ 5.25 (bs, 1'-H anomeric), 5.05 (bs, 1''-H anomeric), 2.7 - 3.9 (c, 19H, H-C-N, H-C-O, 2-H ax, 2''-H ax, 3''-CH=N, CH₂), 1.7 - 2.3 (c, 7H, 2''-H eq, -CH₂N, CHOH). Anal. calcd. for C₂₅H₄₄N₆O₁₁·½H₂CO₃: C, 46.07; H, 7.51; N, 14.02; found: C, 46.11; H, 7.20; N, 14.05.

Methyl 3-Trifluoroacetamido-2,3-dideoxy-α-D-arabinohexopyranoside (80)

Methyl 3-trifluoroacetamido,4,6-O-benzylidene-2,3-dideoxy-α-D-arabinohexopyranoside (95, 214, 215) (5.0 g, 13.8 mmol) was mixed with
an acetic acid solution (44 ml AcOH, 112.5 ml water) and the mixture was stirred at 80°C for 3 h. The reaction mixture was filtered and the filtrate was evaporated to dryness. Traces of acetic acid were removed by coevaporation with toluene (10 ml) and finally by drying in vacuum. The free sugar was crystallized from ethyl acetate to give 80, 3.4 g (90%): mp 173-4°C, $[\alpha]_{D}^{25} + 131.7^\circ$ (c 1.0, CH$_3$OH); IR cm$^{-1}$ 3300, 3100, (OH, NH), 1700 (NHC)I, 1550 NHCO II; NMR (D$_2$O) $\delta$ 3.3 (s, 3H, 1-0CH$_3$), 5.1 (b, 1H, H-1), 6.2 (b, 1H, NH); Anal. calcd for C$_9$H$_{14}$F$_3$N$_5$O$_5$: C, 39.55; H, 5.12; N, 5.12; found: C, 39.52; H, 5.27; N, 5.07.

Methyl 3-Trifluoroacetamido-2,3-dideoxy-4,6-O-(p-nitrobenzoyl)-α-D-arabinohexopyranoside (81)

To a cold (0°C) solution of methyl 3-trifluoroacetamino-2,3-dideoxy-α-D-arabinohexopyranoside 80 (2.0 g, 7.3 mmol) in pyridine (22 ml) was added p-nitrobenzoyl chloride (2.8 g, 15 mmol) and the mixture was allowed to warm to 25°C (room temperature). The reaction mixture was stirred overnight at room temperature, the pyridine was removed under vacuum and the residue was extracted with methylene chloride. This methylene chloride extract was washed with a cold (0°C) saturated solution of sodium bicarbonate followed by cold water. The methylene chloride layer was dried over anhydrous sodium sulfate. On removal of solvent 81 was obtained as a light yellow solid. It was recrystallized from methylene chloride-n-hexane to give 81, 3.3 g (80%); mp 210-12°C; $[\alpha]_{D}^{25} + 121.6^\circ$ (c, 0.9, CH$_3$OH); IR (KBr) cm$^{-1}$, 3300 (s, NH), 1740, 1700 (C = O), 1520, 1350 (NO$_2$). NMR (CDCl$_3$),
δ 8.2 (s, 8H, aromatic), 6.7 (d, 1H, NH) 3.4 (s, 3H, 1-CH₃); Anal. calcd. for C₂₃H₂₀FN₃O₁₁: C, 48.5; H, 3.5; N, 7.3; found: C, 48.26; H, 3.69; N, 7.29.
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