259 nm (ε 14 400). Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot 1.5H_2O$: C, 43.13; H, 5.27; N, 22.87. Found: C, 43.84; H, 4.86; N, 22.33.

9-(α-L-Rhamnofuranosyl)adenine[19] (0.23 g, 0.76 mmol) was treated with paraperiodic acid (0.2 g, 0.9 mmol) to yield 0.19 g (81%) of dialdehyde **4**: R_f 0.57 (A), 0.57 (B), 0.70 (C); UV λ_{max} 259 nm (ε 14 200). Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot H_2O$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.53; H, 4.87; N, 22.46.

9-(6-Deoxy-α-L-talofuranosyl)adenine[20] (0.12 g, 0.43 mmol) was oxidized with paraperiodic acid (0.13 g, 0.57 mmol) to afford 0.1 g (77%) of dialdehyde **5**: R_f 0.62 (A), 0.54 (B), 0.71 (C); UV λ_{max} 259 nm (ε 14400). Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot H_2O$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.39; H, 5.36; N, 26.11.

9-(5,6-Dideoxy-β-L-*ribo*-hex-5-enofuranosyl)adenine[21] (0.19 g, 0.63 mmol) was treated with paraperiodic acid (0.15 g, 0.68 mmol) to give 0.16 g (91%) of dialdehyde **6**: R_f 0.70 (A), 0.83 (B), 0.72 (C); UV λ_{max} 259 nm (ε 13 400). Anal. Calcd for $C_{11}H_{11}N_5O_3 \cdot H_2O$: C, 47.31; H, 4.69; N, 25.08. Found: C, 47.03; H, 4.75; N, 26.00.

9-(5,6-Deoxy-β-L-*ribo*-hex-5-enofuranosyl)adenine[21] (1.2 g, 4 mmol) was treated with paraperiodic acid (0.93 g, 4.1 mmol) to give 0.91 g (82%) of dialdehyde **7**: R_f 0.69 (A), 0.83 (B), 0.74 (C); UV λ_{max} 259 nm (ε 13 700). Anal. Calcd for $C_{11}H_{11}N_5O_3 \cdot H_2O$: C, 47.31; H, 4.69; N, 25.08. Found: C, 46.53; H, 4.77; N, 26.10.

Enzyme Kinetics. Adenosine aminohydrolase (adenosine deaminase, EC 3.5.4.4) from calf intestinal mucosa (type 1) was purchased from Sigma Chemical Co. The preparation had a specific activity of 220 units/mg of protein. One unit is defined as the amount of enzyme that will deaminate 1 μmol of adenosine to inosine per minute at pH 7.5 and 25 °C. The assay method was that of Kaplan.[22] The rate of change in absorbance at 265 nm was measured on a Beckman DK-2 spectrophotometer. The reactions were run at 25 °C in 0.05 M sodium phosphate buffer. Substrate activity was determined by adding adenosine aminohydrolase (60 units) to 3 mL of buffer containing the nucleoside dialdehyde. The solution was allowed to stand for 24 h at room temperature. A shift in the absorption maximum from about 260 nm to about 249 nm indicated the transformation of an N^9-substituted adenine to the corresponding hypoxanthine dialdehyde. Compounds not deaminated were treated with another 60 units of enzyme for an additional 24 h to be certain that they were not substrates. The double-reciprocal plot method of Lineweaver and Burk[23] was used to determine the values of K_m and V_{max}. A weighted least-square analysis based upon that of Wilkinson[24] was used to obtain the best lines. Initial velocities were measured at concentrations of the dialdehydes ranging from 70 to 200 μM. The quantity of enzyme added to a 3-mL solution ranged from 25 to 50 units, depending on the activity of the compound. The K_m for adenosine was 50 μM; the V_{max} was 212 μmol min^{-1} (mg of protein)$^{-1}$. Inhibitor constants were determined by addition of 0.1 mL of enzyme solution (0.25 units) to 3 mL of buffer solution containing adenosine and the nucleoside dialdehyde. The adenosine concentration ranged from 25 to 75 μM; the concentration of dialdehyde ranged from 3 to 200 μM, depending on the activity of the compound. For each determination of K_i, five substrate concentrations and two inhibitor concentrations were used. The K_i values were also determined by the double-reciprocal plot method and weighted least-square analysis. The slopes of all inhibition lines were statistically different from the line representing no inhibition and the intercepts of all lines on the $1/V$ axis were the same.

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Aminoglycoside Antibiotics. 3. Epimino Derivatives of Neamine, Ribostamycin, and Kanamycin B

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2′,3′-Epimino analogues of neamine, ribostamycin, and kanamycin B possessing little or no intrinsic antimicrobial activity were designed to enhance the activity of kanamycin A against bacterial strains that elaborate aminoglycoside 3′-phosphotransferases. Routes were devised for their synthesis from the parent antibiotics and the 2″,3″-epimino analogue of kanamycin B also was prepared. None of these analogues was active against phosphotransferase-producing bacteria, although the kanamycin B derivatives showed very weak activity against nonresistant strains. At 8 and 32 μg/mL, the 2′,3′-epimino analogue of neamine produced a small synergistic effect on the activity of kanamycin A against a strain of *Escherichia coli* that produces aminoglycoside 3′-phosphotransferase II. The N^3-(carbobenzyloxy) derivative of this analogue produced a small effect against the same strain, and it, as well as the 2″,3″-epimino analogue of kanamycin B, slightly enhanced the activity of kanamycin A against a strain of *Proteus rettgeri* that elaborates a similar enzyme.

Aminoglycosides hold an important place in the treatment of bacterial infections, especially with respect to Gram-negative species that are resistant to other types of antibiotics. The effectiveness of earlier aminoglycosides, such as streptomycin and kanamycin, has become limited by the emergence of resistant strains of bacteria that inactivate these antibiotics through enzymatic reactions. These reactions include the phosphorylation, acetylation, and adenylylation of specific hydroxyl and amino groups.[1-3] The production of the enzymes is mediated by R factors, including plasmids.[4] Certain new aminoglycosides, such

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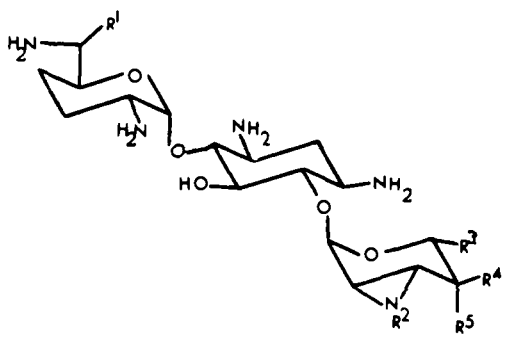
as tobramycin and the gentamicins, are less susceptible to bacterial inactivation than the kanamycins because they lack some of the functional groups attacked by the bacterial enzymes.[5] Other highly effective new compounds are semisynthetics, including 3′,4′-dideoxykanamycin B (dibekacin), which has a site of phosphorylation removed, and amikacin, in which the addition of a 4-amino-2-hydroxybutyryl group hinders binding of the molecule to inactivating enzymes.[5] Despite these notable successes, the problem of bacterial inactivation of aminoglycosides has not been completely solved, and it is not certain that it can be. Strains resistant to gentamicin and tobramycin are encountered occassionally in clinical practice, and resistance even to amikacin is known.[6,7] Furthermore, there are limitations to the structural manipulations of aminoglycosides. Thus, the removal of certain functional groups can lead to decreased potency (e.g., the 2″-hydroxyl group of gentamicin C_1)[8] or increased toxicity (e.g., tobramycin and 3′,4′-dideoxykanamycin B).[9] For these reasons, it seems worthwhile to investigate other methods for overcoming bacterial inactivation of aminoglycosides.

One possible new approach to overcoming the inactivation of aminoglycosides by bacterial enzymes is to develop specific inhibitors of these enzymes. This kind of approach has achieved a measure of success in the β-lactam area, wherein compounds such as clavulanic acid have proven to be inhibitors of certain β-lactamamses.[10] The situation is somewhat more complicated with aminoglycosides because of the larger number of inactivating enzymes. However, we thought that the development of inhibitors still merited investigation. We chose aminoglycoside 3′-phosphotransferases as the initial target because they are widely distributed in clinically important bacteria and have been shown to inactivate antibiotics such as kanamycin, ribostamycin, and neomycin, which are otherwise very valuable.[2] As their name implies, these enzymes transfer a phosphoryl group from adenosine triphosphate to the 3′-hydroxyl group of the aminoglycosides.

Although it was not obvious how to design a reversible inhibitor of aminoglycoside 3′-phosphotransferases, the preparation of potential irreversible inhibitors seemed clear. Because the 3′ position of the aminoglycoside must bind or at least be close to the enzyme, we thought that an alkylating group at this position might form a covalent bond with the enzyme. The epimino (aziridino) group was chosen for this purpose, partly because many aminoglycosides have *trans*-2′,3′-aminohydrins that would lead to its synthesis. Thus, our goal was to synthesize and test the 2′,3′-epimino analogues (**6**, **15**, and **19**, respectively) of neamine, ribostamycin, and kanamycin B. The 2″,3″-epimino analogue (**22**) of kanamycin B was prepared to test the possibility that the enzyme might be inhibited by alkylation at a place remote from the phosphorylation site.

Prior to the initiation of this project, two epimino derivatives of aminoglycosides were known. One was the 2″,3″-epimino analogue (**1**) of 3′,4′-dideoxykanamycin B, which was inadvertently obtained in a synthesis of 3′,4′-



1; $R^1=R^2=R^5=H$, $R^3=CH_2OH$, $R^4=OH$

2; $R^1=R^2=R^4=CH_3$, $R^3=H$, $R^5=OH$

dideoxykanamycin B.[11] The other was the 2″,3″-epimino analogue (**2**) of gentamicin C_1. It was prepared as an intermediate in the synthesis of 2″-deoxygentamicin C.[8] Neither compound was conceived or tested as an inhibitor of an aminoglycoside-inactivating enzyme. Some additional epimino derivatives of aminoglycosides became known in the patent literature during the course of this investigation.[12] The synthesis of the 2′,3′-epimino analogue (**19**) of kanamycin B from the 3′-*O*-phosphate of kanamycin B, bis(trimethylsilyl)acetamide, trimethylsilyl chloride, and triphenylphosphine was described, and related preparations of the corresponding epimino derivatives of neomycin and butirosin were claimed.

The synthesis of the 2′,3′-epimino analogue **6** of neamine began with our previously reported bis(cyclic carbamate) **3**, an intermediate in the partial synthesis of ribostamycin (Scheme I).[13] Treatment of **3** with sodium hydride in *N*,*N*-dimethylformamide resulted in formation of compound **4**, which has the 2′,3′-epimino group present as its *N*-(carbobenzyloxy) derivative. Hydrolysis of **4** with barium hydroxide afforded cleavage of the cyclic carbamates and the carbobenzyloxy group on the aziridine; however, the 3-amino group retained its carbobenzyloxy group in product **5**. This group was removed by sodium in liquid ammonia to give analogue **6**. As discussed below, the carbobenzyloxy group of **5** was compatible with a synergistic effect on the activity of kanamycin A against a resistant strain of *Escherichia coli*. In order to determine if this group had any effect in the absence of the 2′,3′-epimino group, we desired N^3-(carbobenzyloxy)neamine (**9**). This compound was prepared from our previously reported tris(cyclic carbamate) **7**[13] by treatment with carbobenzyloxy chloride and hydrolysis of the resulting intermediate **8** with barium hydroxide.

For the synthesis of the 2′,3′-epimino analogue of ribostamycin, prepared as its N^3-(carbobenzyloxy) derivative **15**, we began with the known tetrakis[*N*-(carbobenzyloxy)]ribostamycin **10** (Scheme II).[14] This compound was converted first into its 5″-*O*-trityl derivative **11**, and then the bis(cyclic carbamate) **12** was formed in the presence of sodium hydride. The latter reaction did not go smoothly if the 5″-hydroxyl group was not protected by tritylation. Following protection of the 2″- and 3″-hydroxyl groups of **12** by cyclohexylidene formation, the remaining 3′-hydroxyl

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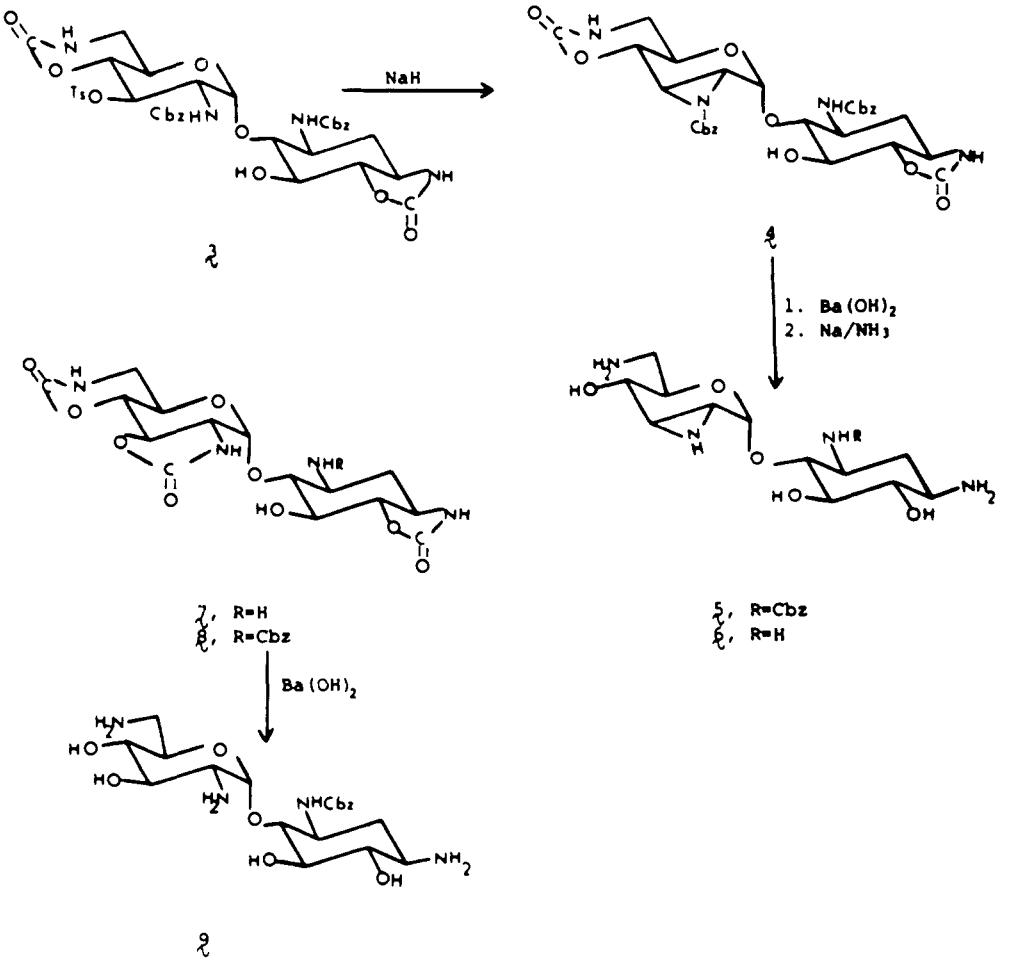
Scheme I



group was converted into its tosylate. The resulting intermediate **13** was treated with acetic acid to remove the trityl and cyclohexylidene groups, and then the *N*-(carbobenzyloxy)aziridine was formed by sodium hydride in *N*,*N*-dimethylformamide. Treatment of the resulting compound (**14**) with barium hydroxide furnished the desired 2′,3′-epimino analogue **15**.

The 2′,3′-epimino analogue **19** of kanamycin B was synthesized from kanamycin B by a sequence of reactions based on those described above (Scheme III). Thus, our previously described 4″,6″-*O*-cyclohexylidenebis(cyclic carbamate) **16**[15] was readily tosylated on its 3′-hydroxyl group to give intermediate **17**. The 5-hydroxyl group of kanamycins such as **16** is hindered and relatively unreactive.[16] Following acetic acid hydrolysis of the cyclohexylidene group of **17**, the *N*-(carbobenzyloxy)aziridine was formed by treatment with sodium hydride. The product **18** of this reaction was converted into analogue **19** by sodium in liquid ammonia, which removed all of the protecting groups.

For the synthesis of the 2″,3″-epimino analogue **22** of kanamycin B, Umezawa's dicyclohexylidene derivative **20** of 2″-*O*-mesylpenta-*N*-tosylkanamycin B[11] was the starting material (Scheme IV). Upon treatment with sodium hydride it gave an *N*-tosylaziridine which afforded compound **21** after hydrolysis of the ketals with acetic acid. Sodium in liquid ammonia removed the protecting groups to give the desired analogue **22**. An alternative route to **22** was based on the closely related dicyclohexylidene derivative **23** of pentakis[*N*-(ethoxycarbonyl)]kanamycin B, also prepared previously by Umezawa's group.[17] This compound was converted into its 2″-*O*-mesylate **24** and then the cyclohexylidene groups were hydrolyzed to furnish compound **25**. Treatment of **25** with barium hydroxide solution afforded a complex mixture of products, none of which was present in appreciable yield. Thin-layer chromatography of this mixture gave, in each of two different solvent systems, a spot that had an R_f value identical with that of 2″,3″-epimino analogue **22** prepared from **20**. Thus, it is probable that the analogue actually was formed. However, the procedure based on compound **23** obviously was not a useful one.

Biological Activity. Our method for determining if the epimino derivatives described above had any inhibiting effects on the enzymes that inactivate aminoglycosides was based on their ability to lower the minimum inhibitory concentration of kanamycin A for bacterial strains otherwise resistant to it. If an enhancement of kanamycin A activity was observed, it would suggest that the enzymatic inactivation of that antibiotic was suppressed. It is conceivable that other mechanisms for such a synergistic effect might exist, and they have not been ruled out at this time.

Before investigating possible synergism with kanamycin A, it was necessary to determine if our epimino analogues had any antibacterial activity of their own. Umezawa and co-workers reported that compound **1** had about one-thirtieth the antibacterial activity of 3′,4′-di-

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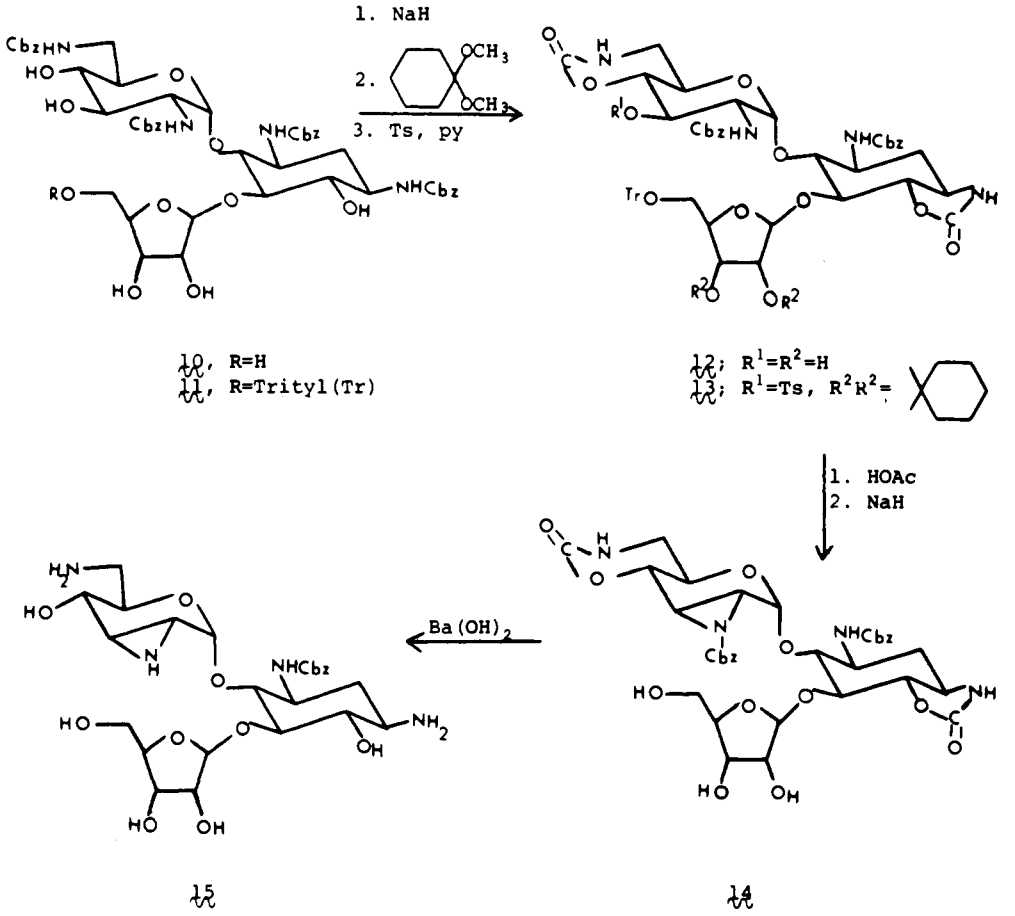
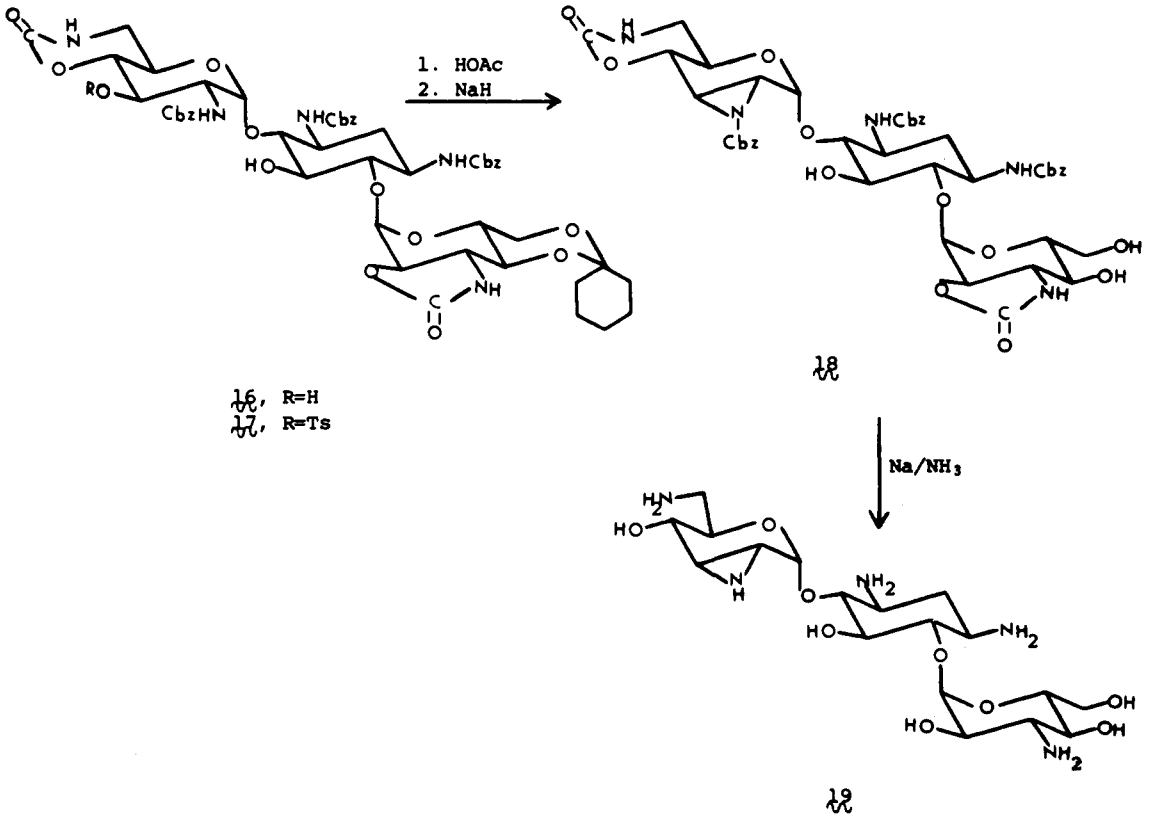
(16) S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *Bull. Chem. Soc. Jpn*, **42**, 537 (1969).
(17) M. Okanishi, S. Kondo, Y. Suzuki, S. Okamoto, and H. Umezawa, *J. Antibiot.*, **20**, 132 (1967).

Scheme II



Scheme III



deoxykanamycin B.[11] Therefore, it was not impossible for analogues **5**, **6**, **9**, **15**, **19**, and **22** to have significant activity. The activity of these analogues in the serial dilution assay is compared with that of kanamycin A in Table I. It is apparent from this table how drastically the potency of kanamycin A is reduced against bacterial species that

Scheme IV

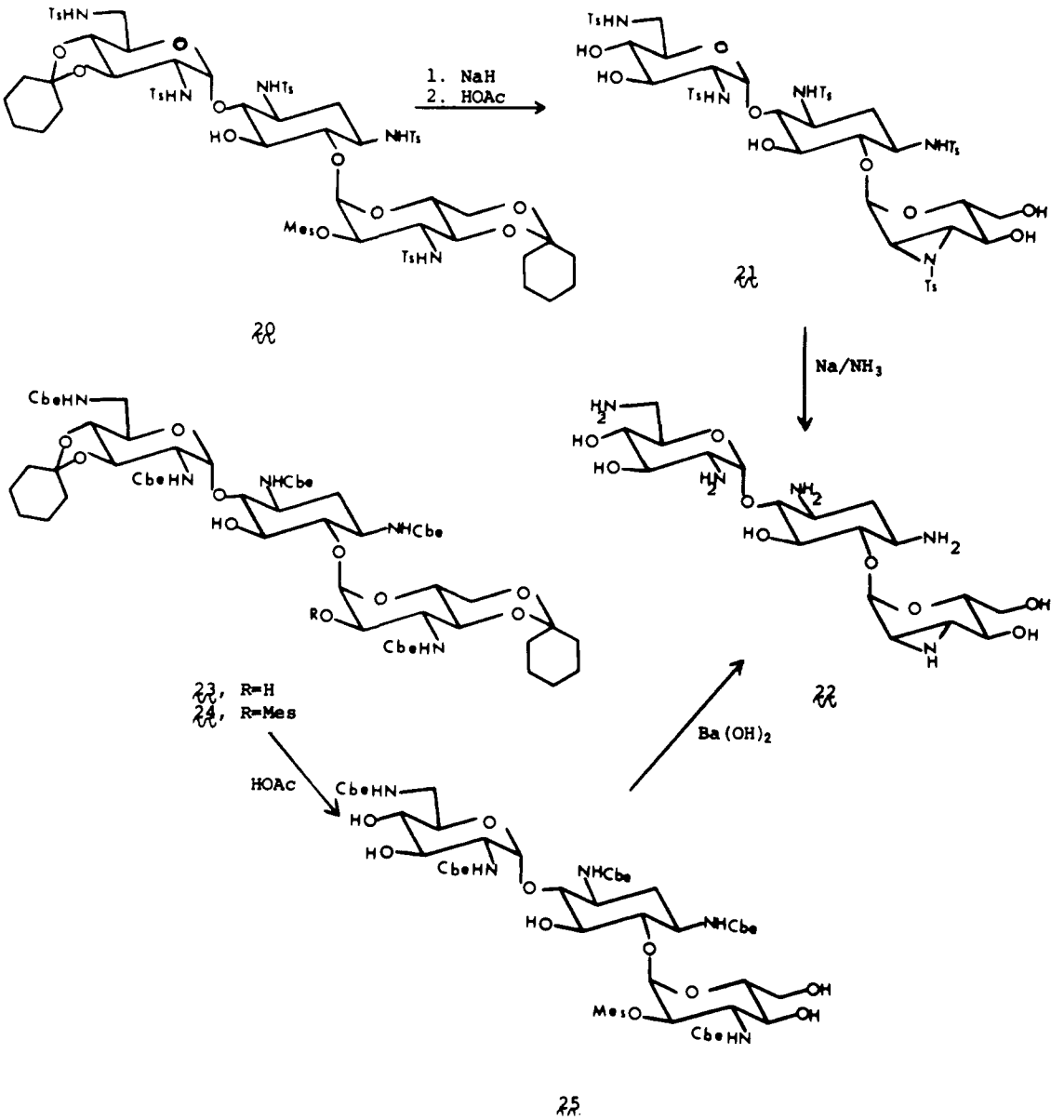


Table I. Antibacterial Activities by Serial Dilution in Mueller–Hinton Broth for Kanamycin A and Epimino Aminoglycosides

bacterial species[a]	Bristol no.	inact enz[b]	kanamycin A	min inhibr concn, μg/mL					
				5	6	9	15	19	22
E. coli	A 20665	APH(3')-I	>125	125		>125	>125	125	>125
E. coli	A 20507	APH(3')-II	>125	>125	>125	>125	>125	125	>125
E. coli	A 9632	none	2	125		>125	>125	8	63
P. rett.	A 21615	APH(3')-II	125	>125		>125	>125	>125	>125
P. rett.	A 9637	none	<0.25	>125		>125	>125	16	>125
P. aerug.	A 20653	APH(3')-I & -II	>125	>125		>125	>125	125	>125
P. aerug.	A 20357	none	1	>125		>125	>125	125	>125

[a] Abbreviations for bacteria: *E. coli, Escherichia coli; P. rett., Proteus rettgeri; P. aerug, Pseudomonas aeruginosa.* [b] Abbreviations for aminoglycoside inactivating enzyme: APH(3'), aminoglycoside 3'-phosphotransferase. For a complete description of the antibacterial assay process, see M. Misiek, T. A. Pursiano, L. B. Crast, F. Leitner, and K. E. Price, *Antimicrob. Agents Chemother.*, 1, 54 (1972).

elaborate aminoglycoside 3'-phosphotransferases I and/or II. The only epimino analogues that show any antibacterial activity are **19** and **22**, the derivatives of kanamycin B. They are active only against strains of *Escherichia coli* and *Proteus rettgeri* that do not elaborate a phosphotransferase and then at much higher concentrations than those of kanamycin A.

Table II shows the effects of combining two different concentrations, 8 and 32 μg/mL, of each of the epimino analogues with serial dilutions of kanamycin A against the same bacterial strains. Although no pronounced synergistic effects were observed, certain analogues appeared to give weak synergism against the *E. coli* and *P. rettgeri* strains that elaborate aminoglycoside 3'-phosphotransferase II. Thus, both the 2',3'-epimino analogue **6** of neamine and its N^3-(carbobenzyloxy) derivative **5** increased the potency of kanamycin A against the *E. coli* strain. Analogues **5** and **22** showed possible very weak synergism at **32** μg/mL

Table II. Antibacterial Activity by Serial Dilution in Mueller-Hinton Broth for Kanamycin A in Combination with Fixed Concentrations of Epimino Derivatives

bacterial species[a]	Bristol no.	inact enz[a]	min inhibr concn, μg/mL, for kanamycin A plus the following compds									
			5		6		9		15		19		22	
			8 μg/mL	32 μg/mL	8 μg/mL	32 μg/mL	8 μg/mL	32 μg/mL	8 μg/mL	32 μg/mL	8 μg/mL	32 μg/mL	8 μg/mL	32 μg/mL
E. coli	A 20665	APH(3′)-I	>125	>125			>125	>125	>125	>125	>125	>125	>125	>125
E. coli	A 20507	APH(3′)-II	>125	63	63	32	125	125	125	63	125	125	125	125
E. coli	A 9632	none					2	2	1	1	0.25		1	1
P. rett.	A 21615	APH(3′)-II	>125	63			125	125	125	125	125	125	63	63
P. rett.	A 9637	none					1	1	0.5	0.5	0.5	0.5	0.5	0.5
P. aerug.	A 20653	APH(3′)-I & -II	>125	>125			>125	>125	>125	>125	>125	>125	>125	>125
P. aerug.	A 20357	none					4	4	4	4	4	4	4	4

[a] See Table I for full names of bacteria and enzymes.

against the *P. rettgeri* strain. Compound **6** was not tested against this strain. The analogue **9** which has the N^3-(carbobenzyloxy) group, but no epimino group, produced no synergistic effect when mixed with kanamycin A. This result shows that the N^3-(carbobenzyloxy) group of analogue **5** is compatible with activity but makes no contribution to it.

In summary, we wish to suggest that we have obtained preliminary evidence supporting the idea that aminoglycosides with epimino groups can potentiate the activity of kanamycin A against certain bacteria that elaborate aminoglycoside 3′-phosphotransferase II, possibly by interfering with this enzyme. It is obvious that the next step is to study the possible interactions between the epimino analogues and the isolated enzyme. Such interactions are already under investigation by Professor Dexter Northrup at the University of Wisconsin. He will report on this investigation, subsequently.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-33 spectrophotometer as KBr pellets. Nuclear magnetic resonance spectra were recorded on Varian EM-360 and T-60 spectrometers in Me_2SO-d_6 (unless otherwise specified) using tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-5-sulfonate as the standards. Optical rotations were taken on a Perkin-Elmer 241MC automatic polarimeter under the indicated conditions. Elemental analyses were performed by the microanalytical laboratory, Department of Chemistry, Purdue University, and Chemalytics, Inc., Tempe, Ariz.

2′,3′-Dideoxy-2′,3′-[*N*-(benzyloxycarbonyl)epimino]-3-*N*-(benzyloxycarbonyl)-1,6:6′,4′-*N*,*O*-dicarbonylneamine (4). The tosylate **3**[13] (0.100 g, 0.125 mmol) was dissolved in 5 mL of dry *N*,*N*-dimethylformamide under a nitrogen atmosphere, and 50% sodium hydride (20 mg, 0.42 mmol) was added. The reaction mixture was stirred at 25 °C for 24 h and neutralized with acetic acid. The clear brown solution was evaporated under reduced pressure, and addition of water resulted in a solid product. This product was filtered and washed thoroughly with water and finally with ether. Recrystallization from *p*-dioxane–ether gave **4**: yield 56 mg (72%); mp 192–194 °C dec; $[\alpha]^{25}_{546}$ +53.0 (*c* 1.2, DMF); IR no SO_2 at 1175 cm^{-1}; NMR δ 2.8 (d, 2 H, *J* = 5.0, 2′,3′ protons). Anal. ($C_{30}H_{32}N_4O_{11}$) C, H, N.

2′,3′-Dideoxy-2′,3′-epimino-3-*N*-(benzyloxycarbonyl)neamine (5). The aziridine **4** (0.250 g, 0.4 mmol) was dissolved in 2.5 mL of *p*-dioxane, heated to 60 °C, treated with 2.5 mL of 0.1 M barium hydroxide octahydrate, and stirred for 7 h. Two aliquots (2 × 2.5 mL) of barium hydroxide were added to keep the reaction mixture alkaline. After neutralization with CO_2 gas, the reaction mixture was filtered and the filtrate was kept on Amberlite IR-45 (OH^-). The resin was removed by filtration, the filtrate was concentrated to a small volume, and addition of acetone gave the product. Recrystallization two times with *N*,*N*-dimethylformamide–acetone gave **5**: yield 0.115 g (65%); mp >250 °C dec; $[\alpha]^{25}_{546}$ + 70.6 (*c* 1.0, DMF); IR 1700 (NHCO I), 1525 cm^{-1} (NHCO II); NMR δ 1.8–2.0 (d, 2 H, 2′ and 3′ protons). Anal. ($C_{20}H_{30}N_4O_7$) C, H, N.

2′,3′-Epiminoneamine (6). The aziridine **5** (0.63 g, 1.44 mmol) was dissolved in 120 mL of dry liquid ammonia, and 0.85 g of sodium metal was added in small pieces. The dark blue mixture was stirred for 2 h at −70 °C and then neutralized by adding water. The ammonia was allowed to evaporate, and the residue was diluted with water and neutralized by the addition of Dowex 50W-X2 (H^+ form). The whole slurry was transferred to a column and washed with water. The column was eluted with 1 N ammonium hydroxide until no ninhydrin-positive product was eluted. The combined eluate was evaporated to dryness and again separated on an Amberlite CG-50 (NH_4^+) column. The major product was isolated and concentrated to a small volume. The addition of methanol–acetone gave **6**: yield 0.110 g (25); mp 225 °C dec; $[\alpha]^{25}_{546}$ +88.6 (*c* 1.0, H_2O); IR 1600 (NH_3^+), 1500 (NH_3^+), 1450 cm^{-1} (CO_3^{-2}); NMR (D_2O) δ 1.75–2.0 (d, 2 H, 2′ and 3′ protons). Anal. ($C_{12}H_{24}N_4O_5 \cdot H_2CO_3 \cdot H_2O$) C, H, N.

3-*N*-(Benzyloxycarbonyl)neamine (9). To an ice-cold solution of **7**[13] (1.0 g 2.5 mmol) in 50% aqueous *N,N*-dimethylformamide (20 mL) was added sodium bicarbonate (0.5 g, 7.14 mmol) and benzyl chloroformate (1 mL). The reaction mixture was stirred at ice-bath temperature for 1 h and then at 25 °C for 20 h. The reaction mixture was evaporated to dryness. The residue was extracted with dry *N,N*-dimethylformamide and filtered. The filtrate was concentrated to a small volume and treated with ether to give **8** as white powder: yield 1.1 g (82%); IR 1790 (five-membered carbamate), 1725 (six-membered carbamate), 1700 (NHCO I), 1540 cm^{-1} (NHCO II).

Compound **8** (0.5 g, 0.93 mmol) was suspended in aqueous *p*-dioxane–water (1:1, 20 mL) and heated to 60–70 °C. Three 10-mL aliquots of 0.1 M aqueous barium hydroxide were added within 3 h, and the reaction mixture was stirred at this temperature for 15 h. The mixture was neutralized with carbon dioxide after cooling to room temperature. It was evaporated to dryness and extracted with dry *N,N*-dimethylformamide and filtered through a Celite pad. The filtrate was concentrated to a small volume and treated with acetone to give a product that was again recrystallized with *N,N*-dimethylformamide–acetone. This procedure gives **9** as the monohydrate carbonate: yield 0.375 g (87%); mp >270 °C dec; $[\alpha]^{25}_{546}$ +55.6 (*c* 1.0, DMF); IR 1700 (NHCO I), 1530 cm^{-1} (NHCO II). Anal. ($C_{20}H_{32}N_4O_8 \cdot H_2CO_3 \cdot H_2O$) C, H, N.

3,2′-Bis[*N*-(benzyloxycarbonyl)]-1,6:6′,4′-*N,O*-dicarbonyl-5″-*O*-(triphenylmethyl)ribostamycin (12). To a solution of tetrakis[*N*-(benzyloxycarbonyl)]ribostamycin[14] (3.0 g, 3.22 mmol) in dry pyridine (30 mL) was added trityl chloride (0.95 g, 3.4 mmol). The reaction mixture was stirred at 25 °C for 24 h and evaporated to a small volume. The residue was titurated with water and the resulting white compound was recrystallized from acetone–ether to give **11** as white powder: yield 3.2 g (84.6%); IR 1710–1685 (NHCO I), 1515 cm^{-1} (NHCO II); NMR δ 7.0–7.8 (m, aromatic protons). The TLC of the product in chloroform–methanol (9:1) indicated essentially a single product. The compound was used in next reaction without further purification.

A solution of **11** (3.2 g, 2.73 mmol) in dry *N,N*-dimethylformamide (50 mL) was cooled at 0 °C under nitrogen and treated with 50% sodium hydride (0.6 g, 12.5 mmol). The mixture was stirred for 30 min at this temperature and then at 25 °C for 2 h. After neutralization with acetic acid, the mixture was evaporated to a small volume and titurated with ice–water to give a solid. This solid was recrystallized from acetone–ether to furnish **12**: yield 2.3 g (88%); mp 272–275 °C dec; $[\alpha]^{25}_{546}$ +7.2 (*c* 1.0, DMF); IR 3700–3150 (OH and NH), 1770 (five-membered carbamate), 1725 (six-membered carbamate), 1700 and 1695 (NHCO I), 1520 cm^{-1} (NHCO II). Anal. ($C_{54}H_{56}N_4O_{16}$) C, H, N.

3,2′-Bis[*N*-(benzyloxycarbonyl)]-1,6:6′,4′-*N,O*-dicarbonyl-2″,3″-*O*-cyclohexylidene-3′-*O*-toluenesulfonyl-5″-*O*-(triphenylmethyl)ribostamycin (13). To a solution of **12** (0.70 g, 0.73 mmol) in dry *N,N*-dimethylformamide (10 mL) were added *p*-toluenesulfonic acid (20 mg) and 1,1-dimethoxycyclohexane (0.8 mL). The mixture was heated between 50 and 60 °C under a water aspirator for 2 h. The mixture was cooled, neutralized with triethylamine, concentrated under reduced pressure, and triturated with water. The resulting product was extracted with chloroform, washed with water, and dried over anhydrous sodium sulfate. Concentration of chloroform and addition of petroleum ether gave the cyclohexylidene derivative: yield 0.75 g (99%); NMR ($CDCl_3$) δ 1.2–2.0 (s, 10 H, cyclohexyl protons).

The above product was dissolved in dry pyridine (10 mL), and *p*-toluenesulfonyl chloride (0.5 g, 2.62 mmol) was added. The mixture was stirred at 25 °C for 48 h and the pyridine was removed under reduced pressure. The residue was triturated with cold dilute sodium bicarbonate solution and extracted with chloroform. The chloroform layer was washed with water and dried over anhydrous sodium sulfate. Concentration of the chloroform gave a colored product, which was separated on a silica gel column to give **13**, after recrystallization from chloroform–hexane: yield 0.775 g (90%); mp 178–180 °C, $[\alpha]^{25}_{546}$ +10.4 (*c* 1.0, $CHCl_3$); IR 3700–3100 (NH), 1775 (five-membered carbamate), 1725 (six-membered carbamate), 1700 (NHCO I), 1520 (NHCO II), 1175 cm^{-1} (SO_2); NMR ($CDCl_3$) δ 1.0–1.8 (s, 10 H, cyclohexyl protons), 2.35 (s, 3 H, CH_3–SO_2 protons). Anal. ($C_{66}H_{70}N_4O_{18}S$) C, H, N, S.

2′,3′-Dideoxy-2′,3′-[*N*-(benzyloxycarbonyl)epimino]-3-*N*-(benzyloxycarbonyl)-1,6:6′,4′-*N,O*-dicarbonylribostamycin (14). Tosylate **13** (1.8 g, 1.51 mmol) was suspended in 80% of glacial acetic acid (20 mL) and heated to 70–80 °C for 12 h. The acetic acid was removed under reduced pressure and remaining traces were coevaporated with toluene. The residue was triturated with water to yield a brownish product which was recrystallized from methanol–ether. This procedure gave a product, 1.15 g (82%), whose NMR spectrum in Me_2SO-d_6 showed no cyclohexylidene or trityl groups. The product was used directly in the next step.

To a solution of the above product (1.15 g, 1.24 mmol) in dry *N,N*-dimethylformamide (65 mL) under nitrogen was added 50% sodium hydride (0.240 mg, 5 mmol). The reaction mixture was stirred at 25 °C for 22 h. The reaction mixture was cooled in an ice bath, neutralized with acetic acid, and filtered. The filtrate was concentrated to a small volume, and addition of ice-cold water gave a product which was further recrystallized from *N,N*-dimethylformamide–ether. This procedure gave **14** as a slightly colored solid: yield 0.65 g (69%); mp >295 °C dec; $[\alpha]^{25}_{546}$ +13.5 (*c* 1.0, DMF); IR 3700–3050 (NH and OH), 1770 (five-membered carbamate), 1725 (six-membered carbamate), 1700 and 1695 (NHCO I), 1520 cm^{-1} (NHCO II); NMR δ 2.71 (d, 2 H, 2′ and 3′ protons). Anal. ($C_{35}H_{41}N_4O_{15}$) C, H, N.

3-*N*-(Benzyloxycarbonyl)-2′,3′-dideoxy-2′,3′-epiminoribostamycin (15). A solution of **14** (0.11 g, 0.083 mmol) in *p*-dioxane (5 mL) was heated to 60–70 °C, and 0.1 M aqueous barium hydroxide octahydrate (5 mL) was added. The mixture was stirred at the same temperature and another two aliquots of barium hydroxide (5 mL) were added after 1 h. The contents were neutralized after 15 h with carbon dioxide, after cooling to 25 °C, and the barium carbonate was removed by filtration through Celite. The residue was finally washed with aqueous *p*-dioxane (1:1), and the combined wash and filtrate was evaporated to dryness. The product was again dissolved in dry *N,N*-dimethylformamide and filtered through a Celite pad. Concentration of the solution and precipitation with dry acetone gave **15**: yield 58 mg (70%); mp 260–263 °C dec; $[\alpha]^{25}_{546}$ +34.8 (*c* 0.5, DMF); IR 3700–3000 (NH and OH), 1695 (NHCO I), 1520 cm^{-1} (NHCO II); NMR δ 1.4–2.3 (m, 4 H, 2′, 3′, and 2 protons), 5.0 (s, benzylic protons), (s, aromatic protons). Anal. ($C_{25}H_{38}N_4O_{11} \cdot 1.5H_2CO_3$) C, H, N.

6′,4′:3″,2″-*N,O*-Dicarbonyl-4″,6″-*O*-cyclohexylidene-3′-*O*-toluenesulfonyl-1,3,2′-tris[*N*-(benzyloxycarbonyl)]kanamycin B (17). To a solution of **16**[15] (1.0 g, 0.98 mmol) in 5 mL of dry pyridine (1.0 g, 5.2 mmol) was added *p*-toluenesulfonyl chloride, and the reaction mixture was kept at 25 °C for 20 h. The pyridine was removed under reduced pressure and the residue was triturated with ether. The residue was dissolved in chloroform and washed with cold sodium bicarbonate solution. The organic layer was dried over sodium sulfate and evaporated to a small volume. Addition of ether gave **17**: yield 0.843 g (73%); mp 195–197 °C dec; $[\alpha]^{25}_{546}$ +52.5 (*c* 1.0, $CHCl_3$); IR 1775 (five-membered cyclic carbamate), 1725 (six-membered cyclic carbamate), 1700 (amide I), 1515 (amide II), 1175 cm^{-1} (SO_3); NMR ($CDCl_3$) δ 1.21 (10 H, cyclohexyl), 2.45 (s, 3 H, methyl). Anal. ($C_{57}H_{65}N_5O_{20}S$) C, H, N, S.

2′,3′-[*N*-(Benzyloxycarbonyl)epimino]-6′,4′:3″,2″-*N,O*-dicarbonyl-1,3-bis[*N*-(benzyloxycarbonyl)]-2′,3′-dideoxykanamycin B (18). A suspension of **17** (0.745 g, 0.63 mmol) in 10 mL of 80% acetic acid was heated at 80 °C for 1 h. The acetic acid was removed under reduced pressure and remaining traces of it were removed by coevaporation with toluene. The residue was triturated with water and washed thoroughly with ether to give the 4″,6″-dihydroxy intermediate: yield 0.684 g; IR 3600–3200 (NH and OH), 1175 cm^{-1} (SO_2). The product was used in the next step without purification.

The above compound (0.585 g, 0.5 mmol) was dissolved in 25 mL of dry *N,N*-dimethylformamide under nitrogen and treated with 50% sodium hydride (0.120 g, 2.5 mol) at 25 °C. The same workup as for compound **4** gave the product **18** after recrystallization from *N,N*-dimethylformamide–acetone: yield 0.305 g (66%); mp >270 °C dec; $[\alpha]^{25}_{546}$ +58.57 (*c* 0.7, DMF); IR 1770 (five-membered carbamate), 1725 (six-membered carbamate), 1700

(amide I), 1520 cm^{-1} (amide II); NMR δ 2.85 (d, 2 H, 2',3' protons). Anal. ($C_{44}H_{51}N_5O_{17}$) C, H, N.

2',3'-Dideoxy-2',3'-epiminokanamycin B (19). Compound 18 (0.42 g, 0.46 mmol) was suspended in 40 mL of dry liquid ammonia and small pieces of sodium metal (0.5 g) were added. The dark blue colored solution was stirred at −70 °C for 2 h. Workup as described for 4 gave a product which was separated on an Amberlite CG-50 (NH_4^+) column. The major component, homogenous on TLC (chloroform–methanol–27% ammonium hydroxide, 1:1:1), was eluted with 0–0.5 N ammonium hydroxide solution. Concentration of the solution and addition of methanol–acetone precipitated **19** as the carbonate–hemihydrate: yield 60 mg (28%); mp >235 °C dec; $[\alpha]^{25}_{546}$ +89.2 (c 1.0, H_2O); IR 1590 (NH_3^+), 1450 cm^{-1} (CO_3^{-2}); NMR (D_2O) δ 1.7–2.1 (d, 2 H, 2' and 3' protons). Anal. ($C_{18}H_{35}N_5O_9 \cdot H_2CO_3 \cdot 0.5H_2O$) C, H, N.

2'',3''-Dideoxy-2'',3''-[*N*-(*p*-toluenesulfonyl)epimino]tetrakis(*N*-*p*-toluenesulfonyl)kanamycin B (21). To a solution the **20** (0.38 g, 0.25 mmol) in dry *N*,*N*-dimethylformamide (25 mL) under nitrogen was added 50% sodium hydride (0.1 g, 20.8 mmol). The mixture was stirred at 25 °C for 48 h, cooled in ice, and neutralized with glacial acetic acid. The solvent was removed under reduced pressure and the resulting syrup was diluted with chloroform. The chloroform solution was washed with water, dried over magnesium sulfate, and concentrated to a small volume. Addition of hexane gave the aziridine as a tan solid: yield 0.202 g (60%); NMR ($CDCl_3$) no mesylate at δ 3.21.

The above aziridine (0.20 g, 0.14 mmol) was dissolved in a very small amount of acetone, treated with 10 mL of 80% acetic acid, and stirred at 70–80 °C for 1 h. The acetic acid was removed under reduced pressure and coevaporated with toluene to remove the last traces. The product was finally purified on a silica gel column with chloroform–methanol as solvent, to give **21** as the trihydrate after recrystallization with chloroform–ether: yield 0.140 g (77%); mp 167–170 °C dec; $[\alpha]^{25}_{546}$ +26.2 (*c* 0.5, DMF); IR 1315, 1150 cm^{-1} (SO_2); NMR δ 2.4 (s 3 H, methyl protons), 2.80 (s 2 H, 2'' and 3'' protons). Anal. ($C_{53}H_{65}N_5O_{19}S_5 \cdot 3H_2O$) C, H, N, S.

2'',3''-Dideoxy-2'',3''-epiminokanamycin B (22). To a solution of **21** (0.11 g, 0.089 mmol) in liquid ammonia–ethylamine (48:10 mL) cooled at dry ice–acetone temperature was added sodium metal (0.253 g, 11 mmol) with stirring. The dark blue reaction mixture was stirred at this temperature for 2 h, and the blue color was neutralized by the addition of methanol. The ammonia was slowly evaporated under reduced pressure. The residue was dissolved in water (25 mL) and neutralized with Dowex-50WX2 (H^+) resin. The solution was then poured over a column of Dowex–50WX2 (NH_4^+) and washed thoroughly with water. The column was eluted with 0–1 M ammonium solution. The main fraction was collected and again purified over Amberlite CG-50 (NH_4^+) column to give **22** as the carbonate monohydrate after precipitation with acetone and methanol: yield 23 mg (47%); mp 235 °C dec; $[\alpha]^{25}_{546}$ +51.0 (*c* 0.2, H_2O); NMR (D_2O) 1.9–2.2 (d, 2 H, 2'' and 3'' protons). Anal. ($C_{18}H_{35}N_5O_9 \cdot H_2CO_3 \cdot H_2O$) C, H, N.

3',4':4'',6''-Di-*O*-cyclohexylidene-2''-*O*-(methanesulfonyl)pentakis[*N*-(ethoxycarbonyl)]kanamycin B (24). A solution of **23**[18] (2.0 g, 2 mmol) in 35 mL of pyridine was treated at room temperature with methanesulfonyl chloride (1.5 g, 13 mmol). After 6 h, the mixture was poured into water and the precipitate that formed was collected, washed thoroughly with water, and air dried. This procedure gave 2.04 g (95%) of **24** as a white powder: mp 219–222 °C dec; IR 1360, 1170 cm^{-1} (SO_2); NMR δ 3.11 (s, 3 H, SO_2CH_3). The analytical sample had mp 226–227 °C dec after recrystallization from methanol–water. Anal. ($C_{47}H_{75}N_5O_{22}S$) C, H, N, S.

2''-*O*-(Methanesulfonyl)pentakis[*N*-(ethoxycarbonyl)]kanamycin B (25). A solution of **24** (2.0 g, 1.8 mmol) in 200 mL of 40% aqueous acetic acid was stirred at 60 °C for 6 h and then concentrated. Residual traces of acetic acid were removed by addition of toluene and reconcentration. The resulting white solid was washed with water and dried in air to give **25** quantitatively: mp 207–210 °C dec; IR 1340, 1170 cm^{-1} (SO_2); NMR δ 3.10 (s, 3 H, SO_2CH_3). Recrystallization from methanol–water gave the analytical sample with mp 220–221 °C dec. Anal. ($C_{34}H_{59}N_5O_{22}S$) C, H, N, S.

Barium Hydroxide Hydrolysis of 25. A mixture of **25** (400 mg, 0.43 mmol), barium hydroxide octahydrate (2.80 g, 9 mmol), and 30 mL of water was placed in a pressure vessel and heated so that the contents boiled vigorously. After 12 h, the mixture was cooled, water (100 mL) was added, and insolubles were removed by filtration. The filtrate was treated with carbon dioxide to precipitate barium salts, and the mixture was refiltered and concentrated. The glassy residue showed at least four spots on thin-layer chromatography. One of these spots had an R_f value identical with that of the 2'',3''-epimino derivative **22**, prepared from **20** as described above, in two different systems: the upper layer of chloroform–methanol–ammonium hydroxide (17%), 2:1:1, on silica gel and 1-butanol–pyridine–acetic acid–water, 15:10:3:12, on silica gel. The glassy residue was chromatographed on Amberlite IRC-50 (NH_4^+) with 0.1 and 0.5 N ammonium hydroxide as eluants, but no resolution of the mixture was obtained according to TLC. Reconcentration of the eluate and treatment with methanol gave 30 mg of an impure solid that was not **22** according to its chromatographic properties. It showed a molecular ion at *m/e* 485 according to field-desorption mass spectrometry and 483 according to electron-impact mass spectrometry. These data suggest an isomer of kanamycin B, but we were unable to further elucidate its structure.

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