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Aminoglycoside Antibiotics. 2. *N,N*-Dialkylkanamycins

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$N^{6'}$,$N^{3''}$-Dialkyl derivatives of kanamycins A and B were prepared regiospecifically from the parent antibiotics. Although the dimethyl and diethyl derivatives of kanamycin A were inactive in standard antibacterial assays, the dimethyl derivative of kanamycin B showed moderate activity, especially against various strains of *Pseudomonas aeruginosa*. A method for the selective dimethylation of the 3″-amino group of kanamycin A also was developed.

The preparation of *N*-alkyl derivatives of the kanamycins has provided compounds with useful antibacterial activity. Thus, the $N^{6'}$-methyl derivatives of kanamycins A and B were similar in potencies to the parent antibiotics but less susceptible to inactivation by strains of bacteria that elaborate aminoglycoside-6′-acetyltransferase.[1,2] The $N^{3''}$-methyl derivatives of kanamycins A and B also were comparable in antibacterial potencies to the parent compounds;[1,3] however, the methyl groups did not afford protection against inactivation by aminoglycoside 2″-nucleotidyltransferase.[1] Larger alkyl groups at either $N^{6'}$ or $N^{3''}$ produced inactive molecules, but a series of N^1,-N^3,$N^{3''}$-trialkyl derivatives of kanamycin A in which the alkyl groups were benzyl or cyclohexylmethyl had low potency.[1] A unique property of the latter compounds was their nearly equal potencies against such diverse bacteria as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

There are no reported examples of $N^{6'}$,$N^{3''}$-dialkylkanamycins. However, this type of compound should be of interest because the same structural feature occurs naturally in gentamicins C_1 and C_{2_b} (sagamycin). Since it appeared that suitable $N^{6'}$,$N^{3''}$-dialkylkanamycins would have appreciable antibacterial activity, we undertook an investigation of their preparation and properties. The main problem in preparing these compounds lies in the regiospecificity of the alkylation reactions. Some selectivity can be obtained for $N^{6'}$, since it is the only amino group on a primary carbon, but $N^{3''}$ is one of the less reactive nitrogens in the kanamycins. As described below, a variety of methods utilizing different blocking groups were developed for $N^{6'}$,$N^{3''}$-dialkylkanamycins.

The synthesis of $N^{6'}$,$N^{3''}$-dimethylkanamycin B (**16**) from kanamycin B (**5**) began with the formation of penta-*N*-carbobenzyloxy derivative **6**.[4] Treatment of **6** with sodium hydride in *N,N*-dimethylformamide gave selectively the bis(cyclic carbamate) **10**, in which both the 6′ and 3″-amino groups are involved in the cyclic carbamates. That the five-membered cyclic carbamates were the 2″,3″-O,N derivative rather than the isomeric 4″,3″ derivatives was shown by the following evidence. Treatment of **10** and **12** with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in dry *N,N*-dimethylformamide gave cyclohexylidene derivatives **21** and **22** in nearly quantitative yields. These cyclohexylidenes could be formed only from the 2″,3″-*O,N*-carbonyl derivatives. Catalytic hydrogenolysis of **10** gave compound **11**, which has only these two amino groups acylated. Lithium aluminum hydride smoothly converted **11** into $N^{6'}$,$N^{3''}$-dimethylkanamycin B (**16**). $N^{6'}$,$N^{3''}$-Dimethylkanamycin A (**17**) was prepared from kanamycin A (**7**) by a route parallel to the one just described. It involved intermediates **8**, **12** and **13**.

Preparation of $N^{6'}$,$N^{3''}$-diethylkanamycins requires a different approach than the one utilized for the corresponding methyl analogues. A convenient synthesis of $N^{6'}$,$N^{3''}$-diethylkanamycin A (**18**) was obtained by a route based on the two O → N acetyl migrations that occurred when hexa-*O*-acetyl-tetra-*N*-carbobenzyloxykanamycin A (**14**) was hydrogenolyzed. This type of migration had been reported previously for $O^{4'} \rightarrow N^{6'}$ in a kanamycin A derivative by Kawaguchi and co-workers.[5] The synthesis of **18** started with the treatment of **8** with acetic anhydride in pyridine, which gave cleanly a hexa-*O*-acetyl derivative. This derivative probably has structure **14** because it is known from many experiments that the 5-hydroxyl group of a kanamycin is the least reactive one. Catalytic hydrogenolysis of **14** gave a tri-*O*-acetyl-$N^{6'}$,$N^{3''}$-di-*N*-acetylkanamycin A as the result of the acetyl migrations. An additional *O*-acetyl group was lost during this reaction or the isolation procedure. Probably this was the 6″-*O*-

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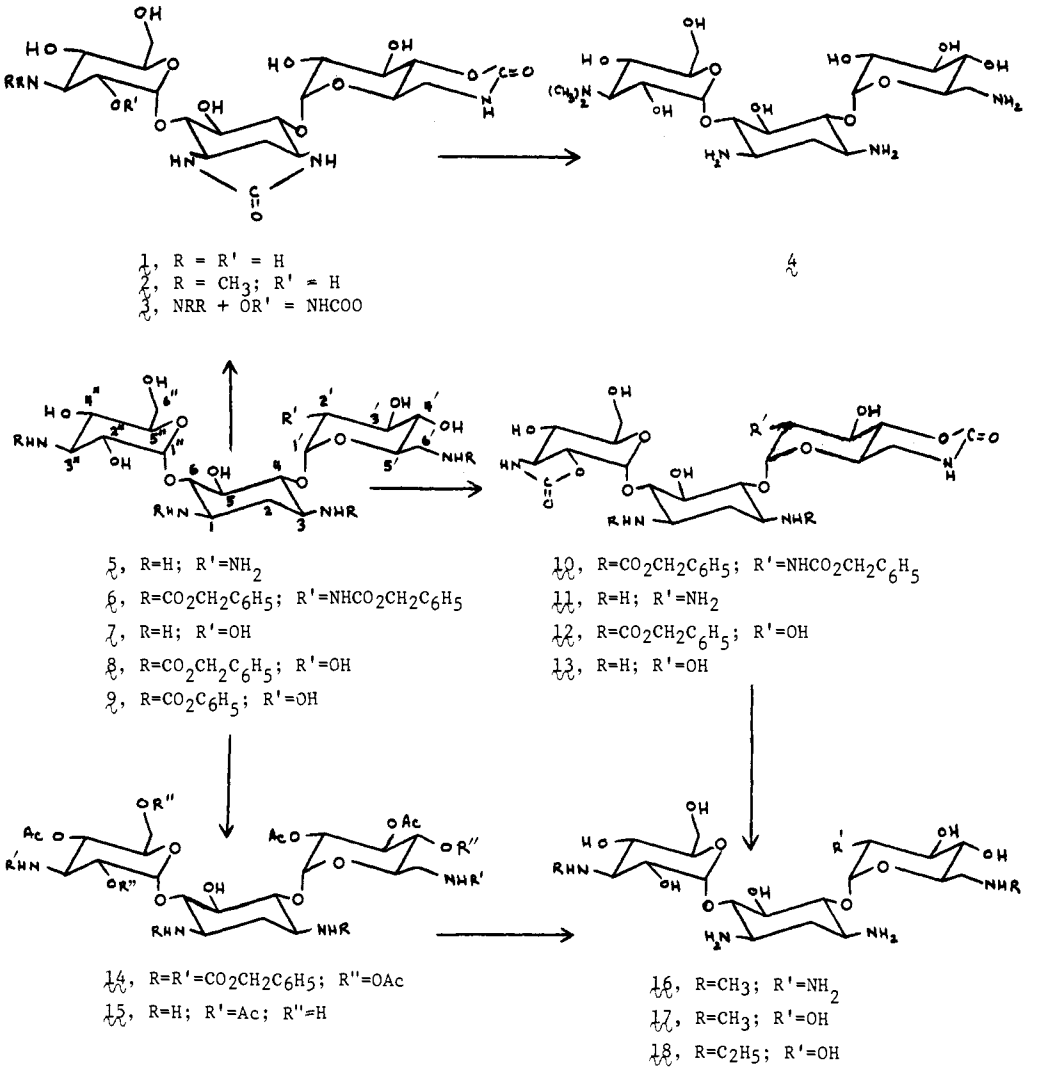
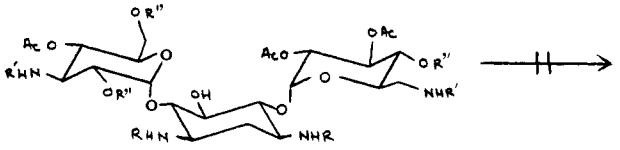


Table I. Antibacterial Activities by Serial Dilution. $N^{6'},N^{3''}$-Dialkylkanamycins Compared with Kanamycins A and B[a]

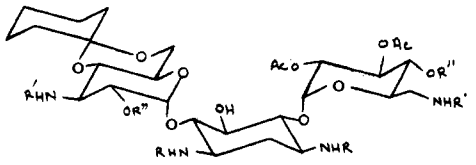
bacterial species	Bristol no.	inact enz	min inhibr concn (μg/mL) kanamycin A	kanamycin B	13	16	17	18
S. aureus	9537	none	1	0.5	32	4	>125	63
S. aureus	20240	APH(3')-IV	>125	>125	32	8	>125	>125
E. coli	9632	none	4	2	32	16	>125	>125
E. coli	20665	APH(3')-I	>125	>125	63	63	>125	>125
E. coli	20732	ANT(2'')	125	32	125	63	>125	>125
E. coli	21218	AAC(6')	>125	32	63	63	125	>125
E. cloac.	9656	none	4	2	63	8	>125	125
E. cloac.	20364	APH(3')-I	>125	>125	125	63	>125	>125
K. pneu.	9977	none	0.25	0.25	63	4	>125	>125
P. rett.	9637	none	0.5	0.5	63	16	>125	32
P. rett.	21207	AAC(2')	8	32	125	63	>125	4
P. aerug.	9843a	none	63	63	32	8	>125	>125
P. aerug.	20610	APH(3') + AAC (3)-I	32	32	32	8	>125	>125
P. aerug.	20653	APH(3')-IV	>125	>125	32	8	>125	>125
P. aerug.	20897	APH(3')-I + AAC(6')	>125	>125	32	8	>125	>125
P. aerug.	21509	Perm. mutant	>125	>125	32	16	>125	>125

[a] All assays were determined at Bristol Laboratories, Syracuse, N.Y. Abbreviations for bacteria: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *E. cloac.*, *Enterobacter cloacae*; *K. pneu*, *Klebsiella pnenumoniae*; *P. rett.*, *Proteus rettgeri*; *P. aerug.*, *Pseudomonas aeruginosa*. Abbreviations for aminoglycoside inactivating enzymes: APH(3'), aminoglycoside-3'-phosphotransferase; ANT(2''), aminoglycoside-2''-nucleotidyltransferase; AAC(6'), aminoglycoside-6'-acetyltransferase; AAC(2'), aminoglycoside-2'-acetyltransferase. For a complete description of the antibacterial assay process, see M. Mislek, T. A. Pursiano, L. B. Crast, F. Leitner, and K. E. Price, *Antimicrob. Agents Chemother.*, 1, 54 (1972).

acetyl group (primary alcohol). The structure of the migration product has not been established unambiguously. However, structure **15** is the most reasonable possibility. Conversion of this product to the corresponding tetra-*N*-acetyl derivative (probably **19**) with acetic anhydride in methanol, followed by treatment with

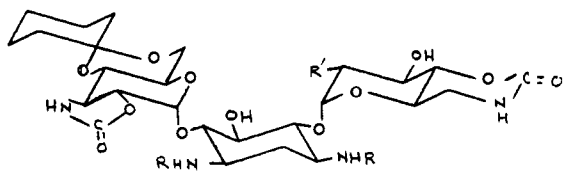


19, R=R'=Ac; R"=H



20, R=R'=Ac; R"=H

1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid under the same conditions used for the preparation of **21** and **22** failed to give a cyclohexylidene derivative (**20**).



21, R=$CO_2CH_2C_6H_5$; R'=$NHCO_2CH_2C_6H_5$
22, R=$CO_2CH_2C_6H_5$; R'=OH

Thus the tetra-*N*-acetyl derivative clearly does not have free 4"- and 6"-hydroxyl groups. It is more likely that it has the free 2"- and 6"-hydroxyl groups of structure **19**. This means that the acetyl group on $N^{3"}$ of **15** would have migrated from the 2"-acetoxy group of **14** rather than from the 4"-acetoxy group. Reduction of compound **15** with sodium bis(2-methoxyethoxy)aluminum hydride (Vitride) gave **18** in 35% yield. This reagent was superior to lithium aluminum hydride because of its greater solubility.

As discussed above, treatment of tetra-*N*-carbobenzyloxykanamycin A (**8**) with sodium hydride gave bis(cyclic carbamate) **12**. In contrast, the corresponding tetra-*N*-phenoxycarbonyl derivative (**9**) of kanamycin A, which has better leaving groups in the substituents, gave a product (**3**) that had a ureide function in addition to the two cyclic carbamates, upon treatment with Amberlite IR-45 (OH^-) resin. Treatment of **9** with Dowex 1-X2 (OH^-) resin gave the corresponding product (**1**) with only one cyclic carbamate group. Compound **1** was unique in that every amino group except 3" was blocked. We briefly investigated $N^{3"}$-alkylation of this compound and found that treatment with formaldehyde and sodium cyanoborohydride gave the dimethyl derivative **2**. It was not possible to obtain a monomethyl derivative by this method. Alkaline hydrolysis of **2** furnished $N^{3"},N^{3"}$-dimethylkanamycin A (**4**) in 23% yield from **1**. Compound **4** had been prepared previously and it was found to be inactive as an antibacterial agent.[1]

Biological Activity. The antibacterial activities of $N^{6'},N^{3"}$-dialkylkanamycins **16–18** are given in Table I. Compound **13**, an intermediate in the syntheses of **14**, also is listed, since it showed weak activity against a variety of bacterial strains. The activity profiles of the dialkylkanamycins were unanticipated and, in the case of the kanamycin A analogues, disappointing. However, a paper published in the course of this research described the synthesis of each of the possible mono-*N*-ethyl derivatives of kanamycins A and B and it revealed that each of these derivatives was less potent than the parent antibiotic. Although the 3"-*N*-ethyl derivative of kanamycin A retained 66% of the parent's activity, the corresponding 6'-*N*-ethyl derivative had only 19% of this activity. In the kanamycin B series the 3"- and 6'-*N*-ethyl derivatives had 87 and 35%, respectively, the activity of the parent compound.[7] $N^{6'},N^{3"}$-Dimethylkanamycin B (**16**) showed the best activity among our compounds. It was about eightfold less potent than the kanamycins against strains of bacteria that do not elaborate aminoglycoside-inactivating enzymes, but it was more potent against some of the enzyme-producing strains. Possibly the difference in profile reflects poorer binding of compound **16** to both bacterial ribosomes and the inactivating enzymes. The relatively good activity of **16** against various strains of *Pseudomonas aeruginosa* is not readily understood, except that its activity against a mutant strain impermeable to kanamycins A and B indicates that **16** penetrates *Pseudomonas* better, perhaps because of its increased lipophilicity. The nearly equal potency of **16** against *S. aureus*, *E. coli*, and *P. aeruginosa* strains resembles the profile of $N^1,N^3,N^{3"}$-trialkyl derivatives of kanamycin A discussed above. Compound **13**, the synthetic precursor to **16**, showed a similar profile, except that it is less active against nearly all bacterial strains.

Table I shows that $N^{6'},N^{3"}$-dimethylkanamycin A (**17**) is inactive against all strains. The corresponding $N^{6'},N^{3"}$-diethyl analogue **18** shows activity against a few strains of bacteria, but overall its activity is insignificant.

Although **16** is more potent than kanamycins A and B against certain bacteria which elaborate inactivating enzymes, no clear structure–activity relationships can be established in this area. One might have predicted that the best enhancement of activity would be against an aminoglycoside 6'-acetyltransferase producing strain such as *E. coli* 21218. However, **16** is less active than kanamycin B against this strain. On the other hand, **16** is more active than kanamycin B against *E. coli* 20665 and *S. aureus* 20240, two aminoglycoside 3'-phosphotransferase producing strains.

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 in Me_2SO-d_6 (unless otherwise specified) on Varian EM-360 and T-60 spectrometers using tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-5-sulfonate as the standard. 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, Arizona. Analytical results were within ±0.4% of theoretical values.

Penta-*N*-benzyloxycarbonylkanamycin B (6). A mixture of kanamycin B sulfate (**5**) (0.5 g) and sodium carbonate (1.5 g, excess) in 16 mL of 75% aqueous methanol was stirred at ice-bath temperature and treated dropwise with benzyl chloroformate (1.25 g, 7.35 mmol) in 2 mL of methanol. After 2 h at this temperature and 18 h at 25 °C, the mixture was concentrated under reduced pressure and the residue was triturated with water. The white solid that formed was washed with ether and water and recrystallized from *N*,*N*-dimethylformamide–ether. This procedure gave 660 mg (85%) of **6**, which decomposed without melting at about 260 °C: $[\alpha]^{24}_{546}$ +81.0 (*c* 0.9, DMF); IR (KBr) 1710 and 1695 (amide I), 1540 cm^{-1} (amide II); NMR δ 5.04 (s, benzylic), 7.34 (br s, aromatic). Anal. ($C_{58}H_{61}N_5O_{20}$) C, H, N.

1,3,2'-Tri-*N*-benzyloxycarbonyl-6',4";3",2"-*N*,*O*-carbonylkanamycin B (10). An ice-cooled solution of **6** (0.70 g, 0.61 mmol) in 15 mL of dry *N*,*N*-dimethylformamide, under nitrogen,

was treated with 50% sodium hydride suspension (0.125 g, 3.1 mmol). In some runs the mixture became gelatinous and more solvent was added. After 2 h at 25 °C, the mixture was neutralized with acetic acid, concentrated under reduced pressure, and treated with water. The resulting white precipitate was washed with ether and water and crystallized from *N,N*-dimethylformamide–ether. This procedure gave 0.49 g (85%) of **10** as the monohydrate, a white powder: mp 258–260 °C dec; $[\alpha]^{24}_{546}$ +102.1 (*c* 1.0, DMF); IR 1775 (five-membered cyclic carbamate), 1725 cm^{-1} (six-membered cyclic carbamate). Anal. ($C_{44}H_{51}N_5O_{18}\cdot H_2O$) C, H, N.

6′,4′:3″,2″-*N,O*-Carbonylkanamycin B (11). A suspension of 1.8 g of **10** in 30 mL of 50% aqueous dioxane and 5 mL of acetic acid was treated with 1.0 g of 10% Pd/C and shaken with hydrogen at 50 psi for 70 h. The mixture was filtered and concentrated under reduced pressure. Toluene was added and the mixture was again concentrated to remove traces of acetic acid. The residue was dissolved in a small volume of water, kept over Amberlite IR-45 (OH^-) for 2 h, filtered, and concentrated partially. Addition of methanol and acetone gave 1.0 g (100%) of **11** as the hemihydrate, a white solid that decomposed above 265 °C: $[\alpha]^{24}_{546}$ +112.0 (*c* 1.0, H_2O); IR 1770 (five-membered cyclic carbamate), 1725 cm^{-1} (6-membered cyclic carbamate); NMR no benzyl groups. Anal. ($C_{20}H_{33}N_5O_{12}\cdot CH_3OH\cdot 0.5H_2O$) C, H, N.

***N*⁶′,*N*³″-Dimethylkanamycin B (16).** A suspension of **11** (1.3 g, 2.5 mmol) in 50 mL of dry tetrahydrofuran was treated with lithium aluminum hydride (0.25 g, 6.6 mmol). The mixture was stirred at reflux for 44 h and treated with additional lithium aluminum hydride (0.15 g, 3.9 mmol). After 6 h, the mixture was cooled and treated carefully with water to destroy excess hydride. It was diluted with water, filtered, concentrated to a small volume, and treated with *p*-anisaldehyde (1.3 g, 10.5 mmol). The Schiff base was collected, washed thoroughly with petroleum ether, and hydrolyzed with 0.1 N HCl. The resulting solution was neutralized with Amberlite IR-45 (OH^-) and evaporated. Chromatography on an Amberlite IRC-50 (NH_4^+) column with 0.1–0.5 M NH_4OH as solvent, followed by concentration to a small volume and addition of methanol and acetone, gave 0.21 g (17%) of **16** as the carbonate salt, a white solid that decomposed above 240 °C: $[\alpha]^{24}_{546}$ +123.00 (*c* 0.9, H_2O); NMR δ 2.5 (3 s, *N*⁶′-methyl group), 2.85 (3 s, *N*³″-methyl group). Anal. ($C_{20}H_{41}N_5O_{10}\cdot H_2CO_3$) C, H, N.

Tetra-*N*-benzyloxycarbonylkanamycin A (8).[8,9] This compound was prepared by the method described for **6**. From 2.0 g of kanamycin A sulfate (**7**) was obtained 3.45 g (94%) of **8** as a white powder: mp 258–260 °C dec, lit. 250–254 °C dec; $[\alpha]^{24}_{546}$ +85.0 (*c* 1.0, DMF).

1,3-Di-*N*-benzyloxycarbonyl-6′,4′:3″,2″-*N,O*-carbonylkanamycin A (12). This compound was prepared by the procedure described for **10**. From 3.0 g of **8** was obtained, after crystallization from methanol–ether, 2.06 g (88%) of **12** as a white powder: mp 240–242 °C dec; $[\alpha]^{24}_{546}$ +78.6 (*c* 1.0, DMF). Anal. ($C_{36}H_{44}N_4O_{17}$) C, H, N.

6′,4′:3″,2″-*N,O*-Carbonylkanamycin A (13). This compound was prepared by the method described for **11**. From 2.8 g of **12** was obtained, after trituration with ethyl acetate, 1.8 g (96%) of **13** as the diacetate hemihydrate, a white powder that decomposed above 260 °C: $[\alpha]^{24}_{546}$ +94.2 (*c* 1.0, H_2O); IR 1765 (five-membered cyclic carbamate), 1715 cm^{-1} (six-membered cyclic carbamate). Anal. ($C_{20}H_{32}N_4O_{13}\cdot 2CH_3CO_2H\cdot 0.5H_2O$) C, H, N.

***N*⁶′,*N*³″-Dimethylkanamycin A (17).** This compound was prepared by the method described for **16**. From 1.2 g of **13** was obtained 0.244 g (21%) of **17** as the hemicarbonate, a white powder: mp 203–206 °C dec; $[\alpha]^{24}_{546}$ +166.0 (*c* 0.6, H_2O); NMR (D_2O) δ 2.4 (3 s, *N*⁶′-methyl groups), 1.7 (3 s, *N*³″-methyl group). Chemical ionization mass spectrometry did not afford a molecular ion, but it gave an intense signal at *m/e* 176, which establishes the presence of a kanosamine moiety with the 3″-*N*-methyl group and/or a 6-amino-6-deoxyglucose moiety with the 6′-*N*-methyl group. Anal. ($C_{20}H_{40}N_4O_{11}\cdot 0.5H_2CO_3$) C, H, N.

1′,2′,3′,2″,4″,6″-Hexa-*O*-acetyl-1,3,6′,3″-tetra-*N*-benzyloxycarbonylkanamycin A (14). A stirred solution of **8** (3.8 g, 3.72 mmol) in 40 mL of dry pyridine was treated at 25 °C with 12 mL of acetic anhydride. The mixture was concentrated under reduced pressure. Ethyl acetate was added and removed by concentration several times to free the product from traces of acetic acid. Trituration with ether gave a white solid that was washed again with ether. This procedure afforded 4.2 g (88%) of **14**: mp 219–222 °C dec; $[\alpha]^{24}_{546}$ +91.4 (*c* 1.0, MeOH); IR 1735 (acetate), 1710 and 1690 (amide I), 1520 cm^{-1} (amide II). Anal. ($C_{62}H_{72}N_4O_{25}$) C, H, N.

6′,3″-Di-*N*-acetyl-1′,2′,4″-tri-*O*-acetylkanamycin A (15). A solution of **14** (4.2 g) in 30 mL of 80% aqueous dioxane and 2 mL of acetic acid was treated with 0.6 g of 10% Pd/C and shaken with hydrogen at 50 psi for 72 h. The mixture was filtered and concentrated. Acetic acid was removed from the product by addition and concentration of ethanol and ethyl acetate, followed by treatment with Amberlite IR-45 (OH^-) in ethanol. Filtration and evaporation gave a solid that was triturated with ethyl acetate. The product was heated at 50 °C in 50 mL of pyridine to ensure complete acetyl migration, and the pyridine was removed under reduced pressure. The residue was dissolved in a small volume of ethanol and precipitated with ethyl acetate. This procedure gave 2.0 g (83%) of **15** as the dihydrate, a white powder: mp 155–158 °C dec; $[\alpha]^{24}_{546}$ +115.2 (*c* 1.0, MeOH); IR 1740 (acetate), 1640 (acetamide), 1550 cm^{-1} (NH_2). Anal. ($C_{28}H_{46}N_4O_{16}\cdot 2H_2O$) C, H, N.

***N*⁶′,*N*³″-Diethylkanamycin A (18).** A solution of **15** (1.7 g, 2.3 mmol) in 50 mL of dry tetrahydrofuran was heated at reflux and treated gradually with a 70% benzene solution of sodium bis(2-methoxyethoxy)aluminum hydride (Vitride).[6] After 48 h at reflux temperature, the solution was cooled and neutralized with sulfuric acid (1.3 mL in 8 mL of water and 25 mL of methanol). The mixture was stirred for 30 min and filtered, and the solids were washed with aqueous methanol. The combined filtrate and washes were concentrated, and the residue was chromatographed on Amberlite IRC-50 (NH_4^+) with 0–0.1 M NH_4OH as solvent. The solid product was crystallized from aqueous methanol to give 0.44 g (35%) of **18** as the carbonate monohydrate, a white powder: mp 180 °C dec; $[\alpha]^{24}_{546}$ +132.3 (*c* 0.5, H_2O); NMR (D_2O) δ 1.9 (6 t, CH_3 of ethyl), 3.1 (4 q, CH_2 of ethyl). Chemical-ionization mass spectrometry gave an intense signal at *m/e* 190, which establishes the presence of a kanosamine moiety with the 3″-*N*-ethyl group and/or a 6-amino-6-deoxyglucose moiety with the 6′-*N*-ethyl group. The molecular ion was not observed. Anal. ($C_{22}H_{44}N_4O_{11}\cdot H_2CO_3\cdot H_2O$) C, H, N.

Tetra-*N*-phenoxycarbonylkanamycin A (9). A mixture of kanamycin A sulfate (**7**; 1.0 g) and sodium carbonate (7.0 g) in 50 mL of 70% aqueous methanol was treated dropwise with phenyl chloroformate (6.0 g) in methanol. After 1 h at room temperature, the mixture was neutralized with 1 N HCl, concentrated under reduced pressure, and extracted with dioxane. The extract was concentrated to a small volume and diluted with ether to give a solid. Thorough washing of this solid with ether and water furnished 1.6 g (95%) of **9** as a white solid: mp 235–237 °C dec; $[\alpha]^{24}_{546}$ +83.0 (*c* 1.0, DMF); IR 1720 and 1710 (amide I), 1520 cm^{-1} (amide II); NMR δ 7.2 (aromatic). Anal. ($C_{46}H_{52}N_4O_{19}$) C, H, N.

1,3-*N,N*-Carbonyl-6′,4′:3″,2″-*N,O*-carbonylkanamycin A (3). A solution of **9** (0.2 g, 0.21 mmol) in 5 mL of dry *N,N*-dimethylformamide was stirred with Amberlite IR-45 (OH^-) (5.0 g) at 25 °C for 20 h and filtered through a Celite pad. The pad was washed with *N,N*-dimethylformamide, and the combined filtrate and wash was concentrated to a small volume. Addition of ether gave a precipitate, which was washed thoroughly with ether and then reprecipitated from *N,N*-dimethylformamide–acetone. This procedure gave 0.105 g (94%) of **3** as a white solid that decomposed above 280 °C: $[\alpha]^{24}_{546}$ +83.4 (*c* 0.7, DMF); IR 1770 (five-membered cyclic carbamate), 1725 (six-membered cyclic carbamate), 1715 cm^{-1} (ureide). Anal. ($C_{21}H_{30}N_4O_{14}$) C, H, N.

1,3-*N,N*-Carbonyl-6′,4′-*N,O*-carbonylkanamycin A (1). A solution of **9** (0.50 g, 0.52 mmol) in 5 mL of dry *N,N*-dimethylformamide was added to a suspension of Dowex 1X2 (OH^-) (10.0 g, prewashed with *N,N*-dimethylformamide) in 10 mL of *N,N*-dimethylformamide. The mixture was stirred 20 h at 25 °C, neutralized with acetic acid, and filtered. The resin was washed two times with *N,N*-dimethylformamide, and the combined filtrate and washes was concentrated to a small volume. Addition of acetone gave a white solid that was recrystallized from aqueous methanol–acetone. This procedure gave 0.22 g (81%) of **1** as the diacetate hemihydrate, a white powder that decomposed above 270 °C: $[\alpha]^{24}_{546}$ +78.66 (*c* 0.9, H_2O); IR 1720 (six-membered cyclic

carbamate), 1700 cm^{-1} (ureide). Anal. ($C_{20}H_{32}N_4O_{13}\cdot 2CH_3CO\text{-}OH\cdot 0.5H_2O$) C, H, N.

$N^{3''}$,$N^{3''}$-Dimethylkanamycin A (4). A solution of 1 (1.1 g, 2.0 mmol) in 6 mL of 37% aqueous formaldehyde, 10 mL of acetonitrile, and 5 mL of water was treated with sodium cyanoborohydride (0.75 g, 11.9 mmol). Acetic acid (1 mL) was added slowly as the mixture was stirred. After 2 h, another 1 mL of acetic acid was added. The mixture was concentrated under reduced pressure after 16 h and the residual solid (compound 2) was dissolved in 10 mL of water. Barium hydroxide (10 mL of a 1 M solution) was added and the mixture was heated at reflux for 48 h. It was neutralized with CO_2 and filtered, and the filtrate was concentrated to a small volume and diluted with acetone. The brown solid that formed was purified by chromatography on Amberlite CG-50 (NH_4^+) with 0–0.2 M NH_4OH as solvent. This procedure was repeated and the product was recrystallized from aqueous ethanol–acetone to give 0.244 g (23%) of 4 as the dicarbonate, a white powder that decomposed above 260 °C: $[\alpha]^{24}_{546}$ +92.0 (*c* 0.4, H_2O); NMR (D_2O) δ 2.5 [6 s, $N(CH_3)_2$]. This sample was identical in its IR spectrum and R_f value (upper layer of chloroform–methanol–27% NH_4OH, 1:1:1, or chloroform–methanol–27% NH_4OH–water, 1:4:2:1) with a sample furnished by Bristol Laboratories.[1] Anal. ($C_{20}H_{40}N_4O_{11}\cdot 2H_2CO_3$) C, H, N.

1,3,6′,3″-Tetra-*N*-acetyl-1′,2′,4″-tri-*O*-acetylkanamycin A (19). A solution of **15** (0.43 g, 0.62 mmol) in 10 mL of dry methanol was treated with 2 mL of acetic anhydride and stirred at 25 °C for 40 h. It was concentrated to dryness with the aid of added ethyl acetate. The residue was precipitated from methanol–ether to give 0.35 g (72%) of **19** as the hemihydrate, a white powder: mp 208–210 °C; $[\alpha]^{24}_{546}$ +145.1 (*c* 1.0, DMF); IR 1730 (acetate), 1640 (amide I), 1540 cm^{-1} (amide II); NMR δ 1.8–2.25 (ms, acetyl), 7.75 (6, amide). Anal. ($C_{32}H_{50}N_4O_{18}\cdot 0.5H_2O$) C, H, N.

1,3,2′-Tri-*N*-benzyloxycarbonyl-6′,4′:3″,2″-*N*,*O*-carbonyl-6″,4″-*O*-cyclohexylidenekanamycin B (21). A solution of **10** (1.05 g, 1.12 mmol) in 2 mL of dry *N*,*N*-dimethylformamide was warmed at 80 °C under reduced pressure until the volume was reduced to 10 mL. It was cooled to 50 °C and treated with *p*-toluenesulfonic acid (40 mg) and 1,1-dimethoxycyclohexane (2.5 g, excess). Then it was again warmed at 80 °C under reduced pressure for 1.5 h. The residue was neutralized with triethylamine, concentrated to a small volume, and treated with water. The solid that formed was reprecipitated from dioxane–ether to give 1.11 g (97%) of **21** as a white solid: mp 238–240 °C dec; $[\alpha]^{24}_{546}$ +53.4 (*c* 1.0, DMF); IR 1770 (five-membered cyclic carbamate), 1725 (six-membered cyclic carbamate), 1700 (amide I), 1520 cm^{-1} (amide II); NMR δ 1.45 (br, cyclohexyl), 5.0 (s, benzylic), 7.3 (br s, aromatic). Anal. ($C_{50}H_{59}N_5O_{18}$) C, H, N.

1,3-Di-*N*-(benzyloxy)-6′,4′:3″,2″-*N*,*O*-carbonyl-6″,4″-*O*-cyclohexylidenekanamycin A (22). This compound was prepared by the procedure described for **21**. From 2.2 g of **12** was obtained, after reprecipitation from dioxane–ether, 2.38 g (98%) of **22** as a white solid: mp 228–230 °C dec; $[\alpha]^{24}_{546}$ +67.0 (*c* 1.0, DMF); IR 1770 (five-membered cyclic carbamate), 1720 (six-membered cyclic carbamate), 1700 (amide I), 1525 cm^{-1} (amide II); NMR δ 1.5 (br, cyclohexyl), 5.05 (br s, benzylic), 7.25 (br s, aromatic). Anal. ($C_{42}H_{52}N_4O_{17}$) C, H, N.

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Novel Application of Proton Nuclear Magnetic Resonance Spectroscopy in the Identification of 2′-Chloronordiazepam Metabolites in the Dog

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The only metabolite of 2′-chloronordiazepam, 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (1), previously identified in the dog is lorazepam (2), which is a product of 3-hydroxylation. Two phenolic metabolites (3 and 4) in the dog corresponding to 4′-hydroxylation of the 5-phenyl ring and 9-hydroxylation of the fused benzene ring, respectively, have now been identified. The structure of the 9-hydroxy isomer 4 is deduced simply from the observed NMR spectral AB (J_{meta} = 2.5 Hz) pattern of the protons of the fused benzene ring. In contrast, since a 2′-chloro substituent is present on the 5-phenyl ring of the parent drug, the usual method of recognizing 4′-hydroxylation of this ring by observation of AA′BB′ multiplets in the proton nuclear magnetic resonance (^1H NMR) spectra is inapplicable. Hence, a novel method is introduced to identify the 4′-hydroxy isomer 3, based on attributing different sets of NMR substituent effect parameters to hydroxyl groups, depending on whether these groups are meta or para to the benzodiazepinimine function. The urinary plus fecal excretion of 2–4 by one dog given a single oral 10 mg/kg dose of ^{14}C-labeled 1 amounted to 20, 5, and 7% of the dose, respectively; the urinary metabolites were excreted predominantly as conjugates of glucuronic acid and/or sulfate.

2′-Chloronordiazepam, 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (1), was reported by Zbinden and Randall[1] to be a potent benzodiazepine in terms of central nervous system activity in

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