in DMF (450 mL) at 10 °C was treated portionwise with a 57% NaH oil dispersion (10.9 g, 0.258 mol). After the solution was stirred at room temperature for 4 h, 2-iodoacetamide (47.5 g, 0.257 mol) was added in one portion. The mixture was stirred, with occasional cooling at 20 ± 2 °C, for 4 h and then poured into 4000 mL of H_2O. The solids were collected by filtration, washed with H_2O, and dried: yield 94 g. The cake was suspended in 740 mL of EtOAc, heated to reflux, and filtered. Upon cooling, the filtrate yielded 62.6 g (64.4%) of **9**: mp 148–149 °C; NMR (Me_2SO-d_6) δ 2.4 (s, Ar CH_3), 4.38 (s, CH_2), 6.72 (dd, J = 8.2 Hz), 7.10 (s, NH), 7.19 (dd, J = 8.2 Hz, H-4), 7.39 (d, J = 8 Hz), 7.65 (d, J = 8 Hz, Ar H), 7.46 (s, Ar H), 7.63 (d, J = 8 Hz, H-5). Anal. ($C_{20}H_{19}N_3O_3S$) C, H, N.

The EtOAc-insoluble portion was nearly pure **10** and weighed 5.08 g (5.2%), mp 223–226 °C dec. Recrystallization from DMF-EtOH yielded analytically pure **10**, mp 225–226 °C dec. NMR (Me_2SO-d_6) 2.33 (s, Ar CH_3), 5.16 (s, CH_2), 6.23 (dd, J = 8 and 2 Hz, H-3), 7.27 and 7.67 (d, J = 8 Hz, tolyl H), 7.17 (dd, J = 8 and 2 Hz, H-5), 7.4–7.6 (m). Anal. ($C_{20}H_{19}N_3O_3S$) C, H, N, S.

The EtOAc filtrates were evaporated to dryness, and the residue was passed through the Prep HPLC to yield an additional 12.9 g (13.2%) of **9** (1.1–1.9 cv; EtOAc–CH_2Cl_2, 7:3). Further elution yielded 0.52 g (0.52%) of **10**.

5-(Phenylthio)-2-(trifluoroacetamido)imidazo[1,2-*a*]pyridine (11). A suspension of **10** (2.6 g, 6.28 mmol) in TFAA (40 mL) was heated at reflux for 90 min. The solvent was removed in vacuo, and the residue was taken up in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and H_2O. The organic layer was dried and evaporated in vacuo to yield 2.02 g of an amorphous residue. Chromatography over 200 g of silica gel and elution with EtOAc–CH_2Cl_2 (1:4) yielded 0.924 g (43.8%) of **11**: mp 115–117 °C (*n*-hexane); NMR ($CDCl_3$) δ 7.03 (dd, J = 7 and 2 Hz), 7.26 (dd, J = 9 and 6 Hz), 7.38 (br s, Ar H), 7.5 (dd, J = 8 and 2 Hz, H-8), 8.25 (H-3), 11.4 (s, NH). Anal. ($C_{15}H_{10}F_3N_3OS$) C, H, N, S.

2-Amino-2-(phenylthio)imidazo[1,2-*a*]pyridine (12). The amide **11** (3.5 g, 10.3 mmol) was stirred in 2.5 N NaOH (40 mL) at room temperature for 24 h. The precipitate was collected by filtration, washed with H_2O, and dried to yield 2.47 g (91%) of **12**, mp 175–179 °C. After recrystallization from EtOH, the melting point was 177–178 °C. Anal. ($C_{13}H_{11}N_3S$) C, H, N.

Methyl 5-(Phenylthio)imidazo[1,2-*a*]pyridine-2-carbamate (1f). The amine **12** (75 mg, 3.1 mmol) and $(C_2H_5)_3N$ (314 mg, 3.1 mmol) in CH_2Cl_2 (40 mL) was treated dropwise with methyl chloroformate (293 mg, 3.1 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h and diluted with ice–H_2O. The organic layer was separated, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed over silica gel and eluted with EtOAc/$MeCl_2$ (1:1) to yield 300 mg of **1f**, mp 228–230 °C. Anal. ($C_{15}H_{13}N_2O_2S$) C, H, N, S.

3-(Trifluoroacetamido)-5-(phenylthio)imidazo[1,2-*a*]pyridine (14). A suspension of **9** (40 g, 96.7 mmol) in TFAA (400 mL) was heated at reflux for 18 h. The cooled mixture was diluted with 400 mL of ether, and the solids were collected by filtration. The cake was vigorously stirred with 500 mL of saturated aqueous $NaHCO_3$. The crude trifluoroacetamide, **14**, was collected by filtration, washed with H_2O, and dried to yield 25 g (76.7%), mp 195–197 °C. After recrystallization from EtOH, the melting point was 202–203 °C. Anal. ($C_{15}H_{10}F_3NOS$) C, H, N.

3-Amino-5-(phenylthio)imidazo[1,2-*a*]pyridine (15). A suspension of **14** (25 g, 0.0741 mmol) was heated in 2.5 N NaOH (190 mL) on the steam bath for 20 min. After cooling, the mixture was extracted with CH_2Cl_2–ether (1:5). The combined extracts were washed with H_2O, dried, and evaporated to dryness in vacuo. The residue, 16.4 g (91.2%), was recrystallized from CH_3CN to yield **15**, mp 102–104 °C. Anal. ($C_{13}H_{11}N_3S$) C, H, N.

Methyl *N*-(Methoxycarbonyl)-*N*-[5-(phenylthio)imidazo[1,2-*a*]pyridin-3-yl]carbamate (16) and Methyl 5-(Phenylthio)imidazo[1,2-*a*]pyridine-3-carbamate (17). A solution of **15** (26.6 g, 0.11 mmol) in C_5H_5N (500 mL) at 0 °C was treated dropwise with methyl chloroformate (28 mL). After allowing the mixture to warm to room temperature, it was heated at 80 °C for 3 h. The cooled reaction mixture was diluted with H_2O (4000 mL) and extracted with ether. The extracts were washed with H_2O, dried, and evaporated in vacuo to a red gum. TLC (EtOAc–CH_2Cl_2, 1:1, on SiO_2) indicated a gross mixture of components. Chromatography on silica gel and elution with EtOAc–CH_2Cl_2 (1:1) yielded 1.7 g (4.3%) of **16**, mp 152–154 °C (EtOH). Anal. ($C_{17}H_{15}N_3O_4S$) C, H, N, S.

Further elution with EtOAc–CH_2Cl_2 (3:1) yielded 1.8 g (5.4%) of **17**, mp 139–140 °C (EtOH). Anal. ($C_{15}H_{13}N_3O_2S$) C, H, N, S.

Preparation of 17 by Partial Hydrolysis of 16. A solution of **16** (120 mg, 0.33 mmol) in MeOH (5 mL) was stirred with $NaOCH_3$ (10 mg, 18 mmol) for 5 h at room temperature. After the solvent was evaporated in vacuo, the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was separated, dried, and evaporated in vacuo to yield 97 mg (96%) of **17**: mp, NMR, IR, and TLC were identical with that obtained above.

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Chemical Modification of Aminoglycosides. 3. Synthesis of 2″-Deoxykanamycins from Neamine[1]

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The preparation of 2″-deoxykanamycin B (**12**) and 2″,3′,4′-trideoxykanamycin B (**14**) from neamine (**1**) is described. Key intermediates in the synthesis of these 2″-deoxyaminoglycoside antibiotics are 3′,4′-bis-*O*-(*p*-nitrobenzoyl)-1,2′,3,6′-tetrakis-*N*-(trifluoroacetyl)neamine (**6**) and 3′,4′-dideoxy-1,2′,3,6′-tetrakis-*N*-(trifluoroacetyl)neamine (**9**). The amino groups of these intermediates are blocked by the trifluoroacetyl group, a blocking group not widely used in aminoglycoside chemistry.

The value of aminoglycoside antibiotics for the treatment of infections caused by Gram-negative bacteria is widely recognized. The quest for novel aminoglycosides which possess good potency to pseudomonads and are not readily inactivated by R-factor-carrying bacteria, but which still possess a favorable toxicity profile, has attracted many investigators. Both chemical and biochemical synthesis of useful aminoglycosides have been rewarding.[2-5]

(1) Presented in part at the 176th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 11–14, 1978. For the previous paper in this series, see Magerlein, B. J. In "Aminocyclitol Antibiotics"; Rinehart, K. L., Jr.; Suami, T., Ed.; American Chemical Society: Washington, DC, 1980; pp 169–182.

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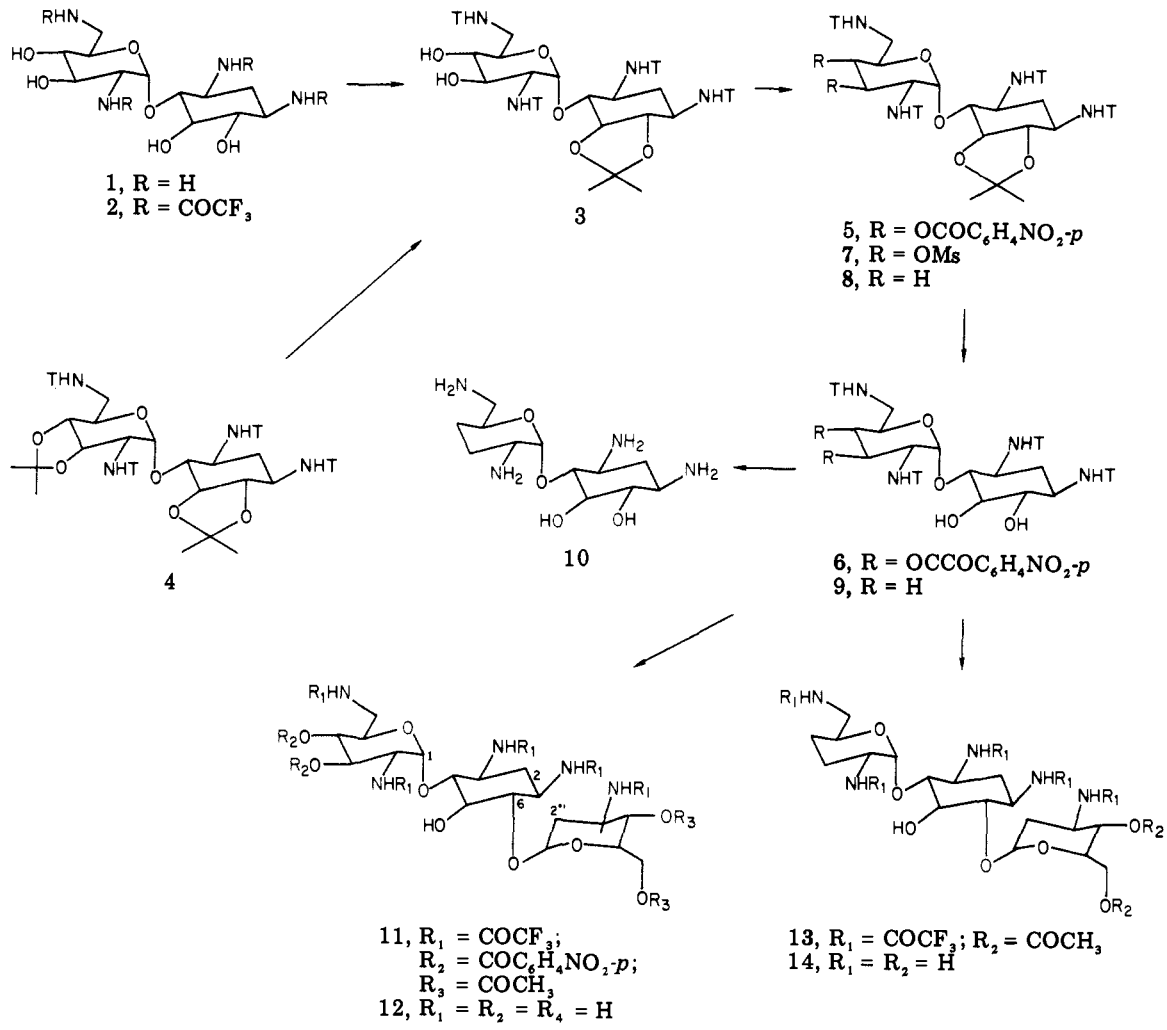
Scheme I[a]



[a] T = $COCF_3$.

One portion of our program to chemically produce semisynthetic aminoglycosides from neamine was directed toward the synthesis of analogues of kanamycin B. This antibiotic is inactivated by a variety of enzymes produced by R-factor-carrying bacteria. These enzymes phosphorylate, adenylate, or acylate susceptible hydroxyl or amino groups. Included among the enzymes which inactivate kanamycin B are NPT I (neomycin–kanamycin phosphotransferase I) and NPT II (neomycin–kanamycin phosphotransferase II), which phosphorylate the 3′-hydroxyl group, and GNT (gentamicin nucleotidyltransferase), which adenylates the 2″-hydroxyl group.[6] Elimination of the functional groups which are subject to enzymatic attack in a number of cases has resulted in antibiotics with improved antibacterial spectra toward resistant bacteria.[7] Therefore, the syntheses of 2″-deoxykanamycin B (12) and 2″,3′,4′-trideoxykanamycin B (14) were of interest both for obtention of the novel aminoglycosides and as a model for the preparation of other analogues.

The starting point in our synthesis was neamine (1). In order to effectively manipulate the various hydroxyl groups of this aminoglycoside, the amino groups must be blocked. The benzyloxycarbonyl group was introduced to aminoglycoside chemistry by Umezawa to block the amino groups of neamine in his classical syntheses of the kanamycins.[8] Subsequently, this blocking group has gained wide acceptance for use with aminoglycosides. However, we elected to block neamine as the tetrakis(trifluoroacetyl) derivative, since in our hands the trifluoroacetyl group imparted several advantages. The trifluoroacetamide intermediates were quite soluble in organic solvents, such as ethyl acetate and acetone, which permitted easy physical manipulation and facilitated TLC and column chromatography. This blocking group was also compatible with GC–MS studies which were carried out on a number of intermediates. Finally, it was readily removed by dilute alkali.

The reaction of neamine (1) with trifluoroacetic anhydride produced tetrakis-*N*-(trifluoroacetyl)neamine (2) in high yield (Scheme I). When treated with 2,2-dimethoxypropane in the presence of trifluoroacetic acid as catalyst, the 5,6-monoketal 3 was obtained in 76% yield, together with a 13% yield of diketal 4. Diketal 4 was readily converted to monoketal 3 when contacted with methanol–trifluoroacetic acid.

While Umezawa et al. have shown that ketalization of tetrakis-*N*-(benzyloxycarbonyl)neamine occurs preferentially at the 5,6-hydroxyl groups,[7] we felt that confirmation

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(6) In ref 2, pp 257 and 310.
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Table I. ^{13}C NMR Data of Substituted Neamines

carbon	chemical shift, δ			
	1[a,c]	2[b]	3[b]	4[b]
1	51.7	51.4	48.8	48.8
2	36.9	32.9	34.5	34.5
3	50.7	50.7	51.0	50.7
4	88.3	82.5	77.0	77.2
5	77.3	77.0	81.8	81.8
6	78.7	74.5	78.3	78.4
1′	102.0	99.7	96.8	96.4
2′	56.6	55.9	55.1	54.1
3′	74.9	72.9	73.1	76.5
4′	72.7	71.6	70.8	75.1
5′	74.0	72.1	71.2	70.6
6′	43.0	41.8	41.5	41.6
CH_3			26.8, 27.0	26.3, 26.8, 26.9 (2)
quat C			112.8	112.7, 112.8
C=O			155–161 (m)	155–160 (m)
CF_3		95.7, 110.0	95.4, 109.8	95.3, 109.8
		124.3, 138.6	124.2, 138.6	124.4, 138.6

[a] In D_2O referenced to external Me_4Si. [b] In Me_2CO-d_6 referenced to internal Me_4Si. [c] Chemical-shift data were recorded in our laboratory; assignments correspond to those of Hanessian, S.; Massé, R.; Ekborg, G. *Can. J. Chem Chem.* 1978, *56*, 1492–1499.

of the structure of ketal **3** was desirable. This was accomplished by examination of the appropriate ^{13}C NMR data in Table I.

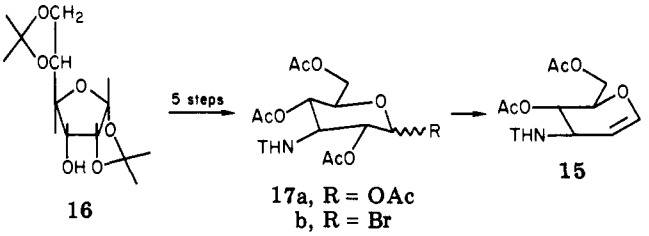
Conversion of neamine (1) to the tetrakis(trifluoroacetyl) derivative **2** resulted in significant upfield shifts of the carbons adjacent to the trifluoroacetamido groups, that is, at C-2, -6, -4, -1′, and -3′. Other carbons remained essentially unchanged. The eight carbons of the four trifluoroacetyl groups appear downfield as shown in Table I and are all coupled to fluorine. When tetrakis(trifluoroacetyl) derivative **2** was converted to monoketal **3**, C-5 and C-6 were shifted downfield while adjacent C-4 and C-1 were shifted upfield, indicating that the ketal was located at C-5,6. Assignments for the methyl groups and the quaternary carbon of the isopropylidene ketal are readily recognized and are also given in Table I. The diketal **4** shows an additional isopropylidene group and also C-3′ and C-4′ are shifted downfield, while adjacent carbons C-2′ and C-5′ have moved upfield.

The 3′ and 4′ hydroxyls of ketal **3** were then esterified with *p*-nitrobenzoyl chloride to afford fully blocked intermediate **5**. Mild acid hydrolysis gave the 5,6-diol **6**, the key intermediate for the synthesis of 2″-deoxykanamycin B (**12**). The overall yield of diol **6** from neamine (1) was 46%.

The comparable intermediate **9** for use in the preparation of 2″,3′,4′-trideoxykanamycin B (**14**) was prepared by deoxygenation of diol **3**, followed by reduction[7] and removal of the 5,6-ketal. A portion of diol **9** was treated with alkali to yield 3′,4′-dideoxyneamine, whose physical constants compared satisfactorily with those previously reported.[9]

Glycosylation of the 6-hydroxyl groups of diols **6** and **9** employed the acid-catalyzed addition of glycal **15**. This glycal glycosylation procedure was shown by Daniels and co-workers to selectively produce a 2″-deoxy-6-α-glycoside when applied to a gentamine intermediate containing both C-5 and C-6-hydroxyl groups.[10]

Scheme II



In order that the blocking groups on nitrogen be consistent throughout the newly formed trisaccharide, the preparation of glycal **15** was undertaken (Scheme II). 1,2,5,6-Di-*O*-isopropylidene-α-D-allofuranose (**16**) was converted to the bromide **17**,[11] which when treated with zinc in acetic acid led to the isolation of crystalline glycal **15** in good yield.

In the synthesis of 2″-deoxykanamycin B (**12**), diol **6** was treated with glycal **15** in the presence of BF_3·Et_2O to form pseudotrisaccharide **11**. The ^{13}C NMR spectrum of this fully blocked aminoglycoside indicated a 6-α-glycoside similar to that found in kanamycin B. Blocking groups on oxygen and nitrogen were readily removed by mild alkaline hydrolysis. Purification over CG-50 resin led to the isolation of 2″-deoxykanamycin B (**12**). The overall yield for the two-step process was 22.0%.

In a similar manner, glycosylation of diol **9** with glycal **15**, followed by alkaline hydrolysis, afforded 2″,3′,4′-trideoxykanamycin B (**14**) in 22.6% yield.

Aminoglycosides **12** and **14** were characterized by ^{13}C NMR and FDMS (field-desorption mass spectrometry) data. Both compounds gave the expected protonated molecular ion ($M^+ + 1$) when subjected to FDMS determination. The ^{13}C NMR chemical shifts for deoxykanamycins **12** and **14**, kanamycin B,[12] and 3′,4′-dideoxykanamycin B[13] are recorded in Table II. The 2″-deoxykanamycins exhibit a strong upfield shift of the 2″ carbon; otherwise, the spectra of the respective pairs are in good agreement.

Antimicrobial Activities. In vitro antibacterial testing data for 2″-deoxykanamycin B (**12**) and 2″,3′,4′-trideoxykanamycin B (**14**) vs. a variety of Gram-positive and Gram-negative bacteria are given in Table III. The minimum inhibitory concentration (MIC) vs. various bacteria was determined by a microplate broth dilution technique. Serial twofold dilutions of the antibiotic were prepared in 50 μL of brain–heart infusion broth medium in the wells of a microplate. Each well was then inoculated with 50 μL of a standardized cell suspension to yield a final concentration of $\sim 10^5$ viable cells per milliliter of drug-supplemented medium. The microplates were incubated at 37 °C for 20 h, and the MIC was read as the lowest concentration of drug that inhibited visible growth of the organism.

The 2″-deoxykanamycins possess interesting antibacterial activity, though they are generally less potent than kanamycin B. 2″,3′,4′-Trideoxykanamycin B (**14**) is

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Table II. ^{13}C NMR Shifts for 2''-Deoxykanamycins

carbon	chemical shift,[a] δ: 2''-deoxykanamycin B (12)	2'',3',4'-trideoxykanamycin B (14)	kanamycin B[b]	3',4'-dideoxykanamycin B[c]	carbon	chemical shift,[a] δ: 2''-deoxykanamycin B (12)	2'',3',4'-dideoxykanamycin B (14)	kanamycin B[b]	3',4'-dideoxykanamycin B[c]
1	50.6	50.9	50.5	51.1	4'	73.9[h]	28.8[f]	73.3[d]	28.1[g]
2	36.8	37.0	36.5	36.3	5'	74.4	74.5	74.7	70.2
3	49.9	49.8	50.3	50.3	6'	42.7	46.3	42.8	45.4
4	87.4	88.2	87.4	86.9	1''	102.1	102.4	101.4	100.7
5	76.1	76.2	75.2[e]	75.4	2''	37.9	38.2	73.0[d]	72.6
6	88.3	88.7	88.6	89.0	3''	51.3	51.3	56.5	55.1
1'	101.4	100.0	100.5	101.1	4''	72.6[e]	71.6	71.3	70.2
2'	56.6	51.3	56.3	50.6	5''	73.8	73.0	74.0	73.1
3'	74.7	27.2[f]	74.9[e]	26.2[g]	6''	62.0	62.0	62.0	61.3

[a] In D_2O referenced to external Me_4Si. [b] Reference 12. [c] Reference 13. [d-h] Assignments with very similar chemical shifts may be reversed.

Table III. Antibacterial Activity of Kanamycin Analogues

organism	min inhibitory concn, μg/mL: 2''-deoxykanamycin B (12)	2'',3',4'-trideoxykanamycin B (14)	kanamycin B sulfate
Staphylococcus aureus UC 76	3.9	<1.0	7.8
Streptococcus pyogenes UC 152	15.6	7.8	15.6
Streptococcus faecalis UC 694	1000	250	250
Streptococcus pneumoniae UC 41	500	250	125
Escherichia coli UC 45	31.2	62.5	3.9
Klebsiella pneumoniae UC 58	3.9	3.9	<1.0
Salmonella schottmuelleri UC 126	15.6	15.6	2.0
Pseudomonas aeruginosa UC 95	>1000	15.6	62.5
Proteus vulgaris UC 93	15.6	31.2	15.6
Proteus mirabilis UC 6671	31.2	62.5	62.5
Serratia marcescens UC 131	125	15.6	7.8
Salmonella flexneri UC 143	62.5	62.5	15.6
Salmonella typhi UC 215	31.2	15.6	

marginally more potent than analogue 12, which possesses 3'- and 4'-hydroxyls. As expected 2'',3',4'-trideoxykanamycin B shows greater potency than 2''-deoxykanamycin B and kanamycin B vs. *Pseudomonas aeruginosa*, since the trideoxy analogue lacks the 3'- and 4'-hydroxyl groups. Table IV indicates the activity of 2'',3',4'-trideoxykanamycin B (14) and kanamycin B vs. a group of antibiotic-resistant clinical isolates of *Pseudomonas aeruginosa*. 2'',3',4'-Trideoxykanamycin B was somewhat more potent than kanamycin B vs. five strains of *Pseudomonas aeruginosa* and equal to kanamycin vs. two strains.

Enzymatic inactivation assays also indicated that 2''-deoxykanamycin B and 2'',3',4'-trideoxykanamycin B were not substrates for 2''-*O*-adenylation, while the latter also was not a substrate for 3'-*O*-phosphorylation.[14].

When administered subcutaneously in the mouse protection assay[15] vs. *Klebsiella pneumoniae* UC58, 2'',3',4'-trideoxykanamycin B sulfate gave a CD_{50} (dose to protect 50% of the animals) of 40 (32–50) mg/kg. Kanamycin B sulfate gave a CD_{50} of 2.3 mg/kg in the same assay.

Table IV. Antibacterial Activity of 2'',3',4'-Trideoxykanamycin B vs. Resistant Strains of *Pseudomonas aeruginosa*

organism	min inhib concn, μg/mL: 2'',3',4'-trideoxykanamycin B sulfate	kanamycin B sulfate
Ps. aeruginosa		
UC 3679	7.8	15.6
UC 3683	7.8	15.6
UC 3680	7.8	31.2
UC 95	7.8	31.2
UC 3682	15.6	62.5
UC 3681	15.6	15.6
UC 6436	>125	>125

Experimental Section

Melting points were taken in a Thomas-Hoover Unimelt apparatus and are uncorrected. TLC was carried out on glass plates coated with silica gel G (Analtech). Silica gel, 0.05–0.2 mm (EM Reagents), was employed for column chromatography. ^{13}C NMR spectra were recorded on a Varian CFT-20 or Varian FT-80A spectrophotometer. Samples were dissolved in acetone-d_6 containing 10% Me_4Si or in D_2O using Me_4Si as an external standard. ^1H NMR spectra were recorded on a Varian A-60 spectrophotometer. Chemical shifts are reported in parts per million (δ) downfield from Me_4Si. Assignments for given carbon atoms with very similar chemical shifts when written in a group may on occasion be interchanged. Optical rotations were measured at *c* 1.0. Elemental analyses were within 0.4% of theoretical values. Organical solutions were dried over Na_2SO_4 and evaporated in vacuo at <50 °C.

1,2',3,6'-Tetrakis-*N*-(trifluoroacetyl)neamine (2). Trifluoroacetic anhydride (33.5 mL, 160 mmol) was added at 15 ± 5 °C over a period of 30 min to a suspension of 9.66 g (30 mmol) of neamine (1) in 100 mL of MeCN and 22.4 mL (160 mmol) of Et_3N. After the solution was stirred at ambient temperature for 1 h, the solvent was evaporated in vacuo. The residue was diluted with EtOAc. The resulting solution was washed several times with 5% $KHCO_3$–saturated NaCl (1:1), dried, and concentrated. The residue crystallized when triturated with ether. Recrystallization from EtOH afforded 15.95 g (75.3%) of trifluoroacetate

(14) The authors are indebted to Professor Julian Davies, University of Wisconsin, for these assays.

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2, mp 286–288 °C dec. An additional 1.2 g (5.2%) of 2, mp 278–280 °C, was obtained by concentration of the mother liquors. A portion twice recrystallized from EtOH melted at 304–306 °C: $[\alpha]^{25}_D$ +63° (EtOH); IR 3600–3300 (NH/OH), 1700 (C=O), 1560 (amide II), 1220, 1180, 1160 (CF/CO) cm^{-1}; ^{13}C NMR, see Table I; MS (Me_3Si derivative), m/z 979 (M − 15), 497, 481. Anal. ($C_{20}H_{22}F_{12}N_4O_{10}$) C, H, N, F.

5,6-Isopropylidene-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (3) and 3',4',5,6-Diisopropylidene-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (4). A solution of 7.06 g (10 mmol) of trifluoroacetate 2 in 30 mL of MeCN and 60 mL of dimethoxypropane containing 0.25 mL of CF_3COOH was heated at reflux for 0.75 h. Amberlite IR-45 (OH^-) (12 g) was added with stirring to the cooled solution. When a neutral reaction to moist indicator paper was obtained (10 min), the solution was filtered and concentrated under vacuum. Chromatography over 500 g of silica gel using $CHCl_3$–CH_3OH (10:1) for elution led to the isolation of 5.68 g (76.3%) of monoketal 3. Rechromatography gave a sample having the following properties: $[\alpha]^{25}_D$ +75° (EtOH); IR 3430, 3300, 3160 (NH/OH), 1705 (C=O), 1560 (amide II), 1215, 1185, 1160 (CF_3/CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.35 (d, anomeric, 3.2–4.7 (cluster), 1.36 [s >$C(CH_3)_2$]; ^{13}C NMR, see Table I; MS (Me_3Si derivative), m/z 890 (M^+), 875 (M − 15) 497, 407. Anal. ($C_{23}H_{26}F_{12}N_4O_{10}$) C, H, N. In addition to monoketal 3, a less polar fraction of 1.02 g (12.95%) was also collected. This material proved to be diketal 4: $[\alpha]^{25}_D$ +70°; ^1H NMR ($CDCl_3$) δ 5.48 (d, anomeric, 3.2–4.5 (cluster), 1.4 [s, 2 >$C(CH_3)_2$]; ^{13}C NMR, see Table I. Anal. ($C_{26}H_{30}F_{12}N_4O_{10}$) C, H, N.

Hydrolysis of Diketal 4 to Monoketal 3. A solution of 5.41 g (6.88 mmol) of diketal 4 and 0.2 mL of trifluoroacetic acid in 10 mL of CH_3OH was heated at reflux for 15 min. IR-45 (OH^-) resin (10 g) was added, and the mixture was stirred for 5 min. The solution was filtered and concentrated. The residue, after chromatography over silica gel [$CHCl_3$–CH_3OH (10:1)], afforded 3.34 g (65.1%) of monoketal 3, identified by TLC and ^{13}C NMR.

5,6-*O*-Isopropylidene-3',4'-bis-*O*-(*p*-nitrobenzoyl)-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (5). *p*-Nitrobenzoyl chloride (34.8 g, 0.19 mol) was added to a solution of 36.0 g (0.048 mol) of monoketal 3 in 420 mL of pyridine while cooling so that the temperature remained below 35 °C. After 2.5 h at ambient temperature, the pyridine was distilled under vacuum. The residue was dissolved in EtOAc and worked up in the usual manner. Chromatography over 5 kg of silica gel using $CHCl_3$–CH_3OH (40:1) for elution afforded 47.5 g (94.2%) of diester 5: $[\alpha]^{25}_D$ −32° (Me_2CO); ^{13}C NMR ($AcCH_3$-d_6) δ 26.95 (2-CH_3), 34.6 (C-2), 40.4 (C-6'), 48.9 (C-1), 51.1 (C-3), 53.0 (C-2'), 68.9 (C-4'), 71.6 (C-5'), 73.1 (C-3'), 77.7 (C-6), 78.5 (C-5), 81.7 (C-4), 96.8 (C-1'), 95.6, 109.8, 124.0, and 138.2 (CF_3), 113.2 (2 quaternary C), 124.4, 131.7, 135.4, 150.1, and 151.7 (aromatic), 164.7 and 165.1 (C=O). Anal. ($C_{37}H_{32}F_{12}N_6O_{16}$) C, H, N.

3',4'-Bis-*O*-(*p*-nitrobenzoyl)-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (6). Ketal 5 (45.5 g, 43.6 mmol) was dissolved in 450 mL of 66% HOAc. The solution was warmed at 65 °C for 4 h and then lyophilized. Chromatography over 3.5 kg of silica gel using $CHCl_3$–CH_3OH (10:1) for elution gave 35.4 g (80.7%) of diol 6: $[\alpha]^{25}_D$ −43° (Me_2CO); ^{13}C NMR ($AcCH_3$-d_6) δ 32.9 (C-4), 40.6 (C-6'), 50.9 and 51.5 (C-3,1), 54.1 (C-2'), 69.9 (C-4'), 71.2 (C-5'), 73.8 (C-3'), 75.0 (C-6), 77.2 (C-5), 83.6 (C-4), 99.8 (C-1'), 95.6, 109.8, 124.0, and 138.3 (CF_3), 124.4, 131.7, 135.3, 135.5, and 151.8 (aromatic), 156-162 ($COCF_3$), 164.6 and 165.1 (C=O). Anal. ($C_{34}H_{28}F_{12}N_6O_{16}$) C, H, N.

5,6-Isopropylidene-3',4'-di-*O*-methanesulfonyl-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (7). Methanesulfonyl chloride (1.1 mL, 14.2 mmol) was added dropwise to 3.73 g (5 mmol) of diol 6 in 15 mL of pyridine cooled in an ice bath. The mixture was subjected to the usual workup after standing overnight at ambient temperature. A residue of 4.40 g (94.4%) of dimesylate 7 was obtained: TLC ($CHCl_3$–CH_3OH, 10:1) R_f 0.63; ^{13}C NMR (Me_2CO-d_6) δ 26.9 (CH_3 of ketal), 34.3 (C-2), 38.9 and 39.2 (CH_3), 40.5 (C-6'), 48.7 (C-1), 50.8 (C-3), 53.0 (C-2'), 68.7 (C-5'), 75.1, 77.1, 77.6, and 78.2 (C-4', -3', -6, and -5), 81.3 (C-4), 96.5 (C-1'), 95.3, 109.7, 124.1, and 138.5 (CF_3), 113.0 (quaternary), 155–161 ($COCF_3$).

3',4'-Dideoxy-5,6-*O*-isopropylidene-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (8). A mixture of 20 g (22.2 mmol) of dimesylate 7, 100 g of NaI, and 50 g of Zn dust in 200 mL of DMF was heated at 95 °C for 1.5 h. The mixture was cooled to 70 °C and diluted with 100 mL of H_2O and 50 mL of EtOAc. The EtOAc solution was washed several times with H_2O, dried, and concentrated. The 13.7 g (86.6%) of crude **3',4'-dideoxy-3'-eno-5,6-*O*-isopropylidene-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine** was dissolved in 200 mL of CH_3OH containing 1.5 g of PtO_2 and shaken under H_2 for 18 h. The catalyst was removed by filtration, and the solvent was distilled to give 12.4 g (90.3%) of ketal 8: TLC ($CHCl_3$–CH_3OH, 10:1) R_f 0.70; $[\alpha]^{26}_D$ +47° (CH_3OH); ^{13}C NMR (Me_2CO-d_6) δ 23.4 (C-3'), 26.7 and 27.0 (CH_3), 28.0 (C-4'), 34.5 (C-2), 44.2 (C-6'), 48.8 (C-1), 49.6 (C-3), 51.0 (C-2'), 67.0 (C-5'), 76.4 (C-5), 78.4 (C-6), 81.8 (C-4), 95.8 (C-1'), 95, 110, 124, and 139 (CF_3), 112.7 (quaternary), 155–160 ($COCF_3$). Anal. ($C_{23}H_{20}F_{12}N_4O_8$) C, H, N.

3',4'-Dideoxy-1,2',3,6'-tetrakis-*N*-(trifluoroacetyl)neamine (9). A solution of 4 g (5.6 mmol) of ketal 8 in 18 mL of trifluoroacetic acid and 2 mL of H_2O was kept at ambient temperature for 15 min. The solvents were evaporated under vacuum and the residue was immediately passed through a column of 250 g of silica gel. The column was eluted with $CHCl_3$–CH_3OH (10.1) to give a crystalline fraction of 1.56 g (41.3%) of diol 9: mp 261–267 °C; $[\alpha]^{25}_D$ 51° (CH_3OH); ^{13}C NMR (Me_2CO-d_6) δ 23.8 (C-3'), 28.1 (C-4'), 32.9 (C-2), 44.4 (C-6'), 50.9 (2) and 51.4 (C-3, -1, and -2'), 68.3 (C-5'), 74.8 (C-5), 77.2 (C-6), 82.6 (C-4), 99.3 (C-1'), 96.5, 109.9, 124.3, and 137.7 (CF_3), 155–162 ($COCF_3$); MS [$(Me_3Si)_2$ derivative], m/e 803 (M − 15), 321, 481. Anal. ($C_{20}H_{22}F_{12}N_4O_8$) C, H, N.

3',4'-Dideoxyneamine (10). A solution of 526 mg (0.63 mmol) trifluoroacetate 9 and 200 mg (5 mmol) of NaOH in 2.5 mL of CH_3OH and 2.5 mL of H_2O was heated under reflux for 15 min. The reaction mixture was cooled, and 20 mL of H_2O and 2.5 mL of 1 N HCl were added. This solution was passed over a column of 50 g of Amberlite resin CG-50 (NH_4^+). The column was eluted with 25 mL of H_2O, followed by a gradient of H_2O–0.5 N NH_4OH. A fraction having an R_f slightly greater than neamine was lyophilized to afford 175 mg (77.4%) of 3',4'-dideoxyneamine 10: $[\alpha]^{25}_D$ 96° (H_2O); ^{13}C NMR (D_2O) δ 27.3 (C-3'), 28.8 (C-4'), 37.0 (C-2), 46.2 (C-6'), 50.8, 51.1, and 51.6 (C-3, -2', and -1), 71.7 (C-5'), 77.3 (C-5), 78.9 (C-6), 88.4 (C-4), 102.6 (C-1').[9]

4,5-Di-*O*-acetyl-1,2,3-trideoxy-3-[(trifluoroacetyl)amino]-D-*arabino*-hex-1-enopyranose (15). While stirring in an ice–water bath, HBr was bubbled into a solution of 41.3 g (93 mmol) of tetraacetate **17a**[11] dissolved in 390 mL of CH_2Cl_2 and 390 mL of HOAc. After 45 min, the CH_2Cl_2 was evaporated in vacuo. The residue was diluted with 82 mL of water and added to a mixture of 82.6 g (1.26 mol) of Zn and 82.6 g (1.01 mol) of NaOAc in 340 mL of 50% aqueous HOAc. The addition was made at 10 ± 5 °C. The reaction mixture was stirred for 2.5 h and then filtered through a pad of filter aid. The filtrate was extracted several times with 500-mL portions of CH_2Cl_2. The extract was washed with water and $KHCO_3$, dried, and concentrated. The residue was chromatographed over 1.3 kg of silica gel using Skellysolve B–EtOAc (2:1) for elution. A fraction of 7.5 g (24.8% yield) of crystalline glycal 15 was obtained. A portion of this material was recrystallized from EtOAc–Skellysolve B to afford glycal 15: mp 114–116 °C, $[\alpha]^{25}_D$ +51° (Me_2CO); ^{13}C NMR ($CDCl_3$) δ 20.6 (2-CH_3), 49.3 (C-6), 61.7 (C-5), 67.6 (C-4), 74.7 (C-3), 99.6 (C-2), 145.7 (C-1), 170.7 (2-CO); MS calcd for $C_{12}H_{14}F_3NO_6$, 325.0773; found, 325.0766. Anal. ($C_{12}H_{14}F_3NO_6$) C, H, N.

***O*-2,6-Dideoxy-2,6-bis[(trifluoroacetyl)amino]-3,4-bis-*O*-(*p*-nitrobenzoyl)-α-D-glucopyranosyl-(1→4)-*O*-[4,6-*O*-diacetyl-2,3-dideoxy-3-[(trifluoroacetyl)amino]-α-D-glucopyranosyl-(1→6)]-2-deoxy-1,3-bis-*N*-(trifluoroacetyl)-D-streptamine (11).** To a solution of 4.0 g (12.3 mmol) of glycal 15 and 6.02 g (6.0 mmol) of diol 6 in 100 mL of EtOAc was added 10 mL of BF_3·Et_2O. After 5 h, TLC ($CHCl_3$–CH_3OH, 10:1) showed only a trace of diol 6 and several faster spots, only one of which was strongly UV positive. The reaction mixture was diluted with CH_2Cl_2 and washed with dilute $KHCO_3$. The residue was chromatographed over 600 g of silica gel ($CHCl_3$–CH_3OH, 25:1) to give 3.0 g (38.4%) of blocked aminoglycoside 11: ^{13}C NMR (Me_2CO-d_6) δ 20.5 and 20.7 (2-CH_3), 32.8, 35.7, and 40.5 (3-CH_2), 47.6, 50.8, 50.9, and 54.0 (4-CHN), 62.8, 69.6, 70.0, 71.1, 73.8, 75.5, 81.3, and 83.7 (9-CHO), 98.2 (C-1'), 99.9 (C-1''), 95.5, 109.8, 124.0 and 138.2 (CH_3), 124.4, 131.7, 135.5, and 151.8 (aromatic), 156–160 ($COCF_3$), 164.6, 165.1, 170.6, and 170.7 (C=O).

O-3-Amino-2,3-dideoxy-α-D-glucopyranosyl-(1→6)-O-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine (12). A solution of 1.6 g (1.23 mmol) of aminoglycoside 11 and 940 mg (23.5 mmol) NaOH in 16 mL of CH_3OH and 16 mL of H_2O was heated at reflux for 10 min. The mixture was diluted with 50 mL of ice–water and 12.1 mL of 1 N HCl was added. The aqueous solution was passed over 65 mL of Amberlite resin CG-50 (NH_4^+). The column was eluted with 100 mL of H_2O, followed by a gradient of 275 mL of H_2O and 275 mL of 0.5 N NH_4OH. Fractions were monitored by TLC using a system of $CHCl_3/CH_3OH/NH_4OH$ (3:4:2). A fraction containing 330 mg (57.5%) of aminoglycoside 12 was obtained: FDMS, m/z calcd 467; observed, 468 ($M^+ + 1$). ^{13}C NMR (D_2O) δ 29.6 (C-2), 34.9 (C-2″), 41.9 (C-6′), 49.8, 50.2, and 51.0 (C-3, -1, and -3″), 55.2 (C-2′), 61.9 (C-6″), 68.4, 69.8, 70.8, 72.3, 75.1, 75.8, and 79.6 (C-4″, -4′, -5″, -3′, -5′, -5, and -4), 84.1 (C-6), 97.7 (C-1′), 99.3 (C-1″).

O-4,6-Diacetyl-2,3-dideoxy-3-[(trifluoroacetyl)amino]-α-D-glucopyranosyl-(1→6)-O-[2,6-bis[(trifluoroacetyl)amino]-2,3,4,6-tetradeoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-1,3-bis-N-(trifluoroacetyl)-D-streptamine (13). In the manner described above, 1.01 g (1.5 mmol) of diol 9 and 500 mg (1.54 mmol) of glycal 15 were reacted and chromatographed to give 391 mg (26.1%) of aminoglycoside 13. ^{13}C NMR (Me_2CO-d_6) δ 20.6 (CH_3), 23.7 and 27.0 (C-3′ and -4′), 32.6, 35.8, 44.2 (3-CH_2), 47.7, 49.8, 50.2, 51.6 (4-CHN), 63.3, 68.0, 70.2, 70.4, 74.1, 77.3, and 86.2 (7-CHO), 95.8 (C-1′), 99.0 (C-1″), 95, 109.9, 124.4, and 139 (CF_3), 156–161 ($COCF_3$), 170.7 and 170.9 (C=O).

O-3-Amino-2,3-dideoxy-α-D-glucopyranosyl-(1→6)-O-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine (14). Hydroxylsis of 345 mg (0.35 mmol) of 13 with 196 mg (4.9 mmol) of NaOH in dilute MeOH, followed by passage through 20 mL of Amberlite resin CG-50 (NH_4^+), led to the isolation of 132 mg (86.5%) of aminoglycoside 14: FDMS, m/z calcd 435; observed, 436 ($M^+ + 1$). ^{13}C NMR (D_2O) δ 27.3 (C-4′), 28.8 (C-3′), 37.0 (C-2), 38.2 (C-2″), 46.3 (C-6′), 49.8, 50.9, and 51.3 (2) (C-3, -1, -3″, and -2′), 62.0 (C-6″), 71.6, 73.0, 74.5, and 76.2 (C-4″, -5″, -5′, and -5), 88.2 and 88.7 (C-4 and -6); 100.0 (C-1′), 102.4 (C-1″).

Synthesis and in Vitro Antimicrobial Property of *o*-Carborane Derivatives

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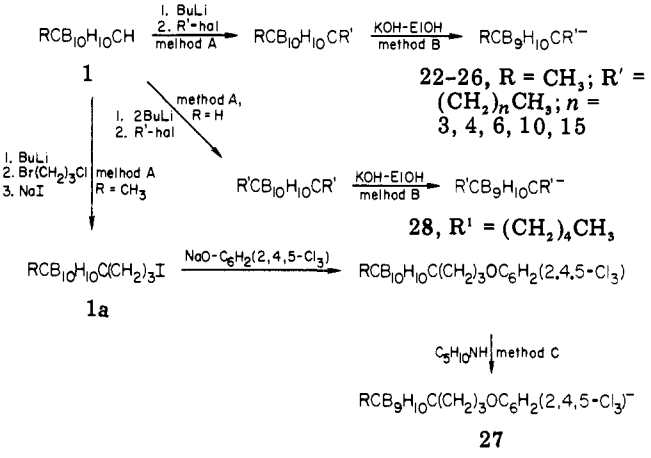
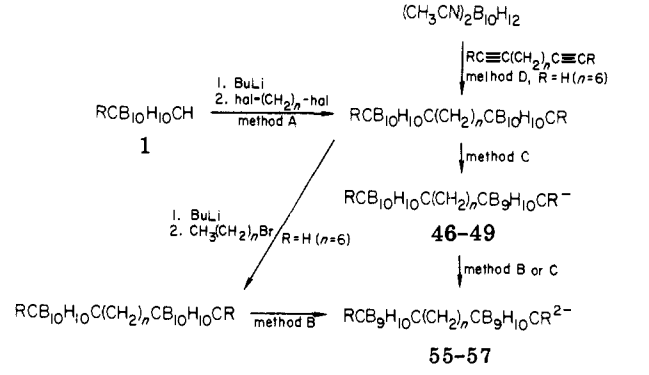
Various *o*-carboranes and nido-type dicarbollide anions have been synthesized and tested for antimicrobial activity. Nearly all of the dicarbollide monoanions investigated were active in vitro against fungi such as *Candida albicans*, *Aspergillus fumigatus*, and *Tricophyton asteroides*, as well as against Gram-positive bacteria. From a consideration of the structure–activity relationships, it seems most reasonable to conclude that the introduction of lipophilic alkyl or *o*-carboranyl groups to the hydrophilic dicarbollide anions leads to the antimicrobial activity.

Pharmacological action of the substances involving the *o*-carborane cage has been little known except for the papers on the application of these to boron-10 neutron-capture therapy.[1] However, there are some instances where the biological effects of the carboranes have been noted. These include the inhibition of certain liver microsomal enzymes by *o*- and *m*-carboranes.[2] Also, the neurotropic property of nitrogen-containing *o*-carborane derivatives[3] and the new findings of carboranylalanine as a chymotrypsin inhibitor[4] are regarded as a development of carboranes in the domain of pharmacology. Since bactericidal and fungicidal properties of 1-(aminoalkyl)-1,2-dicarba-*closo*-dodecaboranes and related compounds were reported in the Japanese patent literature from this company,[5] we describe here the synthesis and antimicrobial property of various types of *o*-carborane derivatives.

Scheme I



Scheme II



Chemistry. Mono- and dicage *o*-carborane derivatives were synthesized by known methods.[6,7] A reaction of

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