CHEMICAL MODIFICATION OF 5,3′,4′-TRIDEOXYKANAMYCIN B

Sir:

From the involvement of 3′- or 2″-phosphotransferase and 4′- or 2″-adenylyltransferase in the resistance mechanism and the successful clinical use of 3′,4′-dideoxykanamycin B[1], we were interested in the structure of the active derivative which had the least number of hydroxyl groups and, as reported in a previous paper[2], we synthesized 3′,4′,4″,6″-tetradeoxykanamycin B and its 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative which had a good antibacterial activity. It was shown by mutasynthesis of 5-deoxygentamicin C complex[3] and chemical syntheses of 5-deoxykanamycin A[4], 5-deoxykanamycin B[5,6], 5,3′,4′-trideoxykanamycin B[8,7] and 5,6″-dideoxykanamycin B[8] that 5-deoxygenation of 2-deoxystreptamine-aminoglycosides improved their activities.

In order to know the active structure with the least number of hydroxyl groups, we have synthesized 5,3′,4′,4″,6″-pentadeoxykanamycin B (**2**), 5,3′,4′,6″-tetradeoxykanamycin B (**3**), 6″-chloro-5,3′,4′,6″-tetradeoxykanamycin B (**4**), 5,3′,4′-tridoxy-6′-*N*-methylkanamycin B (**5**) and 6″-chloro-5,3′,4′,6″-tetradeoxy-6′-*N*-methylkanamycin B (**6**) starting from 5,3′,4′-trideoxykanamycin B[7] (**1**). Their 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivatives **7**～**12** prepared from compounds **1**～**6**, respectively, have a strong activity against Gram-positive and -negative bacteria including *Pseudomonas*.

Compound **2** was synthesized by the method used for preparing 3′,4′,4″,6″-tetradeoxykanamycin B[2]. The five free amino groups of **1** were protected with the *tert*-butoxycarbonyl (Boc) group by reaction with *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate in a mixture of methanol and triethylamine at room temperature for 22 hours to afford 1,3,2′,6′,3″-penta-*N*-Boc-5,3′,4′-trideoxykanamycin B (**13**) in a quantitative yield. Compound **13** was treated with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid at 60°C for 6 hours to yield the 4″,6″-*O*-isopropylidene **14** in 96% yield. Acetylation of **14** with acetic anhydride in pyridine at room temperature for 20 hours gave the 2″-*O*-acetyl derivative **15** in 91% yield. Removal of the *O*-isopropylidene group in **15** with a mixture of acetic acid, methanol and water (1:4:1) at 50°C for 5 hours afforded 2″-*O*-acetyl-1,3,2′,6′,3″-penta-*N*-Boc-5,3′,4′-trideoxykanamycin B (**16**) in a quantitative yield.

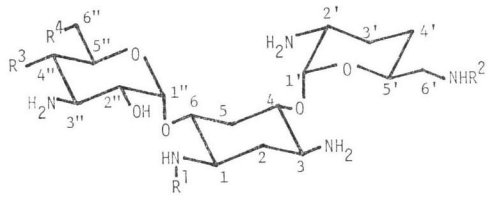
Mesylation of **16** with 3.9 equivalents of methanesulfonyl chloride in pyridine at room temperature for 4 hours followed by silica gel (Wakogel C-200) column chromatography (chloroform - methanol, 100:1) afforded the 4″,6″-di-*O*-mesyl derivative **17** in 81% yield. Iodination of **17** with an excess of sodium iodide in *N*,*N*-dimethylformamide at 94°C for 1 hour followed by catalytic hydrogenation with RANEY-Ni in dioxane for 6 hours in a Parr apparatus (3.6 kg/cm^2) gave 2″-*O*-acetyl-1,3,2′,6′,3″-penta-*N*-Boc-5,3′,4′,4″,6″-pentadeoxykanamycin B (**18**, 94% yield). Deacetylation of **18** with 13% ammonia in methanol, removal of the Boc groups with 90% trifluoroacetic acid, followed by adsorption on a column of Amberlite CG-50 (NH_4^+) resin and elution with 0.5 M ammonia afforded 5,3′,4′,4″,6″-pentadeoxykanamycin B (**2**) in 42% yield.

Tosylation of **16** with 1.5 equivalents of *p*-toluenesulfonyl chloride in pyridine at room temperature for 15 hours yielded the 6″-*O*-tosylate **19** in 91% yield. Iodination of **19** with an excess of sodium iodide in *N*,*N*-dimethylformamide at 95°C for 2 hours, catalytic hydrogenation with RANEY-Ni in dioxane, removals of the *O*-acetyl and *N*-Boc groups, followed by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with 0.3 M ammonia gave 5,3′,4′,6″-tetradeoxykanamycin B (**3**, 35% yield from **19**).

Chlorination of **19** with an excess of lithium chloride in *N*,*N*-dimethylformamide at 90°C for 6 hours, removal of the *O*-acetyl and *N*-Boc groups, followed by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with 0.5 M ammonia afforded 6″-chloro-5,3′,4′,6″-tetradeoxykanamycin B (**4**, 57% yield from **19**).

The 6′-amino group of **1** was methylated by the preferential 6′-*N*-benzyloxycarbonylation[9] followed by reduction with lithium aluminum hydride in tetrahydrofuran[10] to give 5,3′,4′-trideoxy-6′-*N*-methylkanamycin B (**5**) in 14% yield. In two steps, unreacted compounds were recovered in 36% and 40% yields. Compound **5** was converted into 6″-chloro-5,3′,4′,6″-tetradeoxy-6′-*N*-methylkanamycin B (**6**) in 20% yield by the 6″-chlorination method described above.

The 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivatives **7**, **9**, **10**, **11** and **12** were prepared



1: R^1, R^2 = H, R^3, R^4 = OH

2: R^1, R^2, R^3, R^4 = H

3: R^1, R^2, R^4 = H, R^3 = OH

4: R^1, R^2 = H, R^3 = OH, R^4 = Cl

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

6: R^1 = H, R^2 = CH_3, R^3 = OH, R^4 = Cl

7: R^1 = AHB, R^2 = H, R^3, R^4 = OH

8: R^1 = AHB, R^2, R^3, R^4 = H

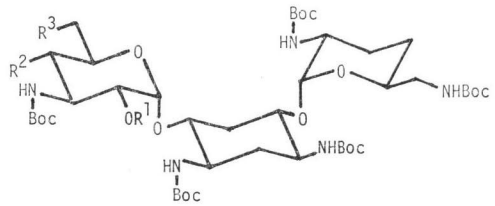
9: R^1 = AHB, R^2, R^4 = H, R^3 = OH

10: R^1 = AHB, R^2 = H, R^3 = OH, R^4 = Cl

11: R^1 = AHB, R^2 = CH_3, R^3, R^4 = OH

12: R^1 = AHB, R^2 = CH_3, R^3 = OH, R^4 = Cl

AHB: $NH_2CH_2CH_2\overset{(S)}{C}H(OH)CO$



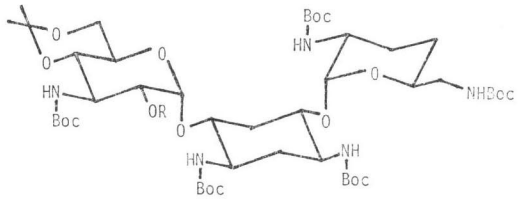
13: R^1 = H, R^2, R^3 = OH

16: R^1 = $COCH_3$, R^2, R^3 = OH

17: R^1 = $COCH_3$, R^2, R^3 = CH_3SO_3

18: R^1 = $COCH_3$, R^2, R^3 = H

19: R^1 = $COCH_3$, R^2 = OH, R^3 = H_3C-C$_6$H$_4$-SO_3



14: R = H

15: R = $COCH_3$

starting from compounds **1**, **3**, **4**, **5** and **6** through 3,2′,6′-tri-*N*-Boc-3″-*N*-trifluoroacetyl derivatives by the method of zinc chelation and *N*-trifluoroacetylation[11] in 36%, 58%, 30%, 53% and 35% yields, respectively. The amino groups of **2** lacking the 4″-hydroxyl group were protected by *tert*-butoxycarbonylation after zinc chelation to yield the tetra-*N*-Boc derivative which was converted into compound **8** by the 1-*N*-acylation with *N*-hydroxysuccinimide ester of (*S*)-4-*p*-methoxybenzyloxycarbonylamino-2-hydroxybutyric acid followed by deprotection in 27% yield.

The properties of new eleven compounds (**2**～**12**) are shown in Table 1. The minimum inhibitory concentrations of compounds **2**～**12** on a nutrient agar plate are shown in Table 2 in comparison with those of compound **1**. Acute intravenous LD_{50} values of **8** and **11** in mice were 50～100 mg/kg and those of **9**, **10** and **12** were 25～50 mg/kg. All 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-5,3′,4′-trideoxy derivatives (**7**～**12**) showed strong antibacterial activities against Gram-positive and -negative bacteria including *Pseudomonas*. The 6″-chlorination improved the activity against Gram-positive bacteria, but increased the toxicity. The 6′-*N*-methylation gave derivatives active against resistant strains having 6′-acetyltransferases. Among these derivatives, compound **11** showed the best antibacterial activity. Moreover, it is interesting that compound **2** having only one hydroxyl group at the C-2″ is still active. It suggests that in case of deoxy derivatives of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] kanamycin B, the amino groups have the main role in the antibacterial activity. The hydroxyl group of the (*S*)-4-amino-2-hydroxybutyryl moiety or the 2″-hydroxyl group may augment the activity.

Table 1. Properties of derivatives of 5,3′,4′-trideoxykanamycin B.

Compound	Mp (decomp.)	$[\alpha]_D$ in H_2O	Molecular formula[a]	MS (m/z)[b]				Rf on TLC[c]	
				$(M+1)^+$	2,6AG	3AG	DSA-3AG	Solvent A	Solvent B
2	125~130°C	+126° at 22°C	$C_{18}H_{37}N_5O_5 \cdot 2H_2CO_3$	404	129	130	304, 276, 258	0.51	0.61
3	128~136°C	+102° at 23°C	$C_{18}H_{37}N_5O_6 \cdot 2H_2CO_3 \cdot H_2O$	420	129	146	320, 292, 274	0.42	0.56
4	162~166°C	+91° at 21°C	$C_{18}H_{36}N_5O_6Cl \cdot \frac{1}{2}H_2CO_3$		129			0.42	0.53
5	137~140°C	+66° at 22°C	$C_{19}H_{39}N_5O_7 \cdot H_2CO_3 \cdot H_2O$	449(M^+)	143	162	336, 308, 290	0.22	0.49
6	163~167°C	+96° at 21°C	$C_{19}H_{38}N_5O_6Cl \cdot \frac{1}{2}H_2CO_3 \cdot H_2O$		143			0.42	0.53
7	163~166°C	+87° at 26°C	$C_{22}H_{44}N_6O_9 \cdot H_2CO_3 \cdot H_2O$		129	162	336, 308, 290	0.05	0.09
8	134~137°C	+91° at 24°C	$C_{22}H_{44}N_6O_7 \cdot 2H_2CO_3$		129	130	304, 276, 258	0.15	0.24
9	131~135°C	+90° at 27°C	$C_{22}H_{44}N_6O_8 \cdot 2H_2CO_3$		129	146	320, 292, 274	0.07	0.23
10	160~167°C	+72° at 21°C	$C_{22}H_{43}N_6O_8Cl \cdot 2H_2CO_3$		129			0.15	0.28
11	162~165°C	+88° at 22°C	$C_{23}H_{46}N_6O_9 \cdot H_2CO_3$		143	162	336, 308, 290	0.05	0.08
12	161~167°C	+71° at 21°C	$C_{23}H_{45}N_6O_8Cl \cdot H_2CO_3 \cdot H_2O$		143			0.15	0.28

[a] Satisfactory elemental analyses were obtained for all compounds.

[b] All compounds showed the fragmentations of the 2-deoxystreptamine (DSA) moiety (m/z 175, 147, 129). 2,6AG: the 2,6-diamino sugar moieties, 3AG: the 3-amino sugar moieties.

[c] TLC on silica gel G (Merck, Art 5721) using solvent A; butanol - ethanol - chloroform - 17% ammonia (4: 5: 2: 5 in volume) and solvent B; chloroform - methanol - 28% ammonia - water (1: 4: 2: 1 in volume).

Table 2. Minimum inhibitory concentrations (μg/ml) of derivatives of 5,3′,4′-trideoxykanamycin B.

Test organism	1	2	3	4	5	6	7	8	9	10	11	12
Staph. aureus FDA 209P	3.13	3.13	3.13	0.78	1.56	0.78	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Staph. aureus Smith	<0.20	<0.20	<0.20	0.20	0.39	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Staph. aureus Ap01[a]	6.25	12.5	6.25	0.78	25	0.78	0.78	<0.20	0.78	<0.20	0.39	0.39
Staph. epidermidis 109[a]	6.25	25	6.25	0.20	6.25	0.39	1.56	<0.20	0.78	<0.20	<0.20	<0.20
Micrococcus flavus FDA16	100	50	50	25	100	25	0.78	6.25	6.25	0.78	1.56	6.25
Micrococcus luteus PCI1001	100	50	50	25	25	12.5	6.25	3.13	0.78	0.39	0.39	3.13
B. anthracis	0.39	0.39	0.39	<0.20	0.78	0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
B. subtilis PCI219	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
B. subtilis NRRL B-558	<0.20	<0.20	<0.20	<0.20	0.78	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
B. cereus ATCC 10702	6.25	6.25	3.13	3.13	12.5	1.56	0.78	0.39	0.39	0.78	0.39	0.78
Corynebact. bovis 1810	100	100	100	25	100	25	6.25	6.25	6.25	0.78	1.56	1.56
Myco. smegmatis ATCC 607	0.78	0.78	0.78	0.78	6.25	0.78	<0.20	<0.20	<0.20	<0.20	<0.20	0.20
E. coli NIHJ	12.5	6.25	6.25	3.13	12.5	3.13	3.13	0.78	0.78	0.78	0.78	0.78
E. coli K-12	12.5	>100	25	3.13	12.5	3.13	1.56	1.56	0.78	1.56	0.78	0.78
E. coli K-12 R5[b]	>100	>100	>100	>100	50	25	100	100	50	50	3.13	6.25
E. coli K-12 R388	6.25	6.25	3.13	3.13	12.5	3.13	0.78	0.78	0.39	0.39	0.39	0.78
E. coli K-12 J5R11-2[c]	12.5	12.5	6.25	3.13	25	6.25	1.56	1.56	0.78	0.78	1.56	1.56
E. coli K-12 ML 1629[c]	12.5	6.25	6.25	3.13	25	3.13	1.56	1.56	0.78	3.13	0.78	3.13
E. coli K-12 ML 1630	25	25	12.5	6.25	25	6.25	6.25	1.56	1.56	3.13	1.56	3.13
E. coli K-12 ML 1410	12.5	6.25	12.5	6.25	25	6.25	3.13	1.56	1.56	1.56	1.56	3.13
E. coli K-12 ML 1410 R81[c]	12.5	6.25	6.25	3.13	25	6.25	1.56	1.56	0.78	1.56	1.56	1.56
E. coli K-12 LA290 R55[d]	>100	>100	>100	>100	100	50	3.13	1.56	1.56	3.13	1.56	1.56
E. coli K-12 LA290 R56	50	100	100	50	25	25	1.56	12.5	0.39	25	0.78	3.13
E. coli K-12 LA290 R64	50	100	100	50	25	12.5	1.56	12.5	0.78	1.56	1.56	3.13
E. coli W677	12.5	6.25	6.25	3.13	12.5	3.13	3.13	0.78	0.78	0.78	1.56	0.78
E. coli JR66/W677[d,e]	>100	>100	>100	>100	100	100	3.13	1.56	1.56	3.13	1.56	3.13
E. coli K-12 C600 R135[f]	12.5	12.5	6.25	6.25	12.5	3.13	1.56	1.56	0.78	1.56	1.56	3.13
E. coli JR225[f]	50	12.5	25	50	100	50	1.56	0.78	0.78	0.78	0.78	1.56

Kl. pneumoniae PCI602	6.25	6.25	3.13	3.13	12.5	3.13	0.78	0.78	0.78	1.56	0.78	1.56
Kl. pneumoniae 22#3038[d,e]	100	>100	100	>100	100	100	3.13	6.25	1.56	12.5	1.56	1.56
Sh. dysenteriae JS11910	25	25	12.5	25	50	12.5	3.13	6.25	1.56	3.13	1.56	3.13
Sh. flexneri 4b JS11811	25	25	25	100	25	6.25	3.13	1.56	1.56	1.56	0.78	3.13
Sh. sonnei JS11746	25	12.5	12.5	12.5	25	6.25	3.13	1.56	3.13	1.56	3.13	1.56
Sal. typhi T-63	25	12.5	12.5	6.25	6.25	1.56	0.78	0.78	25	3.13	1.56	1.56
Sal. enteritidis 1891	25	25	25	25	25	6.25	12.5	6.25	3.13	6.25	0.78	1.56
Proteus vulgaris OX19	6.25	3.13	1.56	1.56	3.13	1.56	0.78	0.39	0.39	0.78	<0.20	0.78
Proteus rettgeri GN311	>100	>100	>100	25	100	25	25	25	25	100	50	100
Proteus rettgeri GN466	25	25	25	3.13	25	3.13	1.56	3.13	1.56	3.13	6.25	1.56
Serratia marcescens	100	100	100	50	100	25	25	3.13	25	25	6.25	25
Serratia sp. SOU	>100	>100	>100	>100	>100	50	100	100	100	50	25	25
Serratia sp. 4	100	50	50	25	50	25	25	6.25	25	25	6.25	12.5
Providencia sp. Pv16[g]	>100	>100	>100	>100	>100	>100	6.25	12.5	25	12.5	6.25	12.5
Providencia sp. 2991[g]	>100	>100	>100	>100	>100	>100	3.13	25	25	12.5	12.5	25
Ps. aeruginosa A3	12.5	6.25	3.13	3.13	3.13	1.56	0.39	0.78	0.78	6.25	0.39	0.78
Ps. aeruginosa No. 12	12.5	100	25	6.25	25	3.13	1.56	1.56	6.25	1.56	1.56	3.13
Ps. aeruginosa H9[e]	50	50	100	>100	25	12.5	3.13	25	3.13	6.25	1.56	12.5
Ps. aeruginosa H11	12.5	50	25	>100	25	12.5	12.5	3.13	12.5	12.5	12.5	12.5
Ps. aeruginosa TI-13[e]	12.5	25	12.5	50	12.5	6.25	1.56	1.56	3.13	6.25	1.56	1.56
Ps. aeruginosa GN315[b]	>100	>100	>100	>100	25	50	25	>100	100	25	3.13	12.5
Ps. aeruginosa 99[f]	25	>100	25	12.5	50	12.5	3.13	6.25	6.25	3.13	3.13	6.25
Ps. aeruginosa B-13[e,e]	50	100	50	50	100	25	6.25	12.5	12.5	12.5	12.5	50
Ps. aeruginosa 21–75[h]	>100	>100	>100	50	>100	50	6.25	25	12.5	6.25	12.5	25
Ps. aeruginosa PSTI[f]	>100	>100	>100	>100	>100	>100	6.25	12.5	6.25	25	12.5	25
Ps. aeruginosa ROS134/PU21[f]	>100	>100	>100	>100	>100	>100	100	>100	100	>100	50	100
Ps. aeruginosa K-Ps102[i]	12.5	50	12.5	12.5	25	6.25	1.56	1.56	3.13	6.25	1.56	6.25
Ps. aeruginosa GN907[i]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Resistance mechanisms: [a] AAD(4′), [b] AAC(6′), [c] APH(3′)-I, [d] AAD(2″), [e] APH(3′)-II, [f] AAC(3), [g] AAC(2′), [h] APH(3′)-III, [i] permeability.

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(Received September 30, 1981)

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