Modification of Aminocyclitol Antibiotics. 8. Preparation of 5,6″-Dideoxykanamycin B[1]

Tetsuo SUAMI* and Katsumi NAKAMURA

Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223

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In order to obtain an effective antibiotic against a resistant strain of bacteria, two hydroxyl groups on C-5 and 6″ of kanamycin B were removed. 5,6″-Dideoxykanamycin B thus prepared was subjected to antimicrobial tests, its structure being established by mass and ^{13}C NMR spectroscopy.

In connection with the preceding paper,[2] a report is given on the preparation and antimicrobial activity of 5,6″-dideoxykanamycin B.

5-Deoxygentamicin complex[3] and 5-deoxysisomicin[4] exhibit improved activity against resistant strains of bacteria, as compared to gentamicin complex and sisomicin, respectively. Considering the structural similarity between these antibiotics and kanamycin B, 5-deoxykanamycin B[5] can be considered to have improved activity against kanamycin resistant strains of bacteria, but this is not the case. This might be attributed to a complicated structural feature of kanamycin B. The characteristic feature of kanamycin B is the existence of a hydroxymethyl group on C-5″, in contrast with gentamicin complex and sisomicin. If the hydroxyl group is removed from 5-deoxykanamycin B, the resulting 5,6″-dideoxykanamycin B (**5**) might show improved activity.

Results and Discussion

When 1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B[6] (**1**) reacted with trityl chloride in pyridine and subsequently acylated with benzoyl chloride, tetra-*O*-benzoyl-*O*-trityl derivative (**2**) was obtained in 77% yield. Since trityl chloride attacks a primary hydroxyl group preferentially,[7] and a hydroxyl group on C-5 in kanamycin B is highly hindered against the acylation,[5] the structure of **2** is assigned to a 3′,4′,2″,4″-tetra-*O*-benzoyl-6″-*O*-trityl derivative. The assignment would be verified by the establishment of the structure of **5** by mass and ^{13}C NMR spectrometries.

Detritylation of **2** with hydrogen bromide, followed by halogenation with sulfuryl chloride in pyridine afforded a 5,6″-dichloro-5,6″-dideoxy derivative (**3**) in 50% yield.

Dehalogenation of **3** with tributylstannane gave a

Table 1. The ^{13}C NMR chemical shifts[a] of 5,6″-dideoxykanamycin B

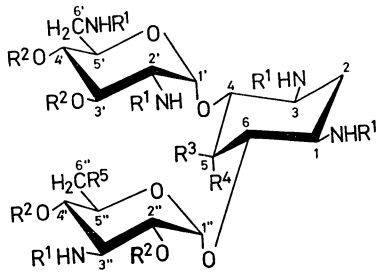
	5,6″-Dideoxykanamycin B (**5**)	
	pD 11	pD 1
C-1	53.4	52.2
C-2	36.9	29.0
C-3	52.6	51.7
C-4	78.0	71.8
C-5	34.7	32.6
C-6	83.9	78.4
C-1′	96.1	91.4
C-2′	54.9	53.9
C-3′	76.1	71.2[b]
C-4′	72.9[b]	71.5[b]
C-5′	74.8	69.8
C-6′	42.5	41.0
C-1″	100.9	100.0
C-2″	72.4[b]	69.5
C-3″	55.7	55.6
C-4″	73.9	69.5
C-5″	68.9	69.0
C-6″	17.6	17.3

a) In parts per million downfield from tetramethylsilane. b) The signals may be reversed.

Table 2. Antimicrobial activity of 5,6″-dideoxykanamycin B (**5**) and kanamycin B

Test organisms	MIC (mcg/ml)	
	Compound **5**	Kanamycin B
Staphylococcus aureus ATCC 6538P	0.39	0.78
Staphylococcus epidermidis ATCC 12228	<0.025	0.1
Diplococcus pneumoniae Type 3	0.1	0.05
Bacillus subtilis ATCC 6633	<0.025	<0.025
Escherichia coli NIH JC-2	6.25	6.25
Klebsiella pneumoniae 602	0.78	1.56
Pseudomonas aeruginosa IAM 1007	100	25
Proteus vulgaris OX-19	0.2	0.2
Salmonella paratyphi A 1015	0.78	0.39
Salmonella paratyphi B	1.56	0.78
Shigella flexneri 2a·SH-74-1	6.25	3.12
Staphylococcus aureus Apo-1[a]	0.28	0.39
Staphylococcus epidermidis 109[a]	50	50
Salmonella sp. D-0001[a]	200	200
Proteus rettgeri J-0026[a]	400	200
Klebsiella pneumoniae 22 #3038[a]	200	400
Klebsiella pneumoniae MB-3841[a]	400	>800
Serratia marcescens MB-3848[a]	12.5	3.13
Serratia marcescens I-0087[a]	25	12.5
Pseudomonas aeruginosa M-0086[a]	>800	200
Escherichia coli K-12 57R391 JJ53[a]	200	400
Escherichia coli K-12 CSH-2 R-6[a]	200	200
Escherichia coli JR66/W677[a]	200	400
Escherichia coli (M) Cast. and Chalm. NIHJ JC-2[a]	0.28	0.28

a) A resistant strain of bacteria.



1 R^1=Cbe, R^2=R^3=H, R^4=R^5=OH
2 R^1=Cbe, R^2=Bz, R^3=OH, R^4=H, R^5=OTr
3 R^1=Cbe, R^2=Bz, R^3=H, R^4=R^5=Cl
4 R^1=Cbe, R^2=Bz, R^3=R^4=R^5=H
5 R^1=R^2=R^3=R^4=R^5=H
6 R^1=Ac, R^2=R^3=R^4=R^5=H

Scheme 1.

5,6″-dideoxy derivative (**4**) in 81% yield.

Hydrolysis of **4** in a sodium hydroxide solution gave **5** in 73% yield. *N*-Acetylation of **5** by the conventional method afforded penta-*N*-acetyl-5,6″-dideoxykanamycin B (**6**).

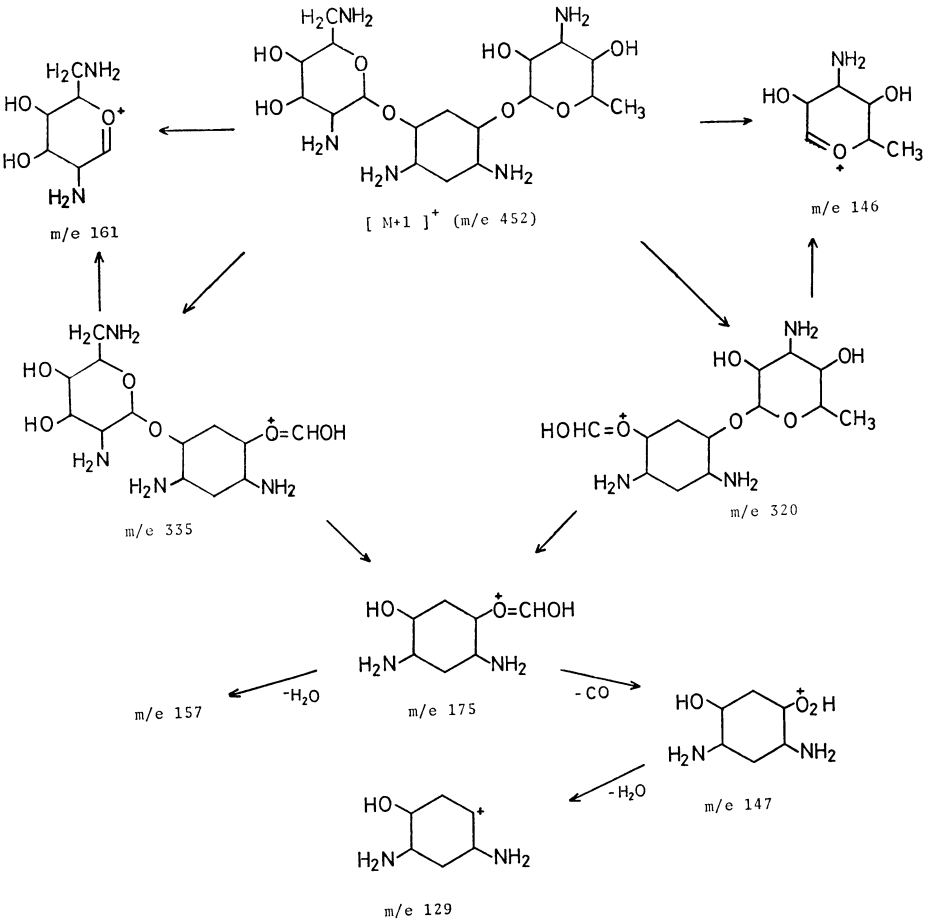
The structure of **5** was established by mass spectroscopy. The mass spectrum of **5** shows $[M+1]^+$ peak at *m/e* 452, corresponding to $[M+1]^+$ of kanamycin B-32. The other peaks are also consistent with the proposed structure of **5** (Scheme 1).

The ^{13}C NMR spectrum of **5** shows eighteen carbon signals (Table 1). In comparison with the spectra of 5-deoxykanamycin B and kanamycin B,[5] large shifts of the signals of C-5 and C-6″, we see in the spectrum of **5**, confirming the structure of **5**.

Compound **5** was subjected to the determination of antimicrobial activity against microorganisms by a dilution method. The MIC (minimum inhibition concentration) values are given in Table 2. As compared to kanamycin B, **5** exhibited improved activity against *Staphylococcus epidermidis* ATCC 12228 and *Klebsiella pneumoniae* 602. Compound **5** was slightly more active than kanamycin B against resistant strains of bacteria: *Staphylococcus aureus* Apo-1, *Klebsiella pneumoniae*, and *Escherichia coli*. Compound **5** showed a marked decrease in activity against *Pseudomonas aeruginosa*.

Experimental

General Method. Melting points were determined in open capillaries in a liquid bath and are uncorrected. Solutions were concentrated under reduced pressure below 40 °C. Optical rotations were measured on a Japan Spectroscopic DIP-SL polarimeter. ^{13}C NMR spectra were determined on a Varian FT-80 spectrometer at 20 MHz, D_2O being used as a solvent with an internal standard of dioxane. The resonance signals are expressed in ppm down-field from the signal of tetramethylsilane ($\delta_c^{TMS}=\delta_c^{dioxane}+67.4$). Mass



Scheme 2.

spectra were recorded with a Hitachi RMU-6M single focusing mass spectrometer. TLC was performed on Wakogel B-10 plates, silica gel (Wakogel C-300) being used in column chromatography.

3',4',2'',4''-Tetra-O-*benzoyl-1,3,2',6',3''-pentakis*-N-*(ethoxycarbonyl)-6''*-O-*tritylkamycin B (2).* Trityl chloride (1.7 g) was added to a stirred solution of 1,3,2',6',3''-pentakis-*N*-(ethoxycarbonyl)kanamycin B[8] (**1**, 1.0 g) in pyridine (50 ml). After 69 h, benzoyl chloride (3 ml) was added to the solution under ice cooling with agitation. After 24 h, the solution was poured into cold water containing $NaHCO_3$. The aqueous mixture was repeatedly extracted with $CHCl_3$, the $CHCl_3$ layers being washed with water, dried over Na_2SO_4 and concentrated. The residue was chromatographed on a SiO_2 column with 1 : 5 (v/v) 2-butanone–$CHCl_3$. From the fractions homogeneous on TLC (R_f 0.72) in 7 : 1 (v/v) benzene–methanol, 1.37 g (77%) of **2** was obtained as amorphous powder, mp 125—131 °C, $[\alpha]_D^{21}$ +85° (*c* 0.56, chloroform).

Found: C, 63.65; H, 5.85; N, 4.66%. Calcd for $C_{80}H_{87}N_5O_{24}$: C, 63.95; H, 5.84; N, 4.66%.

3',4',2'',4''-Tetra-O-*benzoyl-5,6''-dichloro-5,6''-dideoxy-1,3,2',6',3''-pentakis*-N-*(ethoxycarbonyl)-5-epikanamycin B (3).* Glacial acetic acid (3 ml) containing 30% HBr was added to a solution of **2** (1.33 g) in $CHCl_3$ (3 ml) and glacial acetic acid (6 ml) under ice cooling with agitation. After 2 min, the resulting mixture was quenched on ice and repeatedly extracted with $CHCl_3$. The combined organic layers were washed with a $NaHCO_3$ solution, water, dried over Na_2SO_4, and concentrated. Sulfuryl chloride (1.5 ml) was added to a solution of the residue in pyridine (15 ml) under ice cooling with agitation. After 19 h, the solution was poured into ice cold water, precipitates being collected by filtration. The product was chromatographed on SiO_2 with 1 : 5 (v/v) 2-butanone–$CHCl_3$. The fractions homogenous on TLC (R_f 0.47) in 7 : 1 (v/v) benzene–methanol were combined and concentrated to give 0.57 g (50%) of **3**, mp 143—149 °C, $[\alpha]_D^{25}$ +69.4° (*c* 0.54, $CDCl_3$).

Found: C, 56.47; H, 5.52; N, 5.27; Cl, 5.61%. Calcd for $C_{61}H_{71}N_5Cl_2O_{22}$: C, 56.48; H, 5.52; N, 5.40; Cl, 5.47%.

3',4',2'',4''-Tetra-O-*benzoyl-5,6''-dideoxy-1,3,2',6',3''-pentakis* N-*(ethoxycarbonyl)kanamycin B (4).* A solution of **3** (460 mg) and tributylstannane (2.0 ml) in toluene (20 ml) was heated at 80 °C in the presence of α,α'-azobisisobutyronitril (20 mg). After 24 h, the resulting solution was concentrated and the residue was chromatographed on SiO_2 with 15 : 1 (v/v) benzene–methanol. The fractions homogeneous on TLC (R_f 0.38) in the same solvent were combined and concentrated to give 350 mg (80%) of **4**, mp 144—150 °C, $[\alpha]_D^{25}$ +87.8° (*c* 0.47, $CHCl_3$).

Found: C, 59.59; H, 5.95; N, 5.43%. Calcd for $C_{61}H_{73}N_5O_{22}$: C, 59.65; H, 5.99; N, 5.70%.

5,6''-Dideoxykanamycin B (5). A solution of **4** (304 mg) in methanol (6 ml) was added to a 2 M NaOH solution (20 ml) the solution being heated under reflux. After 12 h, the solution was neutralized with HCl and concentrated. The residue was purified on a CG-50 (NH_4^+) resin column. The fractions eluted with 0.3 M NH_4OH gave 81 mg (73%) of **5**, mp 172—175 °C (dec), $[\alpha]_D^{23}$ +136° (*c* 0.44, H_2O). The product showed a single spot (R_f 0.2) on TLC in 5 : 8 : 10 : 7 (v/v) 28% NH_4OH–1-butanol–ethanol–water.

1,3,2',6',3''-Penta-N-*acetyl-5,6''-dideoxykanamycin B (6).* Acetic anhydride (0.1 ml) was added to a solution of **5** (11 mg) in methanol (2 ml), precipitates being collected by filtration to give 13 mg (79%) of **6**, mp 250 °C, $[\alpha]_D^{25}$ +113° (*c* 0.35, H_2O).

Found: C, 48.00; H, 6.67; N, 10.01%. Calcd for $C_{28}H_{47}N_5O_{13}\cdot 2H_2O$: C, 48.20; H, 7.36; N, 10.04%.

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