AMINOGLYCOSIDE ANTIBIOTICS. XI SYNTHESIS AND ACTIVITY OF 4′-DEOXYKANAMYCIN B

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In a previous paper[1] we reported the synthesis and activity of 4′-deoxykanamycin A and showed that some strains of *Pseudomonas aeruginosa* and the aminoglycoside-resistant organisms which produced aminoglycoside-3′-phosphotransferase II were inhibited by 4′-deoxykanamycin A. Recently, a new type of aminoglycoside inactivation has been disclosed in strains of *Staphylococcus epidermidis*[2], *Bacillus brevis*[3] and *Staphylococcus aureus*[4] which involves adenylylation of the antibiotics at the 4′-hydroxyl group, thus calling further attention to the role played by the 4′-hydroxyl group in the resistant mechanisms of aminoglycoside antibiotics.

This paper reports the synthesis of 4′-deoxykanamycin B and its activity against aminoglycoside-resistant organisms including those which are known to produce aminoglycoside-4′-adenylyltransferase.

Synthesis (Chart 1)

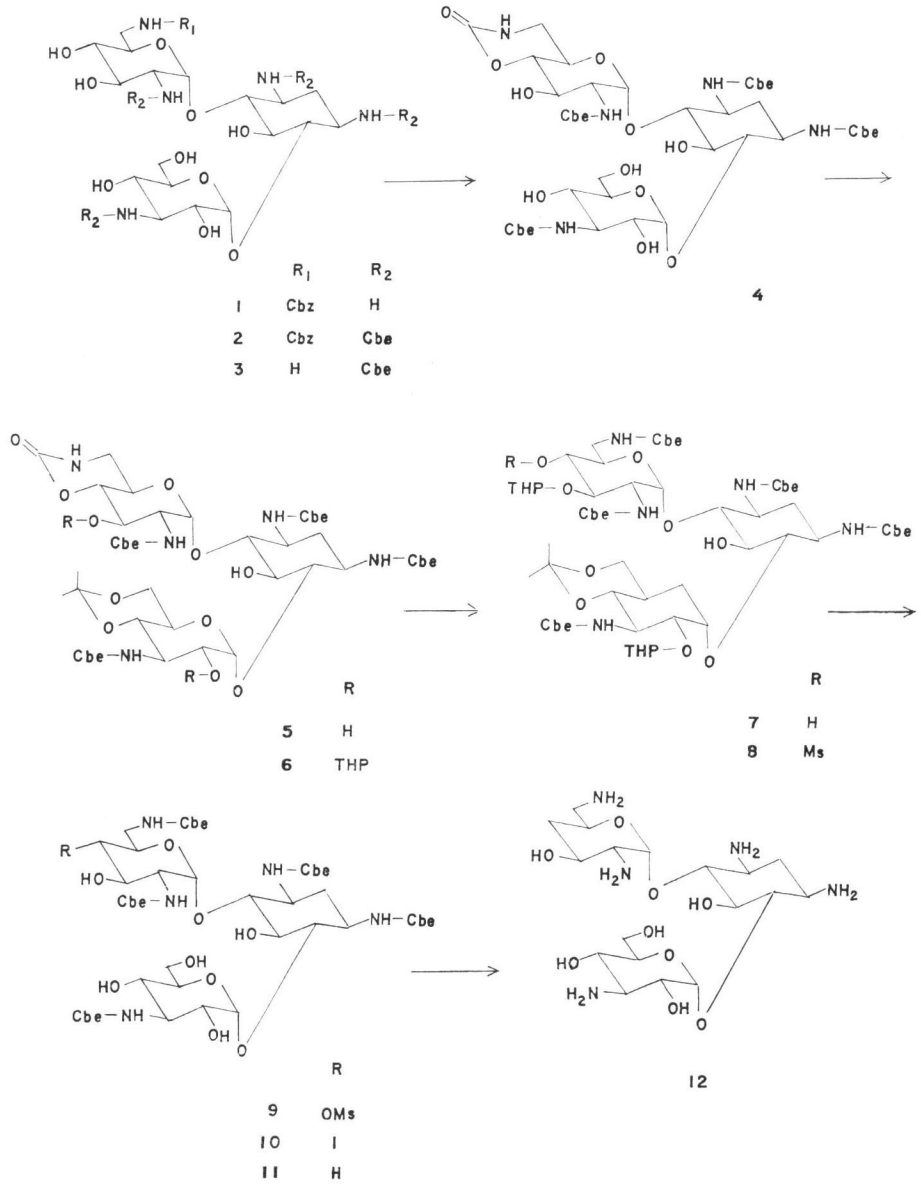
6′-N-Benzyloxycarbonylkanamycin B (**1**)[5] was treated with ethyl chloroformate in the presence of sodium carbonate to give the tetra-N-ethoxycarbonyl (Cbe) derivative (**2**) in 90% yield, m.p. >300°C, $[\alpha]_D^{22}$ +80° (*c* 0.5, DMF). Anal. Calc'd for $C_{38}H_{59}N_5O_{20}$: C 50.38, H 6.56, N 7.73. Found: C 50.46, H 6.55, N 7.37. The benzyloxycarbonyl (Cbz) group of **2** was removed by catalytic hydrogenation to afford the 6′-amino derivative (**3**) in 98% yield, m.p. >300°C $[\alpha]_D^{22}$ + 90° (*c* 0.3, DMF). Silica gel TLC*: Rf 0.58 (anthrone). Anal. calc'd for $C_{30}H_{53}N_5O_{18}\cdot\frac{1}{2}H_2O$: C 46.15, H 6.97, N 8.97. Found: C 46.14, H 7.00 N 8.43. Compound **3** was reacted with phenyl chloroformate[6] in the presence of excess sodium carbonate in THF-water to give the 4′-6′-cyclic carbamate **4** in a quantitative yield, m.p. >300°C, $[\alpha]_D^{23}$ +63.3° (*c* 0.3, DMF). TLC*: Rf 0.90. Anal. Calc'd for $C_{31}H_{51}N_5O_{19}$: C 46.67, H 6.44, N 8.78. Found: C 46.86, H 6.59, N 8.32. Treatment of **4** with 2,2-dimethoxypropane afforded the 4″,6″-mono-O-isopropylidene derivative (**5**) in 89% yield, m.p. >300°C, $[\alpha]_D^{23}$ +59° (*c* 0.5, DMF). Anal. Calc'd for $C_{34}H_{55}N_5O_{19}$: C 48.74, H 6.62, N 8.36. Found: C 48.75, H 6.92, N 7.87. Two hydroxyl groups of **5** at C-2″ and C-3′ were then blocked with a tetrahydropyranyl (THP) group to give **6** in a quantitative yield, m.p. >320°C, $[\alpha]_D^{23}$ +46.3° (*c* 0.4, DMF). NMR (DMSO-d_6, δ in ppm): 0.8~2.1 (14H, m), 4.3~4.85 (2H, broad, anomeric protons of THP). Anal. Calc'd for $C_{44}H_{71}N_5O_{21}$: C 52.53, H 7.11, N 6.96. Found: C 52.73, H 7.22, N 6.77.

Selective cleavage of the cyclic carbamate group in **6** with sodium ethoxide afforded **7**, the key intermediate having a free hydroxy group at C-4′, in 76% yield, m.p. 282~285°C (dec.), $[\alpha]_D^{23.2}$ +83° (*c* 0.5, DMF). Anal. Calc'd for $C_{46}H_{77}N_5O_{22}$: C 52.51, H 7.38, N 6.66. Found: C 52.24, H 7.37, N 6.43. The NMR of **7** indicated the presence of an additional ethoxycarbonyl group on N-6′ at δ 1.21 ppm. **7** was reacted with mesyl chloride in pyridine to give **8** in 92% yield, which, incidentally, on refluxing in methanol for crystallization afforded **9** in 77% yield, m.p. 217.5~218°C (dec.) $[\alpha]_D^{23.2}$ +78.3° (*c* 0.3, DMF). Anal. Calc'd for $C_{34}H_{59}N_5O_{22}S$: C 44.29, H 6.45, N 7.60, S 3.48. Found: C 44.32, H 6.40, N 7.39, S 3.33. The NMR spectrum of **9** indicated the presence of a mesyl group (3H, s, δ 3.13 ppm) but gave no signals for isopropylidene methyl and tetrahydropyranyl methylene protons. Iodination of **8** by heating with sodium iodide in acetone in a sealed tube resulted in a concurrent removal of both of the isopropylidene and THP groups to give **10** in good yield, m.p. 205~207°C, $[\alpha]_D^{23.2}$ +73.3° (*c* 0.3, DMF). Anal. Calc'd for $C_{33}H_{56}N_5O_{19}I$: C 41.56, H 5.92, N 7.34. Found: C 42.28, H 6.25, N 6.83.

Hydrogenation of **10** in the presence of palladium on charcoal and sodium bicarbonate afforded **11** in 89% yield, m.p. 228~230°C, $[\alpha]_D^{23}$ +100° (*c* 0.3, DMF). Anal. Calc'd for $C_{33}H_{57}N_5O_{19}\cdot H_2O$: C 46.86, H 7.03, N 8.28. Found: C 46.61, H 6.84, N 8.03. Hydrolysis of **11** by heating with hydrazine hydrate in a sealed tube, followed by purification on columns of Amberlite CG-50 (NH_4^+ and cupra-ammonium forms), gave 4′-deoxykanamycin B (**12**) in 15% yield, m.p. 224~227°C (in a sealed tube),

* MeOAc - *n*-PrOH - NH_4OH (45: 105: 60).

Chart 1. Synthesis of 4′-deoxykanamycin B



$[\alpha]_D^{24.5}+122°$ (*c* 0.25, water). Anal. Calc'd for $C_{18}H_{37}N_5O_9 \cdot H_2O$: C 44.53, H 8.10, N 14.42. Found: C 44.47, H 8.06, N 14.28. As shown in Fig. 1, the C-4′ axial proton of **12** resonated at 3.37 ppm (q, J=*ca.* 10.9 Hz) high-field from the HOD signal and the C-4′ equatorial proton at around 2.5~3.0 ppm (multiplet) which overlapped with the C-2 equatorial proton.

Compound **12** was hydrolyzed with dil.HCl for structural verification: the hydrolyzate gave two ninhydrin-positive spots at Rf 0.50 and 0.60 by silica gel TLC*, which were shown to be identical with authentic speciemens of 4′-deoxyneamine[7] and 3-amino-3-deoxy-D-glucose, respectively.

* $CHCl_3$ - MeOH - 28% NH_4OH - H_2O (1: 4: 2: 1)

Fig. 1. The NMR spectrum of 4′-deoxykanamycin B in D_2O at 60 MHz

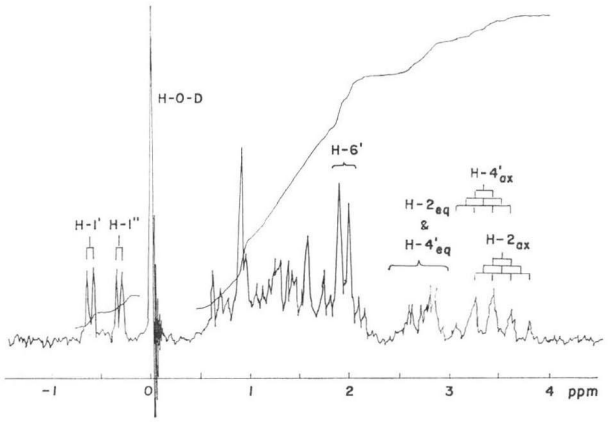


Table 1. Antibacterial activity of 4′-deoxykanamycin B (**12**) and related antibiotics

Organism	Inactivating enzyme	MIC*(mcg/ml)			
		4′-Deoxy-kanamycin B (**12**)	Kanamycin B	Kanamycin A	4′-Deoxy-kanamycin A
S. aureus Smith	—	0.2	0.2	0.4	0.8
S. epidermidis	—	0.2	0.4	0.8	1.6
B. brevis ATCC 8185	—	0.8	0.4	0.8	1.6
E. coli K12	—	0.8	0.8	1.6	3.1
K. pneumoniae D11	—	0.2	0.2	0.4	0.8
Pr. vulgaris A9436	—	0.4	0.2	0.4	0.8
Ps. aeruginosa D15	—	6.3	12.5	100	12.5
Ps. aeruginosa A9930	—	3.1	12.5	25	3.1
E. coli ML 1630	APH(3′)-I	>100	>100	>100	>100
E. coli A20107	″ -II	25	>100	>100	6.3
E. cloacae A21006	″ -II	25	>100	>100	3.1
E. coli A20895	AAC(3)-I	0.8	0.8	1.6	3.1
P. aeruginosa A20741	″ -II	>100	>100	>100	>100
E. coli NR79/W677	AAC(6′)-I	12.5	>100	>100	3.1
P. aeruginosa GN4925	″ -III	50	50	100	>100
P. aeruginosa GN315	″ -IV	>100	>100	>100	>100
E. coli A20732	ANT(2′)	25	25	50	100
S. epidermidis A22033	ANT(4′)	3.1	50	50	6.3
B. brevis IFO 12334	″	3.1	>100	>100	25
S. aureus A22054	″	50	>100	>100	100
S. aureus A22059	″	3.1	25	50	12.5

* determined by STEERS' method[9] on MUELLER-HINTON agar plates; inoculum size: 10^4 dilution of overnight culture.

Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of 4′-deoxykanamycin B (**12**) were determined against both aminoglycoside-sensitive and -resistant organisms by the two-fold agar dilution method. Kanamycin B, kanamycin A and 4′-deoxykanamycin A were tested comparatively as reference antibiotics and the results are shown in Table 1.

The activity of 4′-deoxykanamycin B against 6

strains of aminoglycoside-sensitive organisms was nearly the same as that of kanamycin B, while 4′-deoxykanamycin A was about one-half as active as kanamycin A against these organisms. Two strains of *P. aeruginosa* which did not produce an aminoglycoside-inactivating enzyme showed greater sensitivity to 4′-deoxykanamycins than to the parent antibiotics.

It has been shown[1] that 4′-deoxykanamycin A inhibits aminoglycoside-resistant organisms which produce aminoglycoside-3′-phosphotransferase II [APH(2′)-II]. 4′-Deoxykanamycin B also inhibits APH(2′)-II-producing organisms though to a lesser extent than 4′-deoxykanamycin A.

Both 4′-deoxykanamycins A and B inhibited *Escherichia coli* NR79/W677 which produces aminoglycoside-6′-acetyltransferase-I [AAC(6′)--I], although the organism was more susceptible to 4′-deoxykanamycin A than 4′-deoxykanamycin B. Two pseudomonal strains which produced AAC(6′)-III or IV were not inhibited by 4′-deoxykanamycins A and B. YAGISAWA *et al.*[8] observed that 4′-deoxykanamycin A was less readily acetylated than kanamycin A by *P. aeruginosa* GN315, an AAC(6′)-IV-producer.

Two strains of *S. aureus* and one strain each of *S. epidermidis* and *B. brevis*, all of which have been reported to produce aminoglycoside-4′-adenylyltransferase [ANT(4′)][2,3,4], showed varied susceptibility to 4′-deoxykanamycins A and B. 4′-Deoxykanamycin B inhibited these organisms at 3.1 mcg/ml except for one staphylococcal strain, A22054, which was inhibited only at 50 mcg/ml. The activity of 4′-deoxykanamycin A against this group of resistant organisms was generally lower and more variable than that of 4′-deoxykanamycin B. The degree and nature of sensitivity of these organisms suggest a possible involvement of resistant mechanisms other than 4′-adenylylation that can affect the 4′-deoxygenated kanamycin derivatives.

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