SYNTHESIS OF 3′-DEOXYKANAMYCIN B

Sir:

We have previously reported the synthesis of 3′, 4′-dideoxy derivatives of neamine[1], kanamycin B[2] and ribostamycin[3], which were active against kanamycin-resistant bacteria. Another theoretically interesting group of compounds in view of the resistance mechanism[4] is 3′-deoxy derivatives. As an example of this group, we synthesized 3′-deoxykanamycin A.[5] By selective 3′-dehydroxylation of kanamycin B, we have succeeded in yielding 3′-deoxykanamycin B, which is synonymous with tobramycin[6], a *Streptomyces* product. Since kanamycin B has already been synthesized[11], this synthesis constitutes the total synthesis of tobramycin.

Penta-N-ethoxycarbonylkanamycin B[2] was treated with cyclohexanone dimethylketal in DMF in the presence of *p*-toluenesulfonic acid at 50°C under reduced pressure (25~30 Torr) to give the 3′, 4′; 4″, 6″-di-O-cyclohexylidene derivative (**1**), mp 213~215°C, $[\alpha]_D^{20}$+99° (*c* 1, methanol). [Calcd. for $C_{45}H_{73}N_5O_{20}$: C 53.83, H 7.33, N 6.98; Found: C 53.50, H 7.32, N 6.77]. Benzoylation of **1** with benzoyl chloride in pyridine gave the 2″-O-benzoyl derivative (**2**) quantitatively, mp 152~154°C, $[\alpha]_D^{20}$+99° (*c* 1.4, methanol). [Calcd. for $C_{52}H_{77}N_5O_{21}$: C 56.36, H 7.00, N 6.32; Found: C 56.46, H 6.76, N 6.07]. Selective removal of the cyclohexylidene group at C-3′ and 4′ was effected by treatment with acidic methanol at 25°C and the 2″-O-benzoyl-4″, 6″-O-cyclohexylidene derivative (**3**) was obtained in a yield of 80 %, mp 233~235°C, $[\alpha]_D^{20}$+147° (*c* 0.56, DMF). [Calcd. for $C_{46}H_{69}N_5O_{21}$: C 53.74, H 6.76, N 6.81; Found: C 53.49, H 6.41, N 6.69].

Treatment of **3** (1 mol equivalent) with *p*-toluenesulfonyl chloride (5 mol equivalents) in pyridine at 25°C overnight gave the 3′-O-tosyl derivative as the major product and 4′-O-tosyl and 3′, 4′-di-O-tosyl derivatives as minor ones.

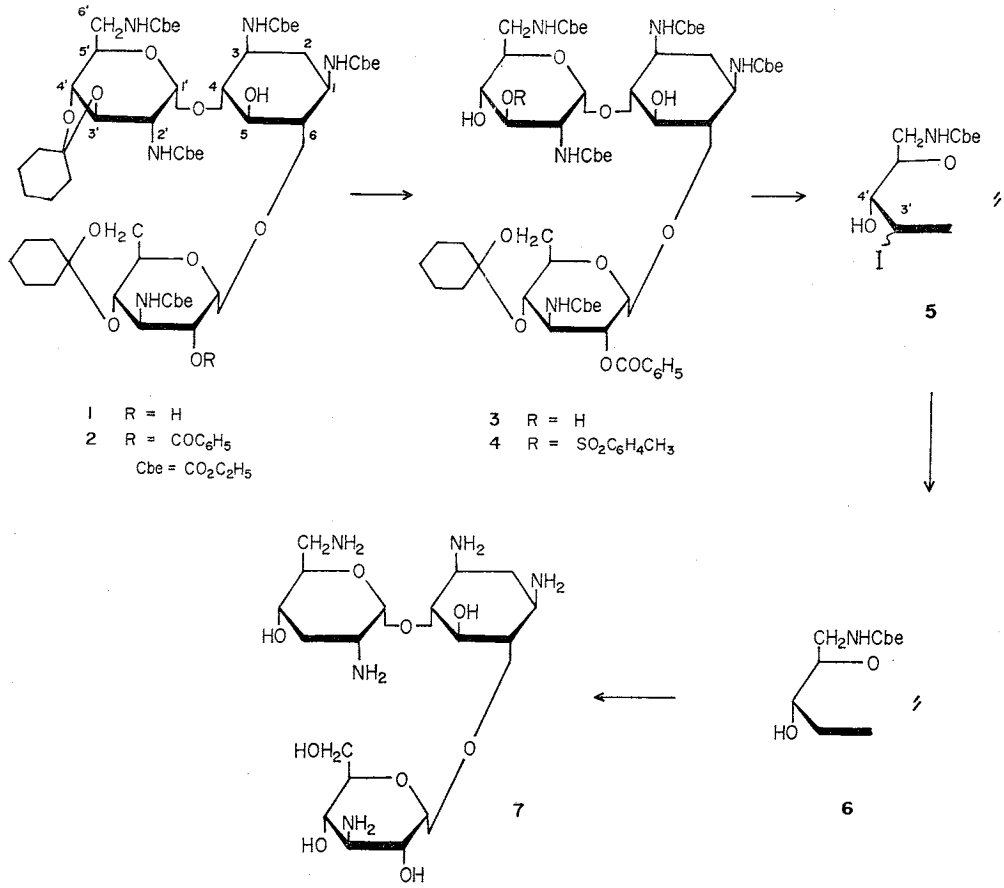

Table 1. Antibacterial spectra of 7, tobramycin, DKB and KMB

Test organisms*	Minimal inhibitory concentration (mcg/ml)			
	7	Tobramycin	DKB	KMB
Staphylococcus aureus FDA 209P	<0.2	<0.2	<0.2	0.39
Sarcina lutea PCI 1001	12.5	25	6.25	1.56
Bacillus subtilis NRRL B-558	<0.2	<0.2	<0.2	<0.2
Klebsiella pneumoniae PCI 602	0.39	0.2	0.39	0.78
〃 type 22 #3038	50	50	100	>100
Salmonella typhosa T-63	0.39	<0.2	<0.2	0.2
Escherichia coli NIHJ	0.78	0.39	0.39	0.78
〃 K-12	0.39	0.39	0.78	0.78
〃 〃 R-5	1.56	3.12	3.12	3.12
〃 〃 ML 1629	1.56	0.78	0.78	>100
〃 〃 ML 1630	1.56	1.56	0.78	>100
〃 〃 ML 1410	1.56	0.78	1.56	0.78
〃 〃 〃 R 81	1.56	3.12	1.56	>100
〃 〃 LA 290 R 55	25	50	50	12.5
〃 〃 〃 R 56	3.12	3.12	12.5	3.12
〃 〃 〃 R 64	3.12	3.12	6.25	3.12
〃 〃 W 677	0.39	0.39	0.2	0.39
〃 〃 JR 66/W 677	50	50	50	>100
Pseudomonas aeruginosa A 3	1.56	0.78	1.56	50
〃 No. 12	0.78	0.78	0.78	12.5
〃 GN 315	100	>100	>100	>100
〃 TI-13	0.78	0.78	1.56	100
〃 99	1.56	3.12	3.12	>100
Proteus rettgeri GN 311	6.25	3.12	3.12	3.12
〃 GN 466	3.12	1.56	0.78	3.12
Mycobacterium smegmatis ATCC 607**	0.39	0.2	0.39	0.78

* Agar dilution streak method (nutrient agar, 37°C, 18 hours).
** 48 hours.

The selective 3′-O-tosylation may be ascribed to the presence of the electron-withdrawing ethoxycarbonylamino group at C-2′ which makes the 3′-hydroxyl group more anionic than the 4′-hydroxyl in the basic medium. The bulky 3′-O-tosyl group is suggested to hinder subsequent 4′-O-tosylation. In contrast to the tosylation, 3′, 4′-di-O-mesylation was easily performed[2]. Successive isolation and purification gave the 3′-O-tosyl derivative (**4**) in a yield of 60 %, mp 149~150°C, $[\alpha]_D^{20}$+88° (*c* 1, methanol). NMR (in $CDCl_3$): τ 7.58 (3H s, $CH_3C_6H_4SO_2$–). [Calcd. for $C_{53}H_{75}N_5O_{23}S$: C 53.84, H 6.40, N 5.93, S 2.71; Found: C 53.90, H 6.58, N 5.67, S 3.00].

Iodination of **4** with excess sodium iodide (4.9 g NaI in 10 ml DMF) in DMF at 100°C for 20 hours gave the 3′-iodo derivative (**5**) in a yield of 70 %. [Calcd. for $C_{46}H_{68}N_5O_{20}I$: C 48.55, H 6.02, N 6.15, I 11.15; Found: C 48.75, H 6.09, N 6.57, I 11.45]. The iodo derivative was unstable, however the immediate hydrogenation with RANEY nickel and hydrogen in dioxane gave the 3′-deoxy derivative (**6**) in a yield of 96 %, mp 248.5~250°C, $[\alpha]_D^{20}$+86° (*c* 0.76, methanol). [Calcd. for $C_{46}H_{69}N_5O_{20}$: C 54.59, H 6.87, N 6.92; Found: C 54.58, H 6.80, N 6.84].

Compound **6** was successively treated with hot 4 N barium hydroxide to remove the ethoxycarbonyl and benzoyl groups and with 50 % acetic acid at 80°C to remove the cyclohexylidene group to give the deblocked product, which was purified by chromatography on CM-Sephadex C-25 (NH_4^+) with 0~0.15 N ammonia. 3′-Deoxykanamycin B (**7**) was obtained as a

monohydrate, $[\alpha]_D^{20}+129°$ (*c* 1, water) (lit[6] $+128°$). $Rf_{kanamycin\ B}$ 1.25 (paper chromatography with 1-butanol-pyridine-water-acetic acid (6:4:3:1)). NMR (in D_2O): τ 7.7~8.9 (4 H m, H-2 and 3'); The whole pattern was different from that of kanamycin B and 3', 4'-dideoxykanamycin B. [Calcd. for $C_{18}H_{37}N_5O_9 \cdot H_2O$: C 44.53, H 8.10, N 14.43; Found: C 44.92, H 8.09, N 14.61].

The structure of 7 was confirmed by its $\Delta[M]_{436(TACu)}$[7] value determination and by acidic hydrolysis. $\Delta[M]_{436(TACu)}$ values of 7, tobramycin, kanamycin (KM), kanamycin B (KMB) and 3', 4'-dideoxykanamycin B (DKB) were $+950°$, $+900°$, $+850°$, $-450°$ and $+800°$, respectively.

Kanamycin, tobramycin and 3', 4'-dideoxykanamycin B are expected to give similar $\Delta[M]_{436(TACu)}$ values, because the 6-O-glycosyl-deoxystreptamine portions are common and the 4-O-glycosyl-deoxystreptamine portions are expected to give no contribution* to the values. Only kanamycin B should be differentiated owing to copper complex formation at the 2'-amino and 3'-hydroxyl groups. Acidic hydrolysis of KMB, DKB, 7 and tobramycin with 6 N hydrochloric acid at 100°C for 2 hours followed by paper-chromatographic examination with 1-butanol-pyridine-water-acetic acid (6:4:3:1) gave 2-deoxystreptamine and 3-amino-3-deoxyglucose ($Rf_{2\text{-deoxystreptamine}}$ 2.7) as common products and the third products having $Rf_{2\text{-deoxystreptamine}}$ 1.3, 1.8, 1.5 and 1.5, respectively. The last two were identical to 2, 6-diamino-2, 3, 6-tri-deoxy-D-*ribo*-hexose.

The synthetic 3'-deoxykanamycin B showed antibacterial activity (Table 1) as strong as that of parent antibiotic, kanamycin B, and moreover showed activity against a variety of resistant bacteria. It showed strong activity against *Pseudomonas* similar to 3', 4'-dideoxykanamycin B and was more active than 3'-deoxykanamycin[1], 3', 4'-dideoxyribostamycin[3], butirosin B[8], 3', 4'-dideoxybutirosin B[9] and BB-K8[10].

* TACu forms complex with vicinal amino and hydroxyl groups when they have ~60° dihedral angle, showing approximately $\Delta[M] \pm 900°$[7], but with two hydroxyl groups, no complexing occurs. For complicated substances such as kanamycin, however, $\Delta[M]$ values often deviate from the anticipated values calculated by the above method. This will be described elsewhere. In this report we compared the $\Delta[M]$ values only among structurally similar substances.

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