Synthesis of 3′-Deoxykanamycin B (Tobramycin)[1]

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3′-Deoxykanamycin B (tobramycin) was prepared from kanamycin B. 3′,4′:4″,6″-Di-*O*-cyclohexylidene derivative (**2**) of penta-*N*-ethoxycarbonylkanamycin B was 2″-*O*-benzoylated and the 3′,4′-*O*-cyclohexylidene group was selectively and quantitatively removed by treatment with 1 mol equivalent of water in *N*,*N*-dimethylformamide (DMF) to give the de-3′,4′-*O*-cyclohexylidene derivative (**4**). Regioselective tosylation in pyridine at −20 °C gave the 3′-*O*-tosyl derivative (**5**) in 70% yield. Despite the 1,3-diaxial interaction in the transition state, replacement of the tosyl group with iodide was successfully performed in high yield with NaI in DMF. Hydrogenolysis of the iodo derivative (**6**) and subsequent removal of the protecting groups gave 3′-deoxykanamycin B (**10**) in ≈25% overall yield from kanamycin B. Confirmation of the 3′-deoxy group was performed by measurement of $\Delta[M]_{TACu}$ of 3′-deoxyneamine which was obtained from **10** by hydrolysis.

In recent papers[2-7] we synthesized a number of deoxy derivatives of aminoglycoside antibiotics and were able to establish the importance of having a 3,4-dideoxy or a 3-deoxy aminohexose moiety at the C-4 of 2-deoxystreptamine portion of aminoglycoside antibiotics to overcome inactivation by phosphorylating enzymes present in resistant bacteria. The present paper lies on the same line and describes the synthesis of 3′-deoxykanamycin B (tobramycin[8]) starting from kanamycin B.

Penta-*N*-ethoxycarbonylkanamycin B[2] (**1**) was treated with 1,1-dimethoxycyclohexane in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid to give 3′,4′:4″,6″-di-*O*-cyclohexylidene derivative (**2**). Benzoylation of **2** gave 2″-*O*-benzoyl derivative (**3**). Selective removal of the 3′,4′-*O*-cyclohexylidene group of **3** was first effected[1] by hydrolysis in methanol in the presence of catalytic amount of *p*-toluenesulfonic acid (25 °C for 24 h). In this case, a mixture of **3**, 4″,6″-*O*-cyclohexylidene (**4**), and decyclohexylidenated derivatives was obtained from which **4** was isolated in a yield of 70—80%. Further experiment showed that when a solution of **3** in DMF was treated with 1 mol equivalent of water in the presence of *p*-toluenesulfonic acid, the 3′,4′-*O*-cyclohexylidene group was selectively removed to give **4** in a yield of 96% without purification by column chromatography. Evidence for the removal of the 3′,4′-*O*-cyclohexylidene group was the fact that **4** was led to 3′-deoxykanamycin B at the end of this synthesis.

Regioselective 3′-*O*-tosylation of **4** was successfully carried out by treating **4** in cold pyridine (−20 °C) with *p*-toluenesulfonyl chloride for 9 days to give the 3′-*O*-tosyl derivative (**5**) in 70% yield.

Replacement of the tosyloxy group with iodide was carried out with 50% sodium iodide in DMF to give the 3′-iodo derivative (**6**) in 91% yield although the configuration at C-3 remains unconfirmed. Generally, the 3-sulfonyloxy group in an α-D-glucopyranoside is known to be resistant to displacement by a nucleophile owing to 1,3-diaxial type interaction[9,10] occured between axial 1-*O*-aglycon group and the nucleophile attacking the C-3 in the transition state. The successful iodination may be due to the neighboring group participation[11] of the 2-acylamino group.

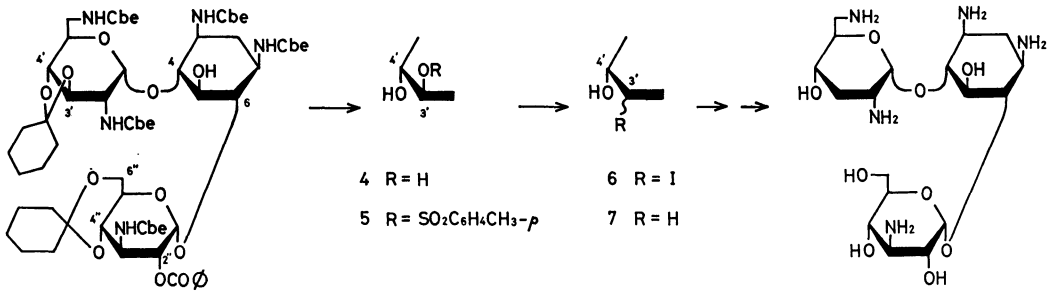
The iodo derivative (**6**) was hydrogenated with Raney nickel to give 3′-deoxy derivative (**7**) quantitatively. Hydrolysis of **7** with barium hydroxide removed its benzoyl and ethoxycarbonyl groups to give 4″,6″-*O*-cyclohexylidene-3′-deoxy derivative (**8**, major) and an unidentified compound (**9**, minor) which is probably a ureido-type compound[12] formed between N-1 and N-3 of 2-deoxystreptamine moiety judging from the mobility in electrophoresis. Finally, treatment of **8** with 50% acetic acid removed the cyclohexylidene group to afford 3′-deoxykanamycin B (**10**).

TABLE 1. THE $\Delta[M]_{TACu}$ VALUES MEASURED AT 20° C

	$\Delta[M]_{TACu}$
Paromamine	−203°[14]
3′-Deoxyparomamine[15]	+988°
Neamine	−183°
3′-*O*-Methylneamine	+730°[4b]
4′-*O*-Methylneamine	−200°[4b]
3′-Deoxyneamine[8] (**11**)	+648°

In order to confirm the structure of **10**,**10** was partially hydrolyzed with 6 M hydrochloric acid. Major components of the hydrolyzate were found to be 3′-deoxyneamine (**11**) and 3-amino-3-deoxy-D-glucose, the latter being identified, on paper chromatogram, by comparison of the R_f value and coloration with those of authentic sample. The structure of **11** was proved on the basis† of $\Delta[M]_{TACu}$[13] values (Table 1). TACu can form complex with vicinal amino and hydroxyl groups when they have ≈60° dihedral angle, showing approximately $\Delta[M]$ ±900°, the sign being decided depending on counterclockwise and clockwise rotation (measured from the front to the rear) in the Newman projection of

† Regarding the $\Delta[M]_{TACu}$ values of 3′-deoxykanamycin B (**10**) and structurally related compounds, we previously reported in a short communication,[1] and the results supported the structure of **10**. The $\Delta[M]_{TACu}$ studies on **11** and related compounds described here were performed in order to confirm the previous conclusion.



3

3'-Deoxykanamycin B (10)

Cbe : $CO_2C_2H_5$

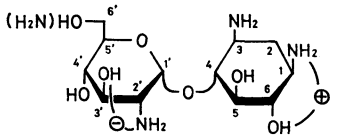


Fig. 1. The complex-forming sites and the signs for contribution to $\Delta[M]_{TACu}$ in paromamine and neamine.

$-(NH_2)CH-CH(OH)-$. TACu can not form complex with two vicinal hydroxyl groups, and, with 6-amino and 4-hydroxyl groups in a glycopyranoside structure, only small contribution in magnitude was observed.[13] As depicted in Fig. 1, paromamine or neamine can form complexes in two sites, but the two contributions are almost cancelled owing to the opposite sign of the values. In the cases of 3'-deoxyparomamine, 3'-*O*-methylneamine and 3'-deoxyneamine (**11**), the complex can be formed only at 1-NH_2 and 6-OH of the 2-deoxystreptamine portion. The value of **11** ($\Delta[M]_{TACu}$ +648°), therefore, substantiated its structure.

Antibacterial spectrum was reported[1] already with other structurally related antibiotics.

Experimental

NMR spectra were recorded at 60 and 100 MHz with Varian A-60D and HA-100 spectrometers, respectively. Infrared spectra were determined in KBr discs with Hitachi 285 grating infrared spectrophotometer. Thin-layer chromatography (TLC) was carried out on Wakogel B-5 with sulfuric acid spray for detection. For column chromatography, silica gel (Wakogel C-200) was used unless otherwise stated. Paper-chromatography (PPC) was carried out on Toyo Roshi No. 50 with 1-butanol–pyridine–water–acetic acid (6:4:3:1, Solvent A) as the developer, descending for 18 h, and colorized with 0.25% ninhydrin in pyridine. Paper electrophoresis was conducted in a Savant LT48A apparatus (Savant Instrument, Inc.) on Toyo Roshi No. 51 in formic acid–acetic acid–water (1:3:36) at 3500 V for 15 min, and after drying the paper in a hood, it was colorized as stated above.

3',4':4'',6''-Di-O-cyclohexylidene-penta-N-ethoxycarbonylkanamycin B (2). To a hot solution (≈50 °C) of **1** (522 mg) in dry DMF (2.5 ml), 1,1-dimethoxycyclohexane (0.5 ml) and anhydrous *p*-toluenesulfonic acid (26 mg) were added and the solution was heated at the temperature under reduced pressure (20—30 Torr) to remove the generated methanol by coevaporation with the solvent. After 1 h, 1,1-dimethoxycyclohexane (0.5 ml) was added and the reaction was continued for further 1 h. TLC (chloroform–ethanol 12:1) then showed two components (monocyclohexylidene derivative, R_f 0.15, minor; and **2**, R_f 0.33, major). Further reaction with additions of the above reagent did not change the ratio of the products. The solution was poured into a mixture of benzene and aqueous sodium hydrogencarbonate and the mixture was stirred vigorously for a while. After centrifugation, the powder appeared between the two layers was collected, washed with water, and dried to give a monocyclohexylidene derivative (27 mg). The benzene layer was concentrated to give a solid, which was dissolved in methanol and **2** was precipitated by addition of water, 506 mg (82%), $[\alpha]_D^{20}$ +99° (*c* 1, methanol).

Found: C, 53.50; H, 7.32; N, 6.77%. Calcd for $C_{45}H_{73}N_5O_{20}$: C, 53.83; H, 7.33; N, 6.98%.

2''-O-Benzoyl-3',4':4'',6''-di-O-cyclohexylidene-penta-N-ethoxycarbonylkanamycin B (3). A solution of **2** (4.95 g) and benzoyl chloride (3.07 g) in pyridine (60 ml) was heated at 50 °C for 1.5 h. After addition of water (0.5 g), the solution was concentrated to a half volume, and poured into aqueous sodium hydrogencarbonate. The resulting precipitate was washed with water and dried to give a solid of **3** (5.44 g, 99%), mp 152—154 °C, $[\alpha]_D^{20}$ +99° (*c* 1.4, methanol).

Found: C, 56.46; H, 6.76; N, 6.07%. Calcd for $C_{52}H_{77}N_5O_{21}$: C, 56.36; H, 7.00; N, 6.32%.

2''-O-Benzoyl-4'',6''-O-cyclohexylidene-penta-N-ethoxycarbonylkanamycin B (4). A solution of **3** (55.1 mg) in DMF (1.1 ml) containing water (0.9 mg, 1 mol equivalent for **3**) and anhydrous *p*-toluenesulfonic acid (1.9 mg, 0.2 mol equivalent for **3**) was kept at room temperature for 43 h. The solution showed on TLC with chloroform–ethanol 12:1 only one major product (R_f 0.26) (*cf.* **3**, R_f 0.45; decyclohexylidene derivative, R_f 0). After addition of 2.5% aqueous sodium hydrogencarbonate (0.1 ml) with stirring, the solution was concentrated *in vacuo* to give a solid, which was thoroughly washed with water to give a solid of **4** (49.0 mg, 96%), $[\alpha]_D^{20}$ +147° (*c* 0.6, DMF).

Found: C, 53.49; H, 6.41; N, 6.69%. Calcd for $C_{46}H_{69}N_5O_{21}$: C, 53.74; H, 6.76; N, 6.81%.

2''-O-Benzoyl-4'',6''-O-cyclohexylidene-penta-N-ethoxycarbonyl-3'-O-tosylkanamycin B (5). To a solution of **4** (106.4 mg) in dry pyridine (2 ml) at −20 °C, *p*-toluenesulfonyl chloride (99.6 mg, 5 mol equivalents for **4**) was added and the solution was kept at −20 °C for 9 days. The solution showed, on TLC (chloroform–2-propanol 10:1), three spots of R_f 0.24 (**5**, major), 0.28 (4'-*O*-tosyl isomer, trace) and 0.3 (di-*O*-tosyl isomer, trace). After addition of water (0.08 ml) followed by standing the solution at room temperature for 1 h, the solution was concentrated *in vacuo* to a syrup, which was dissolved in chloroform. The solution was washed successively with 5% aqueous potassium hydrogensulfate, water, 5% aqueous sodium hydrogencarbonate, and water, dried over $MgSO_4$,

and concentrated. The resulting solid (113.8 mg) was triturated with acetone (2 ml×2) and the acetone-soluble material (104.2 mg) was chromatographed on a column of silica gel with chloroform–2-propanol (15: 1) to give **5** (85.6 mg, 70%) and a mixture of 4′-*O*-tosyl and 3′,4′-di-*O*-tosyl isomers (18 mg). The acetone-insoluble component was found to be 4′-*O*-tosyl isomer[16] (9.6 mg). Compound **5**, mp 149—150 °C, $[\alpha]_D^{20}$ +88° (*c* 1, methanol); IR (KBr): 1720, 1530; 1175 cm^{-1} ($\nu_s SO_2$).

Found: C, 53.90; H, 6.58; N, 5.67; S, 3.00%. Calcd for $C_{53}H_{75}N_5O_{23}S$: C, 53.84; H, 6.40; N, 5.93; S, 2.71%.

NMR ($CDCl_3$) δ: 2.42 (3H, s, CH_3 of Ts).

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-3′-deoxy-penta-N-ethoxycarbonyl-3′-iodokanamycin B (**6**).* A mixture of **5** (282 mg) and sodium iodide (3 g) in DMF (6 ml) was stirred at 100 °C for 20 h. The resulting reddish brown solution, which soon solidified on cooling, was extracted with chloroform and the chloroform solution was concentrated with aid of toluene. The solid obtained was dissolved in chloroform and the solution was washed successively with saturated aqueous sodium chloride, aqueous sodium thiosulfate, and water, dried over $MgSO_4$, and concentrated to give a brown solid (247 mg, 91%). Slight impurities were removed by chromatography on a column of silica gel with chloroform–2-propanol (14: 1) to give a solid of **6** (195 mg, 72%).

Found: C, 48.75; H, 6.09; N, 6.57; I, 11.45%. Calcd for $C_{46}H_{68}N_5O_{20}I$: C, 48.55; H, 6.02; N, 6.15; I, 11.15%.

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-3′-deoxy-penta-N-ethoxycarbonylkanamycin B (**7**).* To a solution of **6** (133 mg) in dioxane (1.3 ml), Raney nickel was added and the mixture was hydrogenated (50 p.s.i.) at room temperature for 1.5 h. After filtration, the solution was treated as above with fresh Raney nickel 3 times more in every 1.5 h intervals. Concentration of the solution gave a solid of **7** (114 mg, 96%), $[\alpha]_D^{20}$ +86° (*c* 0.76, methanol).

Found: C, 54.58; H, 6.80; N, 6.84%. Calcd for $C_{46}H_{69}N_5O_{20}$: C, 54.59; H, 6.87; N, 6.92%.

*3′-Deoxykanamycin B (**10**).* A mixture of **7** (90.6 mg) and barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$, 650 mg) in decarbonated water (0.7 ml) was stirred at 100 °C for 10 h. The solution showed, on PPC, two spots of R_f 0.16 (**8**, major) and 0.22 (**9**, minor), and, on paper electrophoresis, also showed two spots (R_f of **9** is 0.79 of that of **8**). After addition of 5% aqueous sulfuric acid (2.0 ml), the mixture was stirred at 50 °C for 1.5 h. Barium sulfate precipitated was removed by filtration and it was washed with hot water thoroughly. The filtrate and washings were combined, concentrated, and, to the concentrate, acetone was added to give a pale brown solid. The crude **8** (64.3 mg) was dissolved in 50% acetic acid (0.65 ml) and the solution was heated at 80 °C for 3 h. The solution was concentrated and then concentrated with additions of water to give a pale brown solid (62 mg). The aqueous solution of the solid was subjected to column chromatography of Dowex 1X2 (OH form, 1.1 ml) resin with water and from the eluate, colorless solid (free base, 37.5 mg) was obtained. The solid was chromatographed on a column of CM-Sephadex C-25 (NH_4 form) with 0—0.15 M ammonia, the concentration being linearly increased. From the faster moving fractions, an unidentified compound (9.3 mg, a ureido-type compound?) was obtained, and from the slower moving fractions, 3′-deoxykanamycin B base (**10**) was isolated as a monohydrate, 28.5 mg (65%), $[\alpha]_D^{20}$ +129° (*c* 1, water) (lit,[8] +128°). $R_{f\ \text{kanamycin B}}$ 1.25 (PPC, by solvent A).

Found: C, 44.92; H, 8.09; N, 14.61%. Calcd for $C_{18}H_{37}N_5O_9 \cdot H_2O$: C, 44.53; H, 8.10; N, 14.43%.

NMR (D_2O) δ: 1.1—2.3 (4H m, H-2 and 3′).

*3′-Deoxyneamine (**11**).* Compound **10** (100 mg as base) was dissolved in 6 M hydrochloric acid (2 ml) and the solution was heated at 100 °C for 2 h. The solution showed, on PPC, five spots of R_f 0.3 (3-amino-3-deoxy-D-glucose), 0.16 (trace, 2,6-diamino-2,3,6-trideoxy-D-ribohexose), 0.12 (trace, 2-deoxystreptamine), 0.06 (**11**), and 0.03 (trace, **10**). After concentration, the resulting syrup was chromatographed on a column of Dowex 1X2 (OH form) resin with water. The free sugars were retained in the column. The eluate was concentrated and the resulting syrup was chromatographed on a column of CM-Sephadex C-25 (NH_4 form) with 0—0.15 M ammonia, the concentration being linearly increased. 2-Deoxystreptamine (0.5 mg), **10** (6.1 mg) and **11** (54 mg, 78%) were eluted in the order of mobility. 3′-Deoxyneamine (**11**), $[\alpha]_D^{20}$ +122° (*c* 0.3, water); $[\alpha]_{436}^{20}$ +230° (*c* 0.3, water), $[\alpha]_{436}^{20}$ +430° (*c* 0.3, TACu[13]); $\Delta[M]_{TACu}$ is +648°.

Found: C, 44.39; H, 8.30; N, 17.26%. Calcd for $C_{12}H_{26}N_4O_5 \cdot H_2O$: C, 44.43; H, 8.70; N, 17.27%.

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