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A SYNTHESIS OF 3′,4′-DIDEOXYKANAMYCIN B*

TOSHIAKI MIYAKE,
Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Yokohama 223 (Japan)

TSUTOMU TSUCHIYA, SUMIO UMEZAWA, AND HAMAO UMEZAWA
Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki 211 (Japan)
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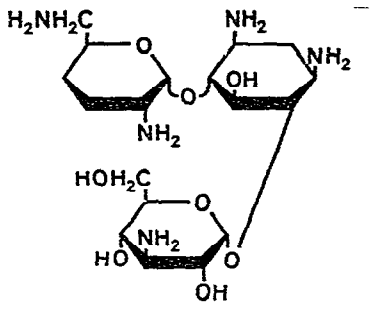
ABSTRACT

3′,4′-Dideoxykanamycin B, the kanamycin B derivative that is active against resistant bacteria, was prepared from kanamycin B *via* *N*-tosylation, 3′,4′-*O*-sulphonylation, 3′,4′-unsaturation, and hydrogenation. The unsaturated intermediate was obtained from the 3′,4′-di-*O*-sulphonyl derivatives by the action of sodium iodide in *N*,*N*-dimethylformamide; if zinc dust was added in this reaction, aziridine derivatives were formed, Removal of the tosyl group was successfully performed by using sodium in ammonia–ethylamine.

INTRODUCTION

Enzymic mechanisms of resistance to aminoglycoside antibiotics have been clarified by Umezawa *et al.*[1]. Resistant organisms produce intracellular enzymes that transfer phosphate from adenosine triphosphate to the hydroxyl groups, or transfer acetyl group to the amino groups of the antibiotics. The most-common mechanism of resistance to kanamycins is transphosphorylation to the 3′-hydroxyl group. In order to overcome the resistance, we have synthesized the kanamycin derivatives in which HO-3′ is removed with or without removal of HO-4′. The resulting 3′-deoxykanamycin[2] and 3′,4′-dideoxykanamycin B[3,11] inhibited growth of resistant *Staphylococci* and resistant Gram-negative bacteria. Moreover, these compounds inhibit *Pseudomonas aeruginosa*, which is also resistant to kanamycins. 3′,4′-Dideoxykanamycin B (dibekacin) showed a strong activity in inhibiting growth of bacteria, and its usefulness in the chemotherapy of resistant infections has been confirmed by a large-scale clinical study. Consequently, we have studied the improvement of the reported synthesis[3]. We now report on a new synthesis of 3′,4′-dideoxykanamycin B.

*Dedicated to the memory of Professor Edward J. Bourne.



3',4'-Dideoxykanamycin B

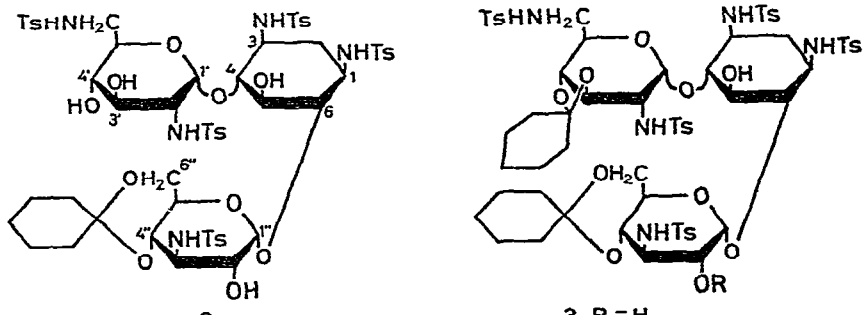
The present synthesis involves a new modification of the Tipson–Cohen reaction[4], and the protection of amino groups was improved by use of tosyl groups. *N*-Tosyl groups are readily removed by treatment with sodium in liquid ammonia–ethylamine without ureide formation[5] at N-1 and N-3 of the 2-deoxystreptamine, which occurs with the urethane-type of amino-group protection and decreases the yield of 3',4'-dideoxykanamycin B. Moreover, the sequence of reactions was shorter than that of the previous synthesis[3], and a higher overall yield was obtained.

RESULTS AND DISCUSSION

The synthesis began with the protection of the amino groups of kanamycin B with tosyl chloride. Penta-*N*-tosylkanamycin B (**1**) was prepared in good yield and is soluble in most organic solvents. *N*-Methanesulphonylation and *N*-benzylsulphonylation were also attempted, but gave poor yields of products.

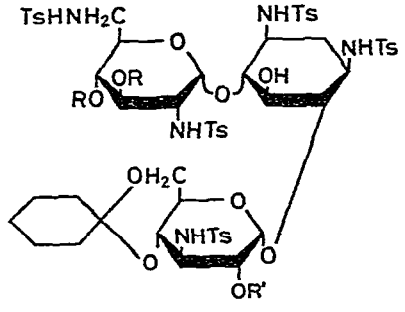
To obtain 3',4'-disulphonyl derivatives of **1**, which are the precursors for 3',4'-unsaturated derivatives, the 4'' and 6''-hydroxyl groups were selectively protected. This was achieved by the use of 1,1-dimethoxycyclohexane[6]. Condensation of **1** with the ketal reagent (~3 mol.) proceeded smoothly in *N*,*N*-dimethylformamide in the presence of an acid catalyst under reduced pressure and gave a mixture of 4'',6''-*O* (**2**) and 3',4';4'',6''-di-*O*-cyclohexylidene (**3**) derivatives. However, addition of water to the reaction mixture selectively removed the 3',4'-cyclohexylidene group to give **2** almost quantitatively. If the amount of the ketal reagent was decreased to avoid the formation of **3**, a mixture of 3',4'- and 4'',6''-*O*-cyclohexylidene derivatives was formed (established by tritylation). Compound **3** was obtained by the use of an excess of the reagent.

Treatment of **2** with 3 equivalents of benzylsulphonyl chloride in pyridine gave mainly the di-*O*-sulphonyl derivative **4**, together with some tri-*O*-sulphonyl derivative **5**. It should be noted that, in this case, the 2''-hydroxyl group is less reactive to sulphonylation and that the 3'- and 4'-hydroxyl groups are selectively sulphonylated. This selective sulphonylation was of great convenience to us. The location of the sulphonyl groups in **4** at C-3' and C-4' was suggested by the subsequent generation of 3',4'-unsaturation, although the n.m.r. spectra of the unsaturated derivatives (**10–12**)



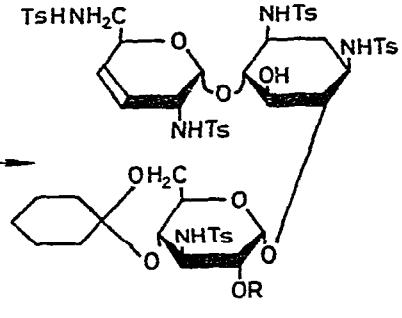
2

3 R = H
8 R = $SO_2CH_2C_6H_5$
9 R = SO_2CH_3



4 R = $SO_2CH_2C_6H_5$ R' = H
5 R = 〃 R' = $SO_2CH_2C_6H_5$
6 R = 〃 R' = SO_2CH_3
7 R = SO_2CH_3 R' = 〃

a

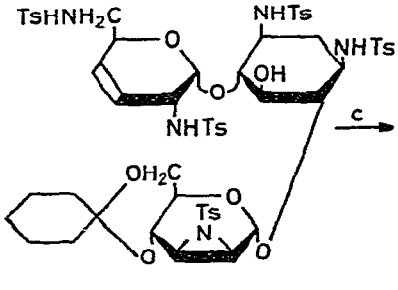


10 R = H 11 R = $SO_2CH_2C_6H_5$ 12 R = SO_2CH_3

c → 15 (R = H), 16 (R = $SO_2CH_2C_6H_5$), 17 (SO_2CH_3)

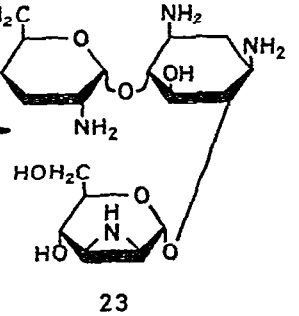
d → 19 (R = H), 20 ($SO_2CH_2C_6H_5$), 21 (SO_2CH_3)

b



13

c → 18 → d → 22 → e



23

a: NaI-DMF, b: Zn-NaI-DMF, c: H_2/Pt, d: 80% AcOH, e: Na-NH_3-$EtNH_2$

derived from **4** and related compounds did not reveal the alkene positions with certainty. Treatment of the 3′,4′-di-*O*-benzylsulphonyl derivative **4** with methanesulphonyl chloride gave the 2″-*O*-methanesulphonyl derivative **6**. In contrast to benzylsulphonylation, methanesulphonylation of **2** was not so selective, as judged by t.l.c. of the reaction mixture. The major product was the 3′,4′,2″-tri-*O*-methanesulphonyl derivative **7**. Benzylsulphonylation and methanesulphonylation of the di-*O*-cyclohexylidene derivative **3** gave the 2″-*O*-benzylsulphonyl (**8**) and 2″-*O*-methanesulphonyl (**9**) derivatives, in good yields.

The generation of 3′,4′ unsaturation was next studied. In the syntheses of 3′,4′-unsaturated derivatives of neamine, kanamycin B, and ribostamycin, we used a mixture of zinc dust and a highly concentrated solution of sodium iodide in *N*,*N*-dimethylformamide (Zn–NaI reagent in the Tipson–Cohen procedure). When this procedure was applied to **4**, **5**, **6**, and **7**, it resulted in the formation of 2″,3″-aziridine derivatives, except for **4**. The epimino structures were confirmed by the following data. (*1*) Compounds **5**, **6**, and **7** were each converted by the Zn–NaI reagent into the *N*-tosylaziridine **13**, which has no sulphonic ester group. (*2*) The di-*O*-cyclohexylidene-2″-*O*-sulphonyl derivatives **8** and **9** also gave the same *N*-tosylaziridine **14**, which has no sulphonic ester group. (*3*) Treatment of **8** and **9** with 0.5M sodium methoxide in methanol also gave compound **14**. (*4*) The 2″-*O*-methanesulphonyl-3′,4′-olefin **12**, described later, was converted into **13**. These results indicated that the 2″-sulphonyloxy groups of these compounds were eliminated. (*5*) In the n.m.r. spectra of **13**, **14**, **18**, and **19**, a slightly broadened, 2-proton singlet appeared at δ 2.75–3.0. The signal pattern indicates that the vicinal couplings are small. The signals are also characteristic for these four compounds, and no such peaks were observed in the spectra of the related compounds **10**, **11**, **12**, **15**, **16**, and **17**. Thus, these signals are reasonably assigned to two *N*-tosylepimino protons at C-2″ and 3″. (*6*) The final sugar derivative (**23**) derived from **13** gave signals characteristic of aziridine[9].

In order to avoid aziridine formation, the zinc dust was omitted from the Zn–NaI reagent. In this modification, use of sodium iodide–*N*,*N*-dimethylformamide effected the transformations **4**→**10**, **5**→**11**, **6**→**12**, and **7**→**12** with concomitant liberation of iodine. The 3′,4′-olefins were obtained in high yields without the formation of aziridines.

Hydrogenation of the olefins **10–13** with platinum oxide afforded the 3′,4′-dideoxy derivatives **15–18**, respectively. Hydrolysis with 80% aqueous acetic acid then removed the cyclohexylidene groups to give **19–22**, respectively. Finally, the remaining *N*- and *O*-sulphonyl groups of **19–22** were removed by treatment with sodium in liquid ammonia or in liquid ammonia–ethylamine. Reaction at −50° in ~3:1 liquid ammonia–ethylamine gave the best yields (~90%). The overall yield of 3′,4′-dideoxykanamycin B from kanamycin B is ~35%.

The characteristic feature of this reaction is that the 2″-*O*-methanesulphonyl and 2″-*O*-benzylsulphonyl groups are readily removed without formation of 2″,3″-aziridine. Nucleophiles usually react with vicinal *trans* acylamino and sulphonyloxy groups to give an aziridine or oxazoline, owing to neighboring-acylamino partici-

pation[10]. The physical constants, n.m.r. spectrum[3], and antibacterial activity[11] of 3′,4′-dideoxykanamycin B synthesized by the improved route were identical to those previously reported.

The final epimino derivative **23** (as the free base in deuterium oxide) showed no clear n.m.r. signals assignable to aziridine. However, introduction of carbon dioxide into the solution resulted in the appearance of a clear AB quartet at $\delta \sim 2.3$ ($J_{A,B}$ 6.5 Hz) without overlapping with other signals. This indicates that $J_{1,2} = J_{3,4} = 0$ and establishes the 2,3-epimino-α-D-mannopyranoside structure[9] of **23**. Compound **23** exhibited antibacterial activity of about 1/30 of that of 3′,4′-dideoxykanamycin B.

EXPERIMENTAL

General. — Melting points were determined on a Kofler block and are uncorrected. Specific rotations were measured, in a 0.1-dm tube, with a Perkin–Elmer Model 241 polarimeter. Infrared spectra were recorded, for potassium bromide pellets, with a Hitachi Model 285 grating spectrophotometer. N.m.r. spectra were recorded at 60 and 100 MHz with Hitachi R-24A and Varian HA-100 spectrometers, respectively. Thin-layer chromatography (t.l.c.) was performed on Wakogel B-5, unless otherwise stated, with sulfuric acid spray for detection. For column chromatography, silica gel (Wakogel C-200) was used with Toyo SF-160K and SF-100P automatic fraction-collectors.

Penta-N-*tosylkanamycin B* (**1**). — To a suspension of kanamycin B (free base, 5.4 g, 11.2 mmol) and anhydrous sodium carbonate (6.7 g) in ice-cold 1:2 aqueous *p*-dioxane (160 ml), tosyl chloride (13 g, 68.2 mmol) was added and the mixture was stirred for 10 h in the cold. Concentration of the reaction mixture gave a residue, which was shaken with water, and the insoluble product was filtered off and washed throughly with water. The crude product (13.8 g) was chromatographed on a short column of silica gel with 10:1 chloroform–ethanol to remove slight impurities. Fractions containing **1** were evaporated to a solid (10.2 g, 73%). After reprecipitation from chloroform–ethanol–ether, **1** had m.p. 164° (dec.), $[\alpha]_D^{25}$ +22.4° (*c* 1.0, *N*,*N*-dimethylformamide); ν_{max} 1310 and 1150 cm^{-1} (SO_2).

Anal. Calc. for $C_{53}H_{67}N_5O_{20}S_5$: C, 50.75; H, 5.38; N, 5.58; S, 12.78. Found: C, 50.97; H, 5.42; N, 5.63; S, 12.68.

4″,6″-O-*Cyclohexylidenepenta*-N-*tosylkanamycin B* (**2**). — To a solution of **1** (1.70 g, 1.36 mmol) in dry *N*,*N*-dimethylformamide (17 ml), anhydrous *p*-toluenesulphonic acid (52.5 mg) and 1,1-dimethoxycyclohexane (0.6 g, 4.16 mmol) were added, and the solution was treated at 50°/30 torr for 30 min, during which time 2–3 ml of liquid were evaporated. T.l.c. (1:3 benzene–ethyl acetate) then showed 2 components (**2**, R_F 0.4; and **3**, R_F 0.55) in ~2:1 ratio by color strength. Water (0.01 ml, 0.56 mmol, as a 10% solution in *N*,*N*-dimethylformamide) was added and the solution was kept at room temperature overnight. Saturated, aqueous sodium hydrogen carbonate (10 ml) was added with vigorous stirring and the mixture was concentrated. The resulting solid was stirred vigorously for 30 min with water (50 ml),

and was then washed with water and dried to give chromatographically homogeneous **2** (1.80 g, 99%), m.p. 175–176° (dec.), $[\alpha]_D^{25}$ +13° (*c* 1.0, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{59}H_{75}N_5O_{20}S_5$: C, 53.10; H, 5.66; N, 5.25; S, 12.01. Found: C, 52.73; H, 5.51; N, 4.83; S, 11.68.

3',4';4",6"-Di-O-cyclohexylidenepenta-N-tosylkanamycin B (**3**). — To a solution of **1** (503 mg, 0.40 mmol) in dry *N*,*N*-dimethylformamide (5 ml), anhydrous *p*-toluenesulphonic acid (15 mg) and 1,1-dimethoxycyclohexane (0.3 g, 2.08 mmol) were added, and the solution was treated at 50°/30 torr. After 2 h, more ketal (0.3 g) was added and the solution was treated as above for 1 h. The procedure was repeated until **3** became the major product; a total of 1.2 g of the ketal was needed. Subsequent purification was carried out as described for **2**. The crude product (568 mg) was chromatographed over silica gel with 2:3 benzene–ethyl acetate to give **3** (543 mg, 96%), m.p. 256–257°, $[\alpha]_D^{22}$ +10.3° (*c* 1.0, chloroform).

Anal. Calc. for $C_{65}H_{83}N_5O_{20}S_5$: C, 55.19; H, 5.91; N, 4.95; S, 11.33. Found: C, 54.83; H, 5.82; N, 4.71; S, 11.30.

3',4'-Di-O-benzylsulphonyl- (**4**) *and 3',4',2"-tri-O-benzylsulphonyl-4",6"-O-cyclohexylidenepenta-N-tosylkanamycin B* (**5**). — To a solution of **2** (1.76 g, 1.32 mmol) in dry pyridine (36 ml), benzylsulphonyl chloride (505 mg, 2.65 mmol) was added and the solution was kept at −3 to −5° for 2 h. More benzylsulphonyl chloride (253 mg) was added, and the solution was kept again at −3 to −5° for 1 h and then at 3° overnight. The resulting solution contained (t.l.c.; 15:1 chloroform–2-propanol) major (R_F 0.25), minor (R_F 0.35), and trace (R_F 0.2) components. After addition of water (~0.4 ml), the solution was concentrated to a brown syrup, which was dissolved in chloroform (120 ml). The solution was washed successively with 10% aqueous potassium hydrogen sulfate, water, 5% aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and concentrated to a brown solid that was then chromatographed over silica gel (140 g) with 3:2 benzene–ethyl acetate. From the earlier fractions, a pale-brown solid (473 mg) was obtained; this still contained (t.l.c.; 2:1 benzene–ethyl acetate) a trace impurity (R_F 0.5; *cf.* **5** R_F 0.6). Column chromatography over silica gel (50 g) with 3:1 benzene–ethyl acetate gave chromatographically homogeneous **5** (252 mg, 11%), m.p. 163–164° (dec.), $[\alpha]_D^{25}$ +20° (*c* 1.0, chloroform).

Anal. Calc. for $C_{80}H_{93}N_5O_{26}S_8$: C, 53.47; H, 5.22; N, 3.90; S, 14.27. Found: C, 53.20; H, 5.20; N, 3.68; S, 13.90.

From the later fractions, **4** was obtained as a chromatographically homogeneous, colorless solid (1.31 g, 61%), m.p. 169–170° (dec.), $[\alpha]_D^{25}$ 0° (*c* 1.0, chloroform).

Anal. Calc. for $C_{73}H_{87}N_5O_{24}S_7$: C, 53.37; H, 5.34; N, 4.26; S, 13.66. Found: C, 53.11; H, 5.25; N, 4.24; S, 13.50.

3',4'-Di-O-benzylsulphonyl-4",6"-O-cyclohexylidene-2"-O-methanesulphonylpenta-N-tosylkanamycin B (**6**). — To a solution of **4** (1.04 g, 0.63 mmol) in pyridine (20 ml), methanesulphonyl chloride (0.15 ml, 1.93 mmol) was added, and the solution was kept at room temperature overnight and then worked-up as described for **4**. Separation of **6** from the unreacted starting material was carried out by column chromatography (2:1 benzene–ethyl acetate) to give **6** (913 mg, 84%), m.p. 168–169°

(dec.), $[\alpha]_D^{22}$ +11.2° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$): δ 0.9–1.8 (~11 H), 2.35 (s, 9 H, 3 PhCH_3), 2.45 (s, 6 H, 2 PhCH_3), 3.35 (s, 3 H, CH_3SO_2).

Anal. Calc. for $C_{74}H_{89}N_5O_{26}S_8$: C, 51.64; H, 5.21; N, 4.07; S, 14.90. Found: C, 51.54; H, 5.27; N, 3.84; S, 14.70.

4″,6″-O-Cyclohexylidene-3′,4′,2″-tri-O-methanesulphonylpenta-N-tosylkanamycin B (7). — To a solution of **2** (4.10 g, 3.1 mmol) in pyridine, methanesulphonyl chloride (1.2 ml, 15.5 mmol) was added and the solution was kept at room temperature for 3 h and then at 60° overnight. Methanesulphonyl chloride (0.6 ml) was added and the solution was heated for a further 3 h. Water (0.5 ml) was added and the reaction mixture was worked-up as described for **4** to give a mixture of crude products, which were fractionated on a column of silica gel (230 g) with 30:1 chloroform–2-propanol. Compound **7** was obtained as a pale-brown solid (2.31 g, 48%) that was decolorized with charcoal in 1:1 *p*-dioxane–methanol, and reprecipitated from *p*-dioxane–water to give a colorless solid, m.p. 182° (dec.), $[\alpha]_D^{22}$ +13° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$): δ 2.4 (s, 15 H, 5 PhCH_3), 3.02, 3.13, and 3.35 (3 s, 9 H, 3 CH_3SO_2).

Anal. Calc. for $C_{62}H_{81}N_5O_{26}S_8$: C, 47.47; H, 5.20; N, 4.46; S, 16.35. Found: C, 47.37; H, 5.25; N, 4.24; S, 16.39.

2″-O-Benzylsulphonyl-3′,4′;4″,6″-di-O-cyclohexylidenepenta-N-tosylkanamycin B (8). — A solution of **3** (201 mg, 0.14 mmol) and benzylsulphonyl chloride (84 mg, 0.44 mmol) in pyridine (4 ml) was kept at −3 to −5° overnight. The solution then contained (t.l.c.; 2:1 benzene–ethyl acetate) one major product (R_F 0.65). Treatment of the solution in the usual manner, as described for **4**, gave a slightly brown solid (251 mg), which was chromatographed on a short column of silica gel with 4:1 benzene–ethyl acetate to give **8** (178 mg, 80%), m.p. 160–161° (dec.), $[\alpha]_D^{25}$ +18° (*c* 1.0, chloroform).

Anal. Calc. for $C_{72}H_{89}N_5O_{22}S_6$: C, 55.12; H, 5.72; N, 4.46; S, 12.26. Found: C, 54.83; H, 5.60; N, 4.24; S, 12.10.

3′,4′;4″,6″-Di-O-cyclohexylidene-2″-O-methanesulphonylpenta-N-tosylkanamycin B (9). — Compound **3** (1 equivalent) was conventionally treated with 4.4 equivalents of methanesulphonyl chloride in pyridine at room temperature overnight, yielding **9** (95%) as a colorless solid (without purification by column chromatography), m.p. 160–161° (dec.), $[\alpha]_D^{25}$ +21° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$): δ 3.22 (s, 3 H, CH_3SO_2).

Anal. Calc. for $C_{66}H_{85}N_5O_{22}S_6$: C, 53.10; H, 5.74; N, 4.69; S, 12.89. Found: C, 52.71; H, 5.55; N, 4.40; S, 13.06.

The reaction of **4**, **5**, **6**, *and* **7** *with sodium iodide.* — A mixture of the 3′,4′-di-*O*-sulphonyl derivative (**4**, **5**, **6**, or **7**; 400 mg, 0.2–0.25 mmol) and sodium iodide (4.0 g) in *N*,*N*-dimethylformamide (8 ml) was stirred at 100°. Dissolution soon occurred, and iodine began to be liberated. After 15 min (for **4**, **5**, and **6**) or 30 min (for **7**), the reaction mixture contained (t.l.c.; 1–2:1 benzene–ethyl acetate or 15–25:1 chloroform–2-propanol) a single product and no starting material. To the resulting reddish violet solution, which soon solidified on cooling, chloroform (30 ml) was added while hot, and, after vigorous stirring, the solvents were evaporated and

further coevaporated with toluene. The residue was then shaken with chloroform (50 ml) and 5% aqueous sodium thiosulfate (10 ml), the colorless chloroform solution was washed with water and dried ($MgSO_4$), and the solvent was evaporated. The solid residue was reprecipitated from *p*-dioxane–water to afford a colorless solid of **10**, **11**, or **12**, described below.

4″,6″-*O*-Cyclohexylidene-3′,4′-dideoxy-3′-eno-penta-*N*-tosylkanamycin B (**10**, 92%), m.p. 168–169°, $[\alpha]_D^{25}$ −14° (*c* 1.0, *N*,*N*-dimethylformamide); i.r. peaks ascribable to unsaturation (~1650 cm^{-1}) were very weak.

Anal. Calc. for $C_{59}H_{73}N_5O_{18}S_5$: C, 54.49; H, 5.66; N, 5.39; S, 12.33. Found: C, 54.55; H, 5.59; N, 5.43; S, 12.07.

2″-*O*-Benzylsulphonyl-4″,6″-*O*-cyclohexylidene-3′,4′-dideoxy-3′-eno-penta-*N*-tosylkanamycin B (**11**, 93%), m.p. 156–157° (dec.), $[\alpha]_D^{23}$ −15° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 0.8–1.7 [11 H, $(CH_2)_5$ and H-2ax), 2.33 (s, 9 H, 3 PhCH_3), 2.42 (s, 6 H, 2 PhCH_3); there were signals for 6 H between 4.5–5.5; 4.6 (2 H, SO_2CH_2Ph?), 4.75 (1 H, anomeric?), 5.0 (2 H, H-3′,4′?), 5.45 (1 H, anomeric?).

Anal. Calc. for $C_{66}H_{79}N_5O_{20}S_6$: C, 54.49; H, 5.47; N, 4.81; S, 13.22. Found: C, 54.17; H, 5.49; N, 4.50; S, 12.95.

4″,6″-*O*-Cyclohexylidene-3′,4′-dideoxy-3′-eno-2″-*O*-methanesulphonylpenta-*N*-tosylkanamycin B (**12**; 94% from **6**, 92% from **7**), m.p. 158° (dec.), $[\alpha]_D^{22}$ −5.4° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 0.8–1.7 (11 H), 2.39 (s, 9 H), 2.45 (s, 6 H), 3.32 (s, 3 H, CH_3SO_2); there were signals for 4 H between 4.5–5.6; 4.65 (1 H, anomeric?), 5.15 (2 H, H-3′,4′?), 5.45 (1 H, anomeric?).

Anal. Calc. for $C_{60}H_{75}N_5O_{20}S_6$: C, 52.27; H, 5.48; N, 5.08; S, 13.95. Found: C, 52.26; H, 5.48; N, 4.91; S, 13.54.

6-O-(*4,6*-O-*Cyclohexylidene-2,3-epimino*-N-*tosyl*-α-D-*mannopyranosyl*)-*2-deoxy-4*-O-(*2,3,4,6-tetradeoxy-2,6-ditosylamino*-α-D-erythro-*hex-3-enopyranosyl*)-*1,3-di*-N-*tosylstreptamine* (**13**). — A mixture of **7** (2.25 g), sodium iodide (22.5 g), and zinc dust (11 g) in *N*,*N*-dimethylformamide (45 ml) was stirred at 80° for 1 h, and then processed as described for **10**. The crude product (1.76 g) was chromatographed on a column of silica gel (85 g) with 25:1 chloroform–2-propanol to give **13** as a a colorless solid (1.48 g, 80%). When reprecipitated from *p*-dioxane–water, **13** had m.p. 149–150° (dec.), $[\alpha]_D^{22}$ +14.5° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 1.0–2.0 (11 H, $(CH_2)_5$ and H-2ax), 2.38 (s, 15 H, 5 PhCH_3), 2.85 (s, 2 H, H-2″,3″); there were signals for 4 H between 4.3–5.5; 4.45 (1 H, anomeric?), 4.9 (2 H, H-3′,4′?), 5.43 (1 H, anomeric?).

Anal. Calc. for $C_{59}H_{71}N_5O_{17}S_5$: C, 55.25; H, 5.58; N, 5.46; S, 12.50. Found: C, 54.93; H, 5.55; N, 5.45; S, 12.09.

4-O-(*3,4*-O-*Cyclohexylidene-2,6-dideoxy-2,6-ditosylamino*-α-D-*glucopyranosyl*)-*6*-O-(*4,6*-O-*cyclohexylidene-2,3-epimino*-N-*tosyl*-α-D-*mannopyranosyl*)-*2-deoxy-1,3-di*-N-*tosylstreptamine* (**14**). — A mixture of **8** (34.9 mg), sodium iodide (300 mg), and zinc dust (150 mg) in *N*,*N*-dimethylformamide (0.6 ml) was stirred at 80° for 25 min. The reaction mixture then contained (t.l.c.; 2:1 benzene–ethyl acetate) a single product (R_F 0.4), and **8** (R_F 0.6) had disappeared completely. The mixture was then

processed as described for **10** to give **14** as a pale-brown solid (26.0 mg, 84%), m.p. 146–147° (dec.), $[\alpha]_D^{25}$ +21° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 1.1–1.8 (~21 H), 2.38 (s, 9 H, 3 PhCH_3), 2.41 (s, 6 H, 2 PhCH_3), 2.79 (s, 2 H, H-2″,3″), 5.05 (d, 1 H, anomeric), 5.27 (d, 1 H, anomeric).

Anal. Calc. for $C_{65}H_{81}N_5O_{19}S_5$: C, 55.90; H, 5.85; N, 5.01; S, 11.48. Found: C, 55.84; H, 5.83; N, 5.14; S, 11.24.

*4″,6″-O-Cyclohexylidene-3′,4′-dideoxypenta-*N*-tosylkanamycin B* (**15**). — To a solution of **10** (147 mg) in 3:1 ethyl acetate–*p*-dioxane (2 ml), platinum oxide (30 mg) was added and the mixture was hydrogenated (50 p.s.i.) at room temperature for 1 h. The resulting suspension (**15** was only partially soluble in the reaction solvent) was concentrated and the residue was dissolved in hot *p*-dioxane. The solution contained (t.l.c.; 10:1 chloroform–2-propanol) a single product (R_F 0.25), and **10** (R_F 0.22) had disappeared. After filtration, the solution was concentrated. Addition of water gave a solid of **15** (124 mg, 84%), m.p. 155–156° (dec.), $[\alpha]_D^{23}$ 0° (*c* 0.4, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{59}H_{75}N_5O_{18}S_5$: C, 54.40; H, 5.80; N, 5.38; S, 12.31. Found: C, 54.65; H, 5.87; N, 5.04; S, 11.90.

*2″-O-Benzylsulphonyl-4″,6″-O-cyclohexylidene-3′,4′-dideoxypenta-*N*-tosylkanamycin B* (**16**). — Hydrogenation of **11**, as described for **10**, gave **16** (98%), m.p. 156–157° (dec.), $[\alpha]_D^{22}$ +16° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 0.9–2.1 [16 H; $(CH_2)_5$, H-3′,4′, and H-2], 2.35 (s, 9 H, 3 PhCH_3), 2.44 (s, 6 H, 2 PhCH_3); there were signals for 4 H between 4.5–5.5; 4.68 (1 H, anomeric), 4.8 (2 H, SO_2CH_2Ph), 5.1 (1 H, anomeric).

Anal. Calc. for $C_{66}H_{81}N_5O_{20}S_6$: C, 54.42; H, 5.60; N, 4.81; S, 13.20. Found: C, 54.56; H, 5.57; N, 4.69; S, 13.01.

*4″,6″-O-Cyclohexylidene-3′,4′-dideoxy-2″-O-methanesulphonylpenta-*N*-tosylkanamycin B* (**17**). — Hydrogenation of **12**, as described for **10**, gave **17** (96%), m.p. 160–161° (dec.), $[\alpha]_D^{22}$ +17.1° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 0.8–1.8 (15 H), 2.39 (s, 9 H, 3 PhCH_3), 2.45 (s, 6 H, 2 PhCH_3), 3.32 (s, 3 H, CH_3SO_2).

Anal. Calc. for $C_{60}H_{77}N_5O_{20}S_6$: C, 52.20; H, 5.62; N, 5.07; S, 13.93. Found: C, 51.91; H, 5.61; N, 4.82; S, 13.53.

*6-O-(4,6-O-Cyclohexylidene-2,3-epimino-*N*-tosyl-α-*D*-mannopyranosyl)-2-deoxy-4-O-(2,3,4,6-tetradeoxy-2,6-ditosylamino-α-*D*-*erythro*-hexopyranosyl)-1,3-di-*N*-tosylstreptamine* (**18**). — Hydrogenation of **13**, as described for **10**, gave **18** (85%), m.p. 150–151° (dec.), $[\alpha]_D^{22}$ +36° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 1.0–2.1 (16 H), 2.38 (s, 15 H, 5 PhCH_3), 2.82 (s, 2 H, H-2″,3″), 4.74 (1 H, anomeric), 5.05 (1 H, anomeric).

Anal. Calc. for $C_{59}H_{73}N_5O_{17}S_5$: C, 55.17; H, 5.73; N, 5.45; S, 12.48. Found: C, 55.32; H, 5.76; N, 5.27; S, 12.26.

*3′,4′-Dideoxypenta-*N*-tosylkanamycin B* (**19**). — A suspension of **15** (39.0 mg) in 80% aqueous acetic acid (1.5 ml) was heated at 80° for 1 h and then filtered, and

the solvent was removed by coevaporation with toluene to give **19** as a colorless solid (31 mg, 85%), m.p. 146–147° (dec.), $[\alpha]_D^{25}$ +28° (*c* 0.1, chloroform).

Anal. Calc. for $C_{53}H_{67}N_5O_{18}S_5 \cdot H_2O$: C, 51.32; H, 5.61; N, 5.65; S, 12.92. Found: C, 51.49; H, 5.58; N, 5.48; S, 12.87.

*2″-O-Benzylsulphonyl-3′,4′-dideoxypenta-*N*-tosylkanamycin B* (**20**). — Compound **16** was treated as described above for **15** to give **20** (93%), m.p. 149–150°, $[\alpha]_D^{25}$ +46° (*c* 1.0, chloroform).

Anal. Calc. for $C_{60}H_{73}N_5O_{20}S_6$: C, 52.35; H, 5.34; N, 5.09; S, 13.97. Found: C, 52.24; H, 5.29; N, 4.97; S, 13.86.

*3′,4′-Dideoxy-2″-O-methanesulphonylpenta-*N*-tosylkanamycin B* (**21**). — Compound **17** was treated as described above for **15** to give **21** (96%), m.p. 144–145° (dec.), $[\alpha]_D^{25}$ +46° (*c* 1.0, chloroform).

Anal. Calc. for $C_{54}H_{69}N_5O_{20}S_6 \cdot H_2O$: C, 49.19; H, 5.43; N, 5.31; S, 14.59. Found: C, 49.24; H, 5.31; N, 4.95; S, 14.35.

*2-Deoxy-6-*O*-(2,3-epimino-*N*-tosyl-α-*D*-mannopyranosyl)-4-*O*-(2,3,4,6-tetradeoxy-2,6-ditosylamino-α-*D*-*erythro*-hexopyranosyl)-1,3-di-*N*-tosylstreptamine* (**22**). — Compound **18** was treated as described above for **15** to give **22** (95%), m.p. 147–148° (dec.), $[\alpha]_D^{22}$ +40° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$–D_2O): δ 1.0–1.9 (5 H, H-3′,4′ and H-2ax), 2.34 (s, 15 H, 5 PhCH_3), 2.95 (s, 2 H, H-2″,3″), 4.90 (1 H, anomeric, H-1′?), 5.32 (1 H, anomeric, H-1″?).

Anal. Calc. for $C_{53}H_{65}N_5O_{17}S_5$: C, 52.85; H, 5.44; N, 5.82; S, 13.31. Found: C, 52.45; H, 5.35; N, 5.35; S. 13.30.

3′,4′-Dideoxykanamycin B. — (*a*) *From* **21**. To a solution of **21** (88.4 mg) in ~3:1 liquid ammonia–ethylamine (40 ml) at −60° (Haake constant temperature circulator KS60W), sodium metal (~160 mg) was added with stirring, and the temperature was raised gradually to −50°, within 10 min, with stirring. The sodium metal liquefied, and floated on the surface of the solution as a brown, thin film. After 1.2 h, the dark blue color of the solution still remained. Methanol (0.6 ml) was added to decompose the excess sodium and the solution was evaporated under a slight vacuum. The residual, colorless solid was dissolved in water (30 ml) and Dowex-50Wx2 (H^+) resin (200–400 mesh, 20 ml) was added to the solution. After agitation, the resin was poured into a column prepacked with the same resin (NH_4^+, 7 ml). The column was washed thoroughly with water and the products were eluted with ammonia, the concentration being linearly increased (0 to 1M). The fraction containing the major product was concentrated and the residue was again chromatographed on a column (20 ml) of the same resin (NH_4^+) with ammonia to give 3′,4′-dideoxykanamycin B free-base as a monohydrate (27 mg, 88%), $[\alpha]_D^{25}$ +130° (*c* 1, water); R_F 0.22 (t.l.c.; Merck silica gel 60F-254; double irrigation with 4:4:2:3 1-butanol–ethanol–chloroform–17% ammonia; *cf.* kanamycin B, R_F 0.1).

Anal. Calc. for $C_{18}H_{37}N_5O_8 \cdot H_2O$: C, 46.04; H, 8.37; N, 14.92. Found: C, 46.15; H, 8.38; N, 14.97.

(*b*) *From* **19** *and* **20**. Compounds **19** and **20** were treated as in (*a*) to give 3′,4′-dideoxykanamycin B (96 and 90%, respectively).

*2-Deoxy-4-O-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranosyl)-6-O-(2,3-dideoxy-2,3-epimino-α-D-mannopyranosyl)streptamine* (**23**). — Compound **22** was treated as described for 3′,4′-dideoxykanamycin B to give **23** (91%), $[\alpha]_D^{25}$ +110° (*c* 0.6, water); R_F 0.3 with the same developing mixture described for 3′,4′-dideoxykanamycin B; n.m.r. data (D_2O): δ 1.0–2.1 (6 H), 5.08 (d, 1 H, *J* 3.5 Hz, H-1′), 5.15 (s, 1 H, H-1″); after CO_2 had been bubbled through the solution: δ 2.3 (2 H AB quartet, $J_{A,B}$ 6.5 Hz, H-2″,3″).

Anal. Calc. for $C_{18}H_{35}N_5O_7 \cdot H_2O$: C, 47.88; H, 8.26; N, 15.51. Found: C, 47.72; H, 8.21; N, 15.93.

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