Carbohydrate Research, 136 (1985) 195–206
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESES OF METHYL 6-DEOXY-6-HYDROXYAMINO-α-D-GLUCOPYRANOSIDE, 6′-*N*-HYDROXYKANAMYCIN A, AND 6′-*N*-HYDROXYDIBEKACIN

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(Received April 30th, 1984; accepted for publication, July 5th, 1984)

ABSTRACT

Methyl 6-deoxy-6-hydroxyamino-α-D-glucopyranoside has been prepared from methyl 6-amino-6-deoxy-α-D-glucopyranoside *via* oxidation with hydrogen peroxide in the presence of sodium tungstate [to give the corresponding 6-aldoxime (**2**)], followed by reduction with sodium cyanoborohydride in an acidic medium. Acetylation of the *Z* isomer of **2** gave a nitrile derivative. The above oxidation–reduction procedure was applied to kanamycin A and dibekacin, starting from the corresponding 6′-amino-*N*-tosyl derivatives. On treatment with sodium in liquid ammonia, 6′-deamino-6′-hydroxyimino-1,3,3″-tri-*N*-tosylkanamycin A gave the corresponding *N*-detosyl derivative in good yield with the 6′-aldoxime group remaining intact, but, with 6′-deamino-6′-hydroxyimino-1,3,2′,3″-tetra-*N*-tosyldibekacin, the *N*-detosyl derivative (**17**) was obtained only by a very short reaction period. The antibacterial activities of **17** and 6′-*N*-hydroxydibekacin were demonstrated.

INTRODUCTION

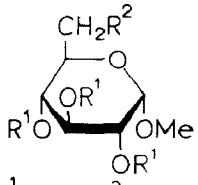
Bacterial resistance to kanamycins has been shown to involve any of several different enzyme inactivations; one of these is 6′-acetyltransferase[1] which acetylates the 6′-amino groups of kanamycins. As part of our studies of functional modifications of members of the kanamycin group of antibiotics, we have undertaken modifications of the 6′-amino group and now describe syntheses of 6′-*N*-hydroxy derivatives of kanamycin A and dibekacin[2] (3′,4′-dideoxykanamycin B), and methyl 6-deoxy-6-hydroxyamino-α-D-glucopyranoside (**5**).

RESULTS AND DISCUSSION

The synthesis of methyl 6-deoxy-6-hydroxyamino-α-D-glucopyranoside (**5**) started from methyl 6-amino-6-deoxy-α-D-glucopyranoside[3] (**1**), which was treated[4] with aqueous hydrogen peroxide in the presence of sodium tungstate dihydrate to give the 6-aldoxime **2** in high yield. From its ^{1}H-n.m.r. spectrum, it was concluded

0008-6215/85/$ 03.30

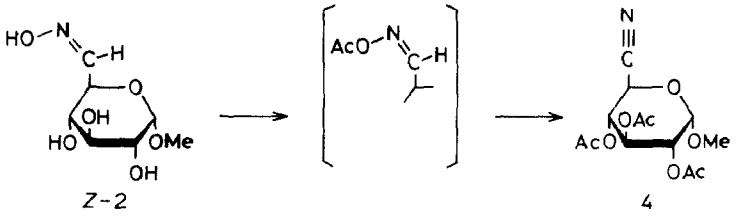
that **2** was an 8:1 *EZ*-mixture. The resonance of an aldoxime methine proton which is *syn* to the hydroxyl group is always downfield[5] relative to that of the corresponding *anti* proton. Thus, the major isomer of **2** (δ 7.56 for H-6) was the *E* isomer, and the minor isomer (δ 6.93) was *Z*. In contrast, the resonance of H-5 of the *E* isomer was upfield (δ 4.18) in comparison with the corresponding resonance of the *Z* isomer (δ 4.90), and the signals, being separated from other signals, were useful for characterising the isomers of 6-aldoximes. The above route to aldoximes, in some instances, may be advantageous over that involving hydroxyimination of sugar 6-aldehydes because of the difficulty in isolating the latter compounds pure. Hydroxyiminations have been reported in syntheses of 1-epitobramycin[6] and 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-1-epikanamycin A[7].



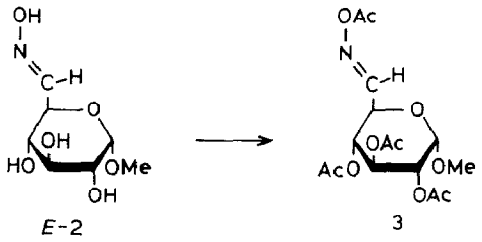
1 $R^1 = H, R^2 = NH_2$

5 $R^1 = H, R^2 = NHOH$

6 $R^1 = Ac, R^2 = N(Ac)OAc$



Z-2 4

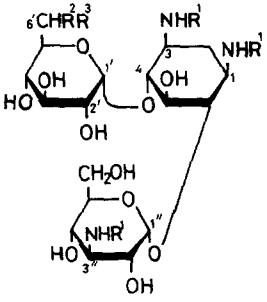


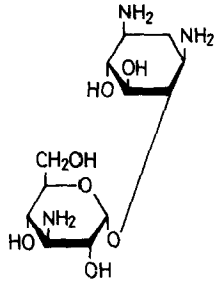
E-2 3

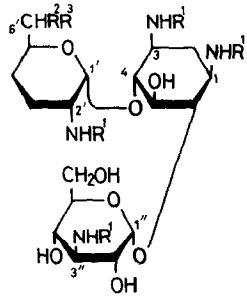
Acetylation of **2** gave the corresponding tetra-acetate **3** together with the nitrile[8] **4** as the minor component, which was formed by loss of acetic acid from **3**. The ^{1}H-n.m.r. spectrum of **3** indicated it to be a single product and to be the *E*-acetoxyimino isomer (H-6 at δ 7.62, and H-5 at δ 4.55). The geometrical purity of **3** suggested that it was formed mainly from the *E* isomer of **2**, and that the nitrile **4** was formed from the *Z* isomer of **2**. This assumption was consistent with the yield (11%) of **4**, and the proportion (~12%) of the *Z* isomer in **2**. The ready formation of **4** from the *Z*-*O*-acetyloxime, the expected intermediate, is explained by the

antiperiplanar relationship of the acetoxyl group and the methine hydrogen at C-6, a situation which facilitates *anti*-elimination[9].

Attempts to convert **2** into the 6-hydroxyamino derivative **5** with sodium borohydride or hydrogen (Pt, Pd, or Ni) as catalyst under neutral or acidic conditions, even for a very short period, gave only methyl 6-amino-6-deoxy-α-D-glucopyranoside[3] (**1**). The conversion of an oxime into the hydroxyamine has been achieved using $NaBH_2S_2$[10], sodium cyanoborohydride[11], $NaBH_4$–AcOH[12], and borane–pyridine[13]. When **2** was treated with sodium cyanoborohydride in slightly acidic methanol, the 6-hydroxyamino compound **5** was obtained in high yield. The structure of **5** was proved by its basic nature (partial salt formation with acid) and the presence of signals for H-6,6′ in the ^{1}H-n.m.r. spectrum. Hydrogenation of **5** in the presence of platinum oxide gave **1**, and acetylation of **5** gave the penta-acetate **6**, a result which also supports the structure assigned. The ^{1}H-n.m.r. spectrum of **6** at 25° contained broadened signals for H-4,5,6,6′ and one of the acetyl groups, but all of these signals were sharp at 50°, indicating that the rotation around the C-6–N (and/or C-5–C-6) bond was sterically hindered.







	R^1	R^2	R^3
Kanamycin A	H	H	NH_2
7	Ts	H	NHZ
8	Ts	H	NH_2
9	Ts	=NOH	
10	H	=NOH	
12	H	H	NHOH

11

Z = $CO_2CH_2C_6H_5$

	R^1	R^2	R^3
Dibekacin	H	H	NH_2
13	H	H	NHZ
14	Ts	H	NHZ
15	Ts	H	NH_2
16	Ts	=NOH	
17	H	=NOH	
18	H	H	NHOH

The synthesis of 6′-*N*-hydroxykanamycin A (**12**) involved 6′-*N*-benzyloxycarbonyl-1,3,3″-tri-*N*-tosylkanamycin A[14] (**7**) which was prepared from kanamycin A *via* selective 6′-*N*-benzyloxycarbonylation using the zinc acetate method[15] followed by *N*-tosylation. Catalytic hydrogenolysis of **7** gave the tri-*N*-tosylkanamycin A (**8**) having NH_2-6 unsubstituted, which was treated with aqueous hydrogen peroxide in the presence of sodium tungstate dihydrate to give the tri-*N*-tosyl-6′-aldoxime (**9**, 47% after column chromatography). Detosylation of **9** with sodium in liquid

TABLE I

^{13}C-N M R CHEMICAL SHIFTS (δ)

Atom	**1**[a]	**2**[a]	**2**[b]	**5**[a]	**10**[b]	**12**[a]	*Kanamycin A*[a]	*Dibekacin*[b]	**17**[b]	**18**[a]	*Dibekacin*
C-1					51.23 (51.31)	50.67	50.63	51.10	51.05 (51.15)	50 39	50.52
C-2					36.62 (36.87)	28.38	28.33	36.35	35.54	28.61	28.55
C-3					50.08 (49.93)	48.55	48.54	50.23	50.02[d]	49.35[d]	49.47[d]
C-4					88.57 (88.95?)	80.28	78.87	87.05	86.77	79.67	77.58
C-5					75.02 (75.10)	73.57	73.60	75.40	75.28	74.87	75.13
C-6					89.15 (89.70?)	84.33	84.64	88 92	88.29 (87.97)	84.40	84.43
C-1′	100.17	100.46 (100.51)	100.63 (100.72)	100.17	100.71	97.42	96.52	101.29	100.76	96.95	95.67[c]
C-2′	71.83	71.84 (71.91)	71.94	71.81	72.20	71.61[d]	71.61[d]	50.59	50 11[d]	49.74[d]	49.56[d]
C-3′	73.50[c]	73.41[c] (73.57)	73.34[c]	73.56[c]	72.63 (72.88?)	73.05	72.98	26.29	24.66 (24.82)	21.64	21.31
C-4′	72.19[c]	72.33[c] (72.39)	72.41[c]	72 19[c]	71.65	71.80[d]	71.73[d]	28.08	28.07 (27.84)	26.49	26.16
C-5′	68.27[c]	69.81[c]	70.49[c]	65.60[c]	73.50 (73.80?)	67.00	69.53	70.44[d]	69.81	64.01[c]	66.87
C-6′	41.40	150.31 (149.38)	148.98 (149.25)	52.50	148.58 (149.01)	52.76	41.25	45.41	152.31 (152.82)	54.40	43.45[c]
C-1″					101.17	101.22	101.19	100.64	99.97 (100.14)	101.21	101.28
C-2″					72.63	69.00	68.98	72.62	72.16	68.80	68.88
C-3″					55.15	55.89	55.83	55.03	55.11 (55.18)	55.76	55.79[c]
C-4″					70.24	66.30	66.40	70.21[d]	67 96	66.36	66.33[c]
C-5″					73.13	73.17	73.20	73.01	73.21	73.85	73.72
C-6″					61.18	60.77	60.87	61.26	61.25	60.86	60.83
OCH_3	56.32	56.20 (56.39)	56.09 (56.27)	56.32							

[a]In D_2O at pD 1. [b]In 20% ND_3 in D_2O [c]Confirmed by selective proton-decoupling. [d]The values in the same column may be reversed.

ammonia (−50°, 2 h) gave 70% of kanamycin A 6′-aldoxime (**10**) without the formation of kanamycin A, but **10** was accompanied by a small proportion of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine[16] (**11**). This result indicated that the 6′-aldoxime moiety was fairly stable under the above reaction conditions, but underwent some cleavage of the C-1′→O-4 glycoside linkage to give **11**. Kanamycin A and dibekacin were not affected by treatment with sodium in liquid ammonia. The ^{1}H-n.m.r. data indicated **10** to be an *EZ* mixture.

Treatment of **10** with sodium cyanoborohydride in an aqueous acidic medium gave 6′-*N*-hydroxykanamycin A (**12**) in good yield. The structure was supported by the n.m.r. data (see Table I for the ^{13}C-n.m.r. data). Thus, the resonance of H-6′a and -6′b had chemical shifts similar to those of **5**. The resonances of C-6′ (δ 52.76) had approximately the same chemical shift as that (δ 52.50) of C-6 in **5**, and was different from those for C-6 in the 6-amino sugar **1** (δ 41.40) and the oxime **2** (δ ~150). The chemical shift of the signal for C-6′ of *N*-hydroxydibekacin (**18**) also had a value (δ 54.40) similar to that for **5**. Reduction of **12** with hydrogen and platinum oxide gave exclusively kanamycin A, which was identical with the naturally occurring compound in chemical characteristics and antibacterial activity. The aldoxime **10** and the hydroxyamino derivative **12** were almost devoid of antibacterial activity.

6′-*N*-Hydroxydibekacin (**18**) was prepared from dibekacin. Thus, selective benzyloxycarbonylation of dibekacin by the zinc acetate method[15] gave 77% of 6′-*N*-(benzyloxycarbonyl)dibekacin (**13**). Tosylation of **13** gave the tetra-*N*-tosyl derivative **14**, hydrogenolysis of which yielded the 6′-amino derivative **15**. Since **15** was more soluble in organic solvents than tri-*N*-tosylkanamycin A (**8**), oxidation with hydrogen peroxide–sodium tungstate was carried out in methanol to give the tri-*N*-tosyl-6′-aldoxime **16** in a moderate yield (51%).

When **16** was treated with sodium in liquid ammonia for 2 h, the desired dibekacin-6′-aldoxime (**17**) was not obtained, but 6-*O*-(3-amino-3-deoxy-α-D-

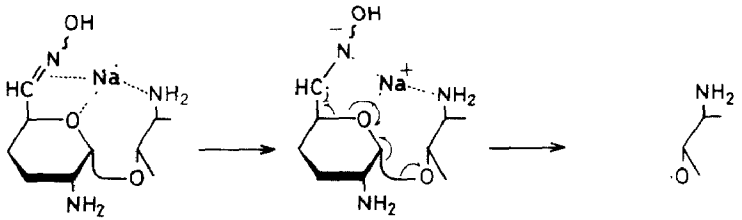
TABLE II

MINIMAL INHIBITORY CONCENTRATIONS (μg/mL) OF DIBEKACIN DERIVATIVES (**17** AND **18**) AND DIBEKACIN

Test organism	**17**	**18**	*Dibekacin*
Staphylococcus aureus 209P	100	3.12	<0.2
Ap01[a]	>100	25	0.78
Sarcina lutea PCI 1001	>100	>100	12.5
Escherichia coli NIHJ	25	3.12	0.2
K-12	100	6.25	0.39
R5[b]	>100	>100	100
ML1629[c]	>100	12.5	0.39
W677	50	6.25	0.39
Pseudomonas aeruginosa A 3	25	1.56	<0.2
T 113[c]	>100	50	1.56

[a]Resistance mechanism: AAD (4′). [b]AAC (6′). [c]APH (3′)-I.

glucopyranosyl)-2-deoxystreptamine[16] (**11**) was the major product. However, when the reaction was stopped after 2 min, 48% of **17** was obtained together with **11**. Thus, the tosyl groups in **16** were removed fairly rapidly, and the product **17** underwent further reaction to give **11**. The kanamycin A derivative **10** is fairly resistant to this kind of transformation. Since kanamycin A and dibekacin are stable and **10** is moderately stable to the action of sodium in liquid ammonia, the reaction may be affected by the presence of the hydroxyimino group at C-6′ and the absence of HO-4′ from **17**. Thus, it is possible that sodium is trapped by π electrons and the oxygen and nitrogen atoms, as depicted in **19**, and that the glycoside linkage is then cleaved by electron transfer from sodium as shown in **20**→**21**. For **10**, HO-4′ may prevent the formation of a complex of the type **19** by, for example, the formation of a similar complex between oxyimino-6′ and HO-4′.



19 20 21

Reduction of **17** with sodium cyanoborohydride gave 6′-*N*-hydroxydibekacin (**18**) in high yield. The antibacterial spectra of **17** and **18** compared with that of dibekacin, shown in Table II, indicate that introduction of the 6′-*N*-hydroxyl group into dibekacin results in a significant decrease in antibacterial activity.

EXPERIMENTAL

General. — Melting points were determined on a Kofler block, and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. I.r. spectra were recorded, for potassium bromide pellets, using a JASCO A202 grating spectrophotometer. ^{1}H-N.m.r. spectra (^{1}H 250 MHz, ^{13}C 62.9 MHz) were recorded in the F.t. mode with a Bruker WM 250 spectrometer at 25° unless otherwise stated. T.l.c. was performed on Kieselgel 60 F_{254} (Merck), and column chromatography on Wakogel C-200.

*Methyl 6-deoxy-6-hydroxyimino-α-*D*-glucopyranoside* (**2**). — To an aqueous solution (20 mL) of **1** (980 mg, hemicarbonate) and sodium tungstate dihydrate (330 mg) was added aqueous 20% hydrogen peroxide (10 mL), and the solution was kept for 6 h at room temperature. Excess of hydrogen peroxide was decomposed using Pd/C, the solution was concentrated, and the residue was eluted from a column of charcoal (150 mL, activated for chromatography, Wako Pure Chemicals Ltd.) with water and then with aqueous 4→10% ethanol. The eluate

was concentrated, and ethanol was repeatedly distilled from the residue to give **2** (880 mg, 97%), $[\alpha]_D^{20}$ +110° (*c* 1, water); ν_{max}^{KBr} 1640 cm^{-1}. Mass spectrum: *m/z* 208.0833 $(M + H)^+$ (Calc. for $C_7H_{14}NO_6$: 208.0821). ^{1}H-N.m.r. data (D_2O): *E* isomer, δ 3.44 (s, 3 H, OMe), 3.53 (dd, 1 H, H-4), 3.63 (dd, 1 H, H-2), 3.71 (dd, 1 H, H-3), 4.18 (dd, 1 H, H-5), 4.85 (d, 1 H, H-1), and 7.56 (d, 1 H, H-6); $J_{1,2}$ 3.5, $J_{2,3}$ 10, $J_{3,4}$ 9.5, $J_{4,5}$ 10, and $J_{5,6}$ 7 Hz; *Z* isomer, δ 3.48 (s, OMe), 4.90 (dd, *J* 7 and 10 Hz, H-5), and 6.93 (d, $J_{5,6}$ 7 Hz, H-6). The integrated intensities indicated an *EZ*-ratio of ~8:1. Irradiation at δ 4.18 (H-5 of *E* isomer) collapsed the signals of H-4 and H-6 to a doublet and a singlet, respectively. Irradiation at δ 4.90 (H-5 of *Z* isomer) collapsed the doublet of H-6 to a singlet. Irradiation at δ 3.57 collapsed the signals for H-5 of both the *E* and *Z* isomers to doublets.

Anal. Calc. for $C_7H_{13}NO_6$: C, 40.58; H, 6.32; N, 6.76. Found: C, 40.75; H, 6.59; N, 6.53.

*Methyl 6-(*N*-acetoxyacetamido)-2,3,4-tri-*O*-acetyl-6-deoxy-α-*D*-glucopyranoside* (**3**) *and (methyl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosid)uruononitrile* (**4**). — A mixture of **2** (100 mg) and acetic anhydride (500 mg) in pyridine (3 mL) was kept overnight at room temperature and then concentrated. The syrupy residue contained (t.l.c., 2:1 benzene–ethyl acetate) two products having R_F 0.38 (major, **3**) and 0.56 (minor, **4**). A chloroform solution of the syrup was washed with aqueous 5% potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel with 4:1 benzene–ethyl acetate to give **3** (98 mg, 54%) and **4** (17 mg, 11%).

Compound **3** had m.p. 119–121° (from acetone–hexane), $[\alpha]_D^{21}$ +84° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.04, 2.10, 2.16 (3 s, each 3 H, 3 Ac), 3.44 (s, 3 H, OMe), 4.53 (dd, 1 H, H-5), 4.89 (dd, 1 H, H-2), 4.98 (d, 1 H, H-1), 5.03 (t, 1 H, H-4), 5.56 (t, 1 H, H-3), and 7.61 (d, 1 H, H-6); $J_{1,2}$ 4.0, $J_{2,3} = J_{3,4} = J_{4,5} = 10$, and $J_{5,6}$ 7.5 Hz.

Anal. Calc. for $C_{15}H_{21}NO_{10}$: C, 48.00; H, 5.64; N, 3.73. Found: C, 48.03; H, 5.57; N, 3.53.

Compound **4** had m.p. 158–159° (needles, from acetone–hexane), $[\alpha]_D^{21}$ +148° (*c* 1, chloroform); lit.[8] m.p. 156–157° (from methanol), $[\alpha]_D^{23}$ +140° (*c* 0.4, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.05, 2.10, 2.13 (3 s, each 3 H, 3 Ac), 3.51 (s, 3 H, OMe), 4.65 (d, 1 H, H-5), 4.90 (dd, 1 H, H-2), 5.07 (d, 1 H, H-1), 5.30 (t, 1 H, H-4), and 5.48 (t, 1 H, H-3); $J_{1,2}$ 3.5, $J_{2,3} = J_{3,4} = J_{4,5} = 10$ Hz.

*Methyl 6-deoxy-6-hydroxyamino-α-*D*-glucopyranoside* (**5**). — To a solution of **2** (117 mg) in dry methanol (5 mL) were added sodium cyanoborohydride (33 mg) and then methanolic 10% hydrogen chloride (1.5 mL). The solution was kept under nitrogen for 30 min at room temperature and then concentrated, and methanol was repeatedly evaporated from the residue. An aqueous solution of the residue was eluted from a column (0.8 × 15 cm) of Sephadex LH-20 with water. The eluate was passed through a column of Dowex 1-X2 (SO_4^{2-}) resin by elution with water, to give **5** as a partial sulfate salt (130 mg, 96%) which was weakly

hygroscopic; $[\alpha]_D^{20}$ +153° (*c* 1, water). Mass spectrum: *m/z* 210.0982 $(M + H)^+$ (Calc. for $C_7H_{16}NO_6$: 210.0978). ^{1}H-N.m.r. data (D_2O, pD 4): δ 3.43 (dd, 1 H, H-4), 3.50 (s, 3 H, OMe), 3.54 (dd, 1 H, H-6a), 3.65 (dd, 1 H, H-2), 3.73 (apparent t, 1 H, H-3), 3.75 (dd, 1 H, H-6b), 4.10 (dt, 1 H, H-5), and 4.91 (d, 1 H, H-1); $J_{1,2}$ 3.5, $J_{2,3}$ 10, $J_{3,4}$ 8.5, $J_{4,5} = J_{5,6a}$ = 9.5, $J_{5,6b}$ 2.5, and $J_{6a,6b}$ 13.5 Hz.

Anal. Calc. for $C_7H_{15}NO_6 \cdot 0.3\ H_2SO_4$: C, 35.23; H, 6.59; N, 5.87; S, 4.03. Found: C, 35.61; H, 6.84; N, 5.48; S, 4.37.

*Methyl 6-amino-6-deoxy-α-*D*-glucopyranoside* (**1**). — An aqueous solution (2 mL) of **5** (20 mg as the 0.3sulfate) was hydrogenated in the presence of platinum oxide at 3.5 atm. for 2 h at room temperature. The crude product was eluted from a column (0.5 × 10 cm) of Dowex 50W-X2 (NH_4^+) resin with M ammonia to give **1** (15 mg, 85% as the hemicarbonate · 0.25 hydrate) as a ninhydrin-positive solid, $[\alpha]_D^{18}$ +139° (*c* 1, water); lit.[3] +147° (*c* 1, water; as the hydrochloride).

Anal. Calc. for $C_7H_{15}NO_5 \cdot 0.5\ H_2CO_3 \cdot 0.25\ H_2O$: C, 39.39; H, 7.27; N, 6.12. Found: C, 39.24 H, 7.41; N, 6.32.

*Methyl 6-(*N*-acetoxy-*N*-acetylamino-2,3,4-tri-*O*-acetyl-6-deoxy-α-*D*-glucopyranoside* (**6**). — A solution of **5** (15 mg, as the 0.3sulfate) and acetic anhydride (0.05 mL) in pyridine (0.5 mL) was kept overnight at room temperature and then concentrated. A solution of the syrupy residue in chloroform was washed with aqueous 5% potassium hydrogensulfate, aqueous sodium hydrogencarbonate (saturated), and water, dried (Na_2SO_4), and concentrated to give **6** as a thick syrup (20 mg, 76%), $[\alpha]_D^{20}$ +121° (*c* 0.8, chloroform). ^{1}H-N.m.r. data ($CDCl_3$ at 50°); δ 1.99, 2.02 (split into several signals at 25°), 2.03, 2.06, and 2.18 (5 s, each 3 H, 5 Ac), 3.41 (s, 3 H, OMe), 3.72 (d with minor complex signals, 1 H, $J_{6a,6b}$ 13 Hz; bd at 25°; H-6a), 3.99–4.11 (m, 2 H; 3.97–4.22 at 25°; H-5,6b), 4.84 (dd, 1 H, H-2), 4.90 (d, 1 H, H-1), 5.00 (t, 1 H; bt at 25°; H-4), and 5.44 (dd, 1 H, H-3); $J_{1,2}$ 3.5, $J_{2,3}$ 10, $J_{3,4}$ 9.5, and $J_{4,5}$ ~10 Hz.

Anal. Calc. for $C_{17}H_{25}NO_{11}$: C, 48.69; H, 6.01; N, 3.34. Found: C, 48.73; H, 5.88; N, 3.43.

*1,3,3″-Tri-*N*-tosylkanamycin A* (**8**). — A solution of **7**[14] (2.02 g) in methanol (80 mL) containing M hydrochloric acid (4 mL) was hydrogenated in the presence of palladium black at atmospheric pressure. T.l.c. (4:3:1 chloroform–methanol–aqueous 28% ammonia) then revealed a single component (R_F 0.28; *cf.*, 0.6 for **7**). The crude product was eluted from a column (2 × 40 cm) of Dowex 50W-X2 (NH_4^+) resin with 50% aqueous methanol containing 0 → M ammonia. Ninhydrin-positive fractions were collected and concentrated to give **8** (1.74 g), $[\alpha]_D^{23}$ +27° (*c* 1, pyridine). ^{1}H-N.m.r. data (pyridine-d_5): δ 2.05 and 2.29 (2 s, 3 and 6 H, 3 Ts), 5.30 (d, 1 H, *J* 3.5 Hz, H-1′ or 1″), 5.78 (d, 1 H, *J* 3.5 Hz, H-1″ or 1′).

*6′-Deamino-6′-hydroxyimino-1,3,3″-tri-*N*-tosylkanamycin A* (**9**). — To a solution of **8** (4.0 g), anhydrous sodium carbonate (860 mg), and sodium tungstate dihydrate (270 mg) in 50% aqueous methanol (160 mL) was added aqueous 30% hydrogen peroxide (2.3 mL, 4.6 mL after 6 h, and 2.3 mL after 12 h). The solution was then kept for 16 h at room temperature, excess of hydrogen peroxide was decomposed using Pd/C, and the mixture was filtered and concentrated. The

resulting syrup contained (t.l.c., 4:3:1 chloroform–methanol–aqueous 28% ammonia) several components [R_F 0.77, 0.72, 0.6, 0.5 (major, **9**), 0.4, and 0.3 (slight, **8**)]. Column chromatography of the mixture on Wakogel C-200 (300 g), with 5:1 chloroform–methanol → acetone → acetone–methanol (10:1 → 10:3) → 1:10 aqueous acetone, gave **9** (2.05 g, 47% based on **7**), $[\alpha]_D^{23}$ +26° (*c* 1, methanol), R_F 0.5. ^{1}H-N.m.r. data (acetone-d_6): δ 2.36 and 2.48 (2 s, 3 and 6 H, 3 Ts), 4.50 (dd, 1 H, $J_{4',5'}$ 10, $J_{5',6'}$ 7 Hz, H-5'), 4.96 (d, 1 H, *J* 3.5 Hz, H-1' or 1''), and 5.12 (d, 1 H, *J* 3.5 Hz, H-1'' or 1').

Anal. Calc. for $C_{39}H_{52}N_4O_{18}S_3 \cdot 3\ H_2O$: C, 46.15; H, 5.71; N, 5.52; S, 9.46. Found: C, 45.93; H, 5.32; N, 5.43; S, 9.46.

6'-Deamino-6'-hydroxyiminokanamycin A (**10**). — To a solution of **9** (210 mg) in liquid ammonia (~20 mL) at −50° was added sodium (~230 mg); the deep-blue solution was kept for 2 h at −50°, then diluted with methanol until colourless, and concentrated by gradually warming to room temperature and finally under diminished pressure. An aqueous solution (5 mL) of the resulting syrup was mixed with Dowex 50W-X2 (NH_4^+) resin (10 mL), which was then packed into a column, washed with water, and eluted with M ammonia. T.l.c. (1:4:3 chloroform–methanol–aqueous 28% ammonia) of the syrupy product revealed two components of R_F 0.55 (major, **10**; *cf.*, kanamycin A, R_F 0.45) and 0.75 (minor, **11**). Column chromatography of the syrup on CM-Sephadex C-25 (NH_4^+ form, 10 mL) with ammonia (0 → 0.1 M) gave **10** as its carbonate (79 mg, 70%), $[\alpha]_D^{23}$ +111° (*c* 1, water). ^{1}H-N.m.r. data (20% ND_3 in D_2O): δ 1.15 and 1.20 (2 q, 1 H, *J* 12.5 Hz, H-2*a* of *Z* and *E* isomer), 1.87–2.0 (m, 1 H, H-2*e*), 2.75–3.0 (3 H, H-1,3,3''), 4.31 (dd, $J_{4',5'}$ 10, $J_{5',6'}$ 7 Hz, H-5' of *E* isomer), 4.98–5.06 (H-5' of *Z* isomer), 5.02 (d, 1 H, *J* 3.5 Hz, H-1' or 1''), 5.23 (d, 1 H, *J* 3.5 Hz, H-1'' or 1'), 6.82 (d, $J_{5',6'}$ 7 Hz, H-6' of *Z* isomer), and 7.38 (d, H-6' of *E* isomer); the *EZ*-ratio was ~6:1.

Anal. Calc. for $C_{18}H_{34}N_4O_{12} \cdot 0.75\ H_2CO_3 \cdot 0.25\ H_2O$: C, 40.98; H, 6.60; N, 10.20. Found: C, 41.18; H, 6.92; N, 10.01.

6'-N-Hydroxykanamycin A (**12**). — To an aqueous solution (3 mL) of **10** (70 mg) and sodium cyanoborohydride (32 mg) under nitrogen was added dropwise 0.1M hydrochloric acid (0.7 mL), the solution was kept in a closed vessel for 3 h at room temperature and then concentrated, and methanol was repeatedly evaporated from the residue to remove boric acid. To an aqueous solution of the residue was added dry CM-Sephadex C-25 (5 g), the mixture was immediately concentrated to dryness, and 9:1 methanol–toluene was repeatedly evaporated from the residue, which, after swelling with water, was packed into a column and eluted with hydrochloric acid (0 → 0.02M). The eluate was neutralised to pH ~3 with Dowex 1-X2 (HO^-) resin, concentrated, and eluted from a column (0.8 × 15 cm) of Dowex 1-X2 (SO_4^{2-}) resin with water to give **12** as the sulfate (70 mg, 77%), $[\alpha]_D^{20}$ +89° (*c* 1, water). ^{1}H-N.m.r. data (D_2O–DCl, pD ~1): δ 1.98 (q, 1 H, *J* 12.5 Hz, H-2*a*), 2.59 (dt, 1 H, *J* 4, 4, and 12.5 Hz, H-2*e*), 3.45 (dd, 1 H, H-4'), 3.53 (dd, 1 H, H-6'a), ~3.72 (H-6'b), 4.29 (dt, 1 H, H-5'), 5.15 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''), and 5.51 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'); $J_{3',4'}$ 9, $J_{4',5'} = J_{5',6'a}$ = 10, and

$J_{5',6'b}$ 3 Hz. Irradiation of H-5′ collapsed the signals of H-4′ and 6′a to doublets. Irradiation at δ 3.72 collapsed the double triplets of H-5′ to a triplet.

Anal. Calc. for $C_{18}H_{36}N_4O_{12} \cdot 1.6\,H_2SO_4 \cdot 3\,H_2O$: C, 30.39; H, 6.40; N, 7.88; S, 7.21. Found: C, 30.34; H, 6.08; N, 7.35; S, 7.11.

Kanamycin A. — When a solution of **12** (20 mg) in 0.05M hydrochloric acid was reduced as described for **5**, kanamycin A (14 mg) was obtained which was identical (^{1}H-n.m.r. and antibacterial spectra) with the naturally occurring compound.

6′-N-(Benzyloxycarbonyl)dibekacin (**13**). — A mixture of dibekacin hemicarbonate hemihydrate (4.25 g) and zinc acetate dihydrate (8.17 g) in dry methyl sulfoxide (100 mL) was heated for 1 h at 50°. To the cooled, clear solution was added during 1 h *N*-(benzyloxycarbonyloxy)succinimide (3.0 g), and the mixture was kept for 2 h at room temperature. Addition of ether gave a syrup that was thoroughly washed with ether to give a solid which was purified by elution from a column (3 × 50 cm) of Amberlite CG-50(H^+) resin (100–200 mesh) with 0.6 → 1.5M ammonia to give **12** as a ninhydrin-positive, zinc ion-free solid (4.24 g, 77%), $[\alpha]_D^{20}$ +102° (*c* 1, water), R_F 0.46 (4:3:1 chloroform–methanol–aqueous 28% ammonia; *cf.*, dibekacin, R_F 0.25).

Anal. Calc. for $C_{26}H_{43}N_5O_{10} \cdot 0.5\,H_2CO_3 \cdot H_2O$: C, 50.15; H, 7.30; N, 11.04. Found: C, 50.23; H, 7.13; N, 10.98.

6′-N-(Benzyloxycarbonyl)-1,3,2′,3″-tetra-N-tosyldibekacin (**14**). — A solution of **13** (1.37 g), anhydrous sodium carbonate (1.0 g), and toluene-*p*-sulfonyl chloride (1.8 g) in 1:3 water–1,4-dioxane (30 mL) was stirred overnight at room temperature and then concentrated. Addition of water gave a precipitate that was thoroughly washed with water, dried, and washed with ether to give **14** (2.44 g, 94%), $[\alpha]_D^{21}$ +40° (c 0.8, methanol). ^{1}H-N.m.r. data (acetone-d_6): δ 2.37, 2.40, and 2.50 (3 s, 3, 3, and 6 H, 4 Ts).

Anal. Calc. for $C_{54}H_{67}N_5O_{18}S_4$: C, 53.94; H, 5.62; N, 5.82; S, 10.67. Found: C, 53.68; H, 5.70; N, 5.51; S, 10.39.

6′-Deamino-6′-hydroxyimino-1,3,2′,3″-tetra-N-tosyldibekacin (**16**). — Compound **14** (1.93 g) was hydrogenated as described for **8**, to give **15** (1.64 g), to a solution of which together with anhydrous sodium carbonate (160 mg) and sodium tungstate dihydrate (190 mg) in methanol (30 mL) was added aqueous 30% hydrogen peroxide (3.4 mL initially, and 1.2 mL after 3.5 h), and the mixture was stirred for 7 h at room temperature. Isolation of the product, as described for **9** (12:1 chloroform–methanol was used in the chromatography), gave **16** (900 mg, 51%), $[\alpha]_D^{21}$ +16° (*c* 1, methanol), R_F 0.5 (t.l.c., 5:1 chloroform–ethanol). ^{1}H-N.m.r. data (acetone-d_6): δ 2.37 and 2.38 (2 s, 6 H, ratio ~2.3:1, Ts), 2.45 and 2.48 (2 s, 3 H, ratio ~1:2.3, Ts), and 2.50 (s, 3 H, Ts).

Anal. Calc. for $C_{46}H_{59}N_5O_{17}S_4 \cdot H_2O$: C, 50.21; H, 5.59; N, 6.37; S, 11.66. Found: C, 50.06; H, 5.39; N, 6.25; S, 11.60.

6-O-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**11**). — To a solution of **16** (40 mg) in liquid ammonia (~5 mL) at −50° was added sodium (~50

mg), and the solution was kept for 2 h at −50°. Evaporation of the ammonia gave a syrup which contained (t.l.c., 3:6:4 chloroform–methanol–aqueous 28% ammonia) a single component, R_F 0.33 (*cf.*, dibekacin: R_F 0.43). Chromatography of the syrup on CM-Sephadex C-25 (NH_4^+) by elution with ammonia (0 → 0.1M) gave **11** (11 mg, 79%), $[\alpha]_D^{23}$ +74° (*c* 0.7, water); lit.[16] $[\alpha]_D^{28}$ +76.1° (*c* 1, water; as the trihydrochloride). ^{1}H-N.m.r. data (20% ND_3 in D_2O): δ 1.18 (q, 1 H, *J* 12.5 Hz, H-2*a*), 1.94 (dt, *J* 4, 4, and 12.5 Hz, H-2*e*), 2.67 (ddd, *J* 4, 10, and 12.5 Hz, H-3), 2.88 (ddd, *J* 4, 10, and 12.5 Hz, H-1), 3.01 (t, 1 H, *J* 10 Hz, H-3″), 3.11 (t, 1 H, *J* 9.5 Hz, H-4), 3.21 (t, 1 H, *J* 9.5 Hz, H-6), 3.32 (t, 1 H, *J* 10 Hz, H-4″), 3.34 (t, 1 H, *J* 9.5 Hz, H-5), 3.48 (dd, 1 H, *J* 4 and 10 Hz, H-2″), 3.76 (apparent d, 2 H, H-6″a,b), 3.92 (dt, 1 H, *J* ~3, ~3, and 10 Hz, H-5″), and 5.02 (d, 1 H, *J* 4 Hz, H-1″).

Anal. Calc. for $C_{12}H_{25}N_3O_7 \cdot H_2CO_3$: C, 40.53; H, 7.06; N, 10.91. Found: C, 40.35; H, 7.06; N, 11.03.

6′-Deamino-6′-hydroxyiminodibekacin (**17**). — To a solution of sodium (~80 mg) in liquid ammonia (~10 mL) at −50° was added with stirring a solution of **16** (116 mg) in oxolane (2 mL) and, after 2 min, ice-cold methanol was added until the solution became colourless. Concentration then gave a syrup which contained (t.l.c., 3:6:4 chloroform–methanol–aqueous 28% ammonia) two components of R_F 0.33 (**11**, minor) and 0.6 (**17**, major). The syrup was eluted from a column (1 × 25 cm) of Dowex 50W-X2 (NH_4^+) resin with M ammonia, and the eluate was freeze-dried to give **17** (30 mg, 48%), $[\alpha]_D^{22}$ +103° (*c* 1, water). ^{1}H-N.m.r. data (20% ND_3 in D_2O; 34°): *E* isomer, δ 1.22 (q, 1 H, *J* 12.5 Hz, H-2*a*), 1.55–1.97 (4 H, H-3′a,3′b,4′a,4′b), 1.96 (dt, *J* ~4, ~4, and 10 Hz, H-2*e*), 2.77–2.95 (3 H, H-1,3,2′), 3.00 (t, 1 H, *J* 10 Hz, H-3″), 3.2–3.36 (3 H, H-4,6,4″), 3.48 (dd, 1 H, *J* 4 and 10 Hz, H-2″), 3.59 (t, 1 H, *J* 9.5 Hz, H-5), 3.67–3.83 (ABq, 2 H, $J_{6''a,6''b}$ 12, $J_{5'',6''a}$ 5, $J_{5'',6''b}$ 2.5 Hz, H-6″a,6″b), 3.90 (ddd, 1 H, *J* 2.5, 5, and 10 Hz, H-5″), 4.50 (m, 1 H, H-5′), 5.03 (d, 1 H, *J* 4 Hz, H-1″), 5.13 (d, 1 H, *J* 3.5 Hz, H-1′), and 7.37 (d, 1 H, $J_{5',6'}$ 5.8 Hz, H-6′); *Z* isomer, δ 6.80 (d, ~0.27 H, *J* 6.0 Hz, H-6′). Irradiation of H-5′ (*E* isomer) and δ 5.08 (H-5′ of *Z* isomer) collapsed the H-6′ doublets of the *E* and *Z* isomers to singlets.

Anal. Calc. for $C_{18}H_{35}N_5O_9 \cdot 2\,H_2CO_3$: C, 40.74; H, 6.67; N, 11.88. Found: C, 40.88; H, 6.78; N, 12.29.

6′-N-Hydroxydibekacin (**18**). — A solution of **17** (28 mg) and sodium cyanoborohydride (12 mg) in methanol (3.4 mL) containing methanolic 10% hydrogen chloride (0.5 mL) was kept under nitrogen for 2 h at room temperature. Addition of dry CM-Sephadex C-25 (H^+, 1.3 g), followed by concentration of the solution, gave a residue that was swollen with water (5 mL), packed into a column, washed with water, and eluted with 0.02M hydrochloric acid. The eluate was neutralised with Dowex 1-X2 (HO^-) resin to pH ~6, concentrated to ~2 mL, and eluted from a column (0.5 × 10 cm) of Dowex 1-X2 (SO_4^{2-}) resin with water. Concentration of the eluate gave **18** (30 mg, 88%), $[\alpha]_D^{21}$ +63° (*c* 1, water), R_F 0.5 (t.l.c., 3:6:4 chloroform–methanol–aqueous 28% ammonia). ^{1}H-N.m.r. data (D_2O–DCl, pD ~1): δ 1.68 (slightly unresolved q, 1 H, H-2*a*), 1.88–2.12 (4 H,

H-3′a,3′b,4′a,4′b), 2.61 (dt, 1 H, J ~4, ~4, and 12 Hz, H-2*e*), 4.53 (dt, 1 H, J ~8, ~8, and ~4 Hz, H-5′), 5.18 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), and 5.67 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_9 \cdot 2\ H_2SO_4 \cdot 3\ H_2O$: C, 30.12; H, 6.60; N, 9.76; S, 8.94. Found: C, 30.29; H, 6.42; N, 9.48; S, 8.66.

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