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TRANSFORMATION OF KANAMYCIN A INTO 3′-DEOXYKANAMYCIN A*

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ABSTRACT

3′-Deoxykanamycin A has been prepared from kanamycin A. 6′-*N*-(Benzyloxycarbonyl)kanamycin A, prepared by applying the zinc chelation method, was *N*-tosylated, 4″,6″-*O*-cyclohexylidenated, and 4′-*O*,6′-*N*-carbamated. Acetonation of the carbamate gave the 2′,3′-*O*- and the 5,2′-*O*-isopropylidene derivative (the key intermediate). Imidazolylthiocarbonylation of the latter afforded the 3′,2″-bis(imidazolylthiocarbonyl) derivative, treatment of which with tributylstannane, followed by deprotection, gave 3′-deoxykanamycin A. It was noted that tributylstannane did not attack the 2″-(imidazolylthiocarbonyl) group.

INTRODUCTION

Studies[2,3] on the mechanism of resistance to aminoglycoside antibiotics have shown that clinically isolated resistant bacteria produce enzymes that phosphorylate, nucleotidylate, or acetylate special positions of the antibiotics, to produce inactive derivatives. In the case of kanamycins, they are, in most instances, phosphorylated at the 3′-hydroxyl group. Replacement of the 3′-hydroxyl group by a hydrogen atom was found to give a substantial improvement in the activity against resistant bacteria that produce 3′-*O*-phosphorylating enzymes, as shown by 3′-deoxykanamycin A (ref. 4), 3′-deoxykanamycin B (ref. 5) (tobramycin[6]), and 3′,4′-dideoxykanamycin B (ref. 7). Another kind of derivatization, which involves the introduction of an (*S*)-4-amino-2-hydroxybutanoyl group onto the 1-amino group involved in the binding[8] to the 3′-*O*-phosphorylating enzymes, was also found to give derivatives active against resistant bacteria, as shown by 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-kanamycin A (amikacin[9]). In a further study[10,11], amikacin was found to be inactivated by enzymes of some resistant strains that phosphorylate or adenylylate O-3′ or O-4′. This observation suggested that a combination of 3′-deoxygenation and the 1-*N*-acylation by the aforementioned amino acid residue would give a derivative of kanamycin A more improved than amikacin, and this concept has recently been verified by the antibacterial spectrum of 3′-deoxyamikacin[12].

*An outline of this work was read as a paper by T. Tsuchiya at the 5th Anniversary Symposium of Bioorganic Chemistry, Nov. 6, 1979, at the Tokyo Prince Hotel, see ref. 1.

From the synthetic point of view in order to prepare 3′-deoxyamikacin, it is necessary to establish for 3′-deoxykanamycin A (**13**) a high-yielding, synthetic process The total synthesis of 3′-deoxykanamycin A (ref 4) is not applicable to industrial processes, but we now describe a transformation of kanamycin A into its 3′-deoxy derivative as an approach to an industrial process

RESULTS AND DISCUSSION

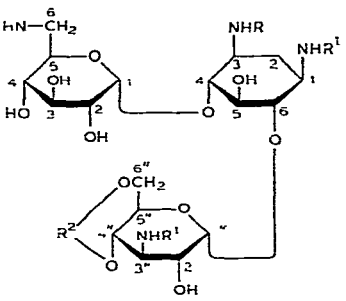
This synthesis of 3 -deoxykanamycin A was initially designed for preparing a derivative having the 3′-hydroxyl group free and all other functional groups protected, followed by dextrous removal of the 3′-hydroxyl group

The synthesis started with the selective 6′-*N*-(benzyloxycarbonyl)ation of kanamycin A Direct treatment of kanamycin A with *N*-(benzyloxycarbonyloxy)succinimide gave a mixture of *N*-benzyloxycarbonyl derivatives, although the 6′-*N*-(benzyloxycarbonyl)kanamycin A (**1**) was mainly obtained (~55%) after tedious separation, as expected from the results of Kawaguchi *et al* [9] Application of the recently developed, zinc chelation method[13] markedly improved the yield of **1** Kanamycin A suspended in dimethyl sulfoxide (Me_2SO) was dissolved therein by addition of zinc acetate and the solution was then treated with *N*-(benzyloxycarbonyloxy)succinimide As zinc ion forms a complex with kanamycin A in such a way as to protect the 1- and 3″-amino groups[13] simultaneously, the succinimide ester reacted mainly with the free 6′-amino group, to give **1** (78%) Removal of the zinc ion was successfully achieved by use of a carboxylic acid, cation-exchange resin The use of hydrogen sulfide was not advantageous in this case

The amino groups on C-1, -3, and -3″ of **1** were then tosylated with *p*-toluenesulfonyl chloride, to give **2** in 98% yield The reasons for the use of *N*-tosyl protection are as follows (*1*) this protection is comparatively stable for most of the chemical transformations, (*2*) the *N*-tosyl groups can be readily removed with sodium metal in liquid ammonia, and (*3*) the tosyl derivative has a higher solubility than the *N*-acyl or *N*-(benzyloxycarbonyl) derivatives in most organic solvents

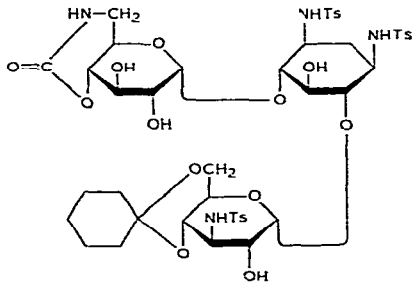
The hydroxyl groups on C-4″ and -6″ of **2** were selectively protected by an acetal-exchange reaction Treatment of **2** with 1,1-dimethoxycyclohexane in *N*,*N*-dimethylformamide (DMF) in the presence of an acidic catalyst at room temperature under strictly anhydrous conditions gave the 4″,6″-*O*-cyclohexylidene derivative (**3**) (98%) The 4′-hydroxyl group of **3** was then protected by formation of the 4′,6′-cyclic carbamate Treatment of **3** with sodium hydride in DMF gave the desired carbamate (**4**) (90%) The structure of **4** was proved by intercomparison of the i r spectra of **4** and **3**, the absorptions at 1700 (carbamate) and 1530 cm^{-1} (Amide II) in the spectrum of **3** changed to a single absorption at 1700 cm^{-1} in that of **4**, indicating that a *cis*-amide (cyclic carbamate) was present in **4**

In the next step, selective protection of the hydroxyl groups of **4** by acetal-exchange reactions was attempted, but this proved to be the most difficult part of the whole task Among the reagents tested, namely, 1,1-dimethoxyethane, benzaldehyde



	R^1	R^2
1	H	H H
2	Ts	H H
3	Ts	

Z = $PhCH_2OCO$



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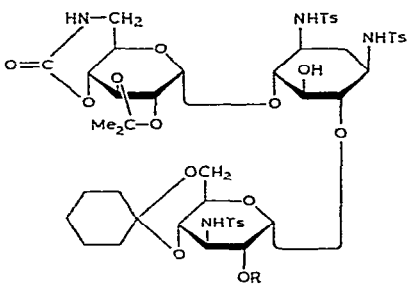
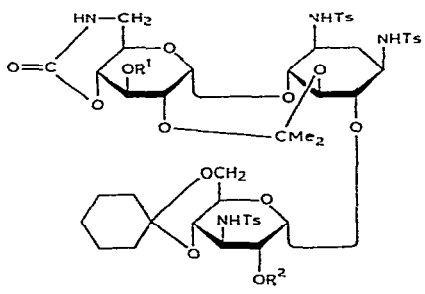


5 R = H

6 R = Ac

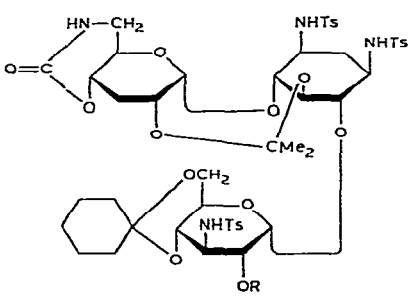


7 R^1 = R^2 = H

8 R^1 = R^2 = Ac

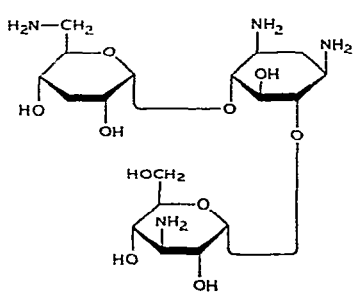
9 R^1 = Ac R^2 = H

10 R^1 = R^2 = 



11 R = 

12 R = H



13

dimethyl acetal, 1,1-dimethoxycyclohexane and 2,2-dimethoxypropane, the last reagent gave a promising product, the 5,2′-*O*-isopropylidene derivative (**7**). After many attempts to raise the yield of **7**, it was found that treatment of **4** with 2,2-dimethoxypropane in refluxing dichloromethane–oxolane (tetrahydrofuran, THF) containing a slight proportion of hydrogen chloride (by a devised procedure[1]) gave the highest yield (~40%) of **7**, although it was always accompanied by the 2′,3′-*O*-isopropylidene derivative (**5**). In this process, prolonged reaction decreased the proportion of **7** and increased that of **5**, and direct addition of molecular sieves to the reaction mixture stopped the reaction. The best experimental procedure found is described in the Experimental section. Separation of **5** from **7** was conveniently performed by treating the mixture with chloroform, because **5** is barely soluble in the solvent.

The structures of **5** and **7** were proved by examination of their acetyl derivatives (**6** and **8**). Acetylation of **5** gave the mono-*O*-acetyl derivative (**6**). In its ^{1}H-n.m.r. spectrum, the methine proton on the carbon atom bearing the acetoxyl group gave a double doublet (J 3.5 and 11 Hz) at δ 5.58. The signal-pattern indicated that acetylation occurred at O-2′ or -2″. If acetylation occurred at the former position, the isopropylidene group should bridge 3′-O and 2″-O or 3′-O and 3″-N, neither of which possibilities is reasonably feasible sterically. Therefore, the 2″-*O*-acetyl-2′,3′-*O*-isopropylidene structure was assigned to **6**. In the case of **7**, a *di*-*O*-acetyl derivative (**8**) was obtained. In its ^{1}H-n.m.r. spectrum, two methine protons, on carbon atoms bearing an acetoxyl group, appeared at δ 5.40 (10-Hz triplet) and 5.60 (3.5- and 11-Hz double doublet), respectively. As there were no signal deformations (as expected in the case of H-2 and -3′), it is clear that the methine groups are situated at separate positions. The acetyl groups of **8** should, therefore, be positioned at O-2′ and -5, O-2″ and -5, or O-3′ and -2″. The first and the second situations are impossible from the steric point of view, because the isopropylidene group could not bridge the relevant oxygen atoms. Thus, the 3′,2″-di-*O*-acetyl-5,2′-*O*-isopropylidene structure is assigned to **8**, indicating a novel acetalation between hydroxyl groups on two rings.

Another proof of the structure of **7** was obtained by partial hydrolysis of the 3′,2″-di-*O*-acetyl derivative (**8**). Brief treatment of **8** with aqueous ethanol containing ammonia gave the 3′-*O*-acetyl derivative (**9**) without formation of the nonacetylated compound (**7**). This finding, that is, the selective removal of the 2″-*O*-acetyl group, also proved valuable in the present synthesis, because **9** can lead to a compound that has the 3′-hydroxyl group free, with all other functional groups protected. However, preparation of this derivative was not necessary, because the 3′,2″-dihydroxy compound (**7**) could successfully be converted into the corresponding 3′-deoxy derivative (**12**) as described later.

There have been only a few reports of compounds having an acetal bridge between two rings. Khan *et al.*[14,15] described the 2,1′-*O*-isopropylidene derivatives of sucrose and 6,6′-dichloro-6,6′-dideoxysucrose, formed by acid-catalyzed, acetal-exchange reactions. Thiem *et al.*[16] reported that the acetals obtained from the reaction of methyl β-cellobioside with benzylidene halide in pyridine have acetal structures

bridging the 3- and 2′-hydroxyl groups Recently, Horton *et al*[17] described a 6,2-*O*-isopropylidenelactose derivative, obtained by treatment of lactose with 2-methoxypropene In the present investigation, formation of the 5,2′-acetal (**7**) is explicable by the steric character of kanamycin A, in which the 5- and 2′-hydroxyl groups come into close proximity, as has been shown by X-ray crystal-structure analysis[18] of kanamycin A monosulfate

Treatment of **7** with thiocarbonylbis(imidazole) in THF gave the 3′,2″-bis-*O*-(imidazolylthiocarbonyl) derivative (**10**) (68%), which was then treated with tributylstannane in THF (instead of toluene[19]), according to the method reported by Barton and McCombie[19] The 3′-deoxy-2-*O*-(imidazolylthiocarbonyl) derivative (**11**) was obtained in high yield (86%) In the ^{1}H-n m r spectrum of **11** the absorptions caused by the methine at C-2″ [bearing the (imidazolylthiocarbonyl)oxy group] remained at δ 6 27, but the absorptions caused by the methine bearing the 3′-(imidazolylthiocarbonyl)oxy group (discerned in the spectrum of **10**) disappeared This observation indicated that the 2″-*O*-(imidazolylthiocarbonyl) group was not affected by tributylstannane On treatment of **11** with aqueous ammonia, the imidazolylthiocarbonyl group was readily removed to give the 3′-deoxy derivative (**12**) in good yield Finally detosylation of **12** with sodium metal in liquid ammonia, followed by deprotection, gave 3′-deoxykanamycin A (**13**) (67%) In the ^{1}H-n m r spectrum, the synthetic 3′-deoxykanamycin A gave signals assignable to the 3′-methene protons at δ 1 5–2 3 The R_F value (2 2 in p p c) and the antibacterial activity of the final compound were the same as those of the compound prepared by total synthesis[4]

EXPERIMENTAL

General methods — Optical rotations were determined with a Perkin–Elmer 241 polarimeter Except for **1** and **13** t l c was performed on Wakogel B-5 silica gel, using a sulfuric acid spray for detection, for **1** and **13**, precoated Kieselgel 60 (Art 5721) was used, with detection by spraying with 0 25% ninhydrin in pyridine followed by heating P p c was performed on Toyo–Roshi paper No 50, with detection with ninhydrin as just described Column chromatography was performed on Wakogel C-200 silica gel U v spectra were recorded with a Hitachi 200-10 spectrophotometer, and i r spectra, with a Hitachi 285 grating spectrometer ^{1}H-N m r spectra were recorded at 90 MHz with a Varian EM-390 spectrometer

6′-N-(*Benzyloxycarbonyl*)*kanamycin A* (**1**) — To a suspension of kanamycin A monocarbonate monohydrate (100 mg) in dry Me_2SO (1 5 mL) was added zinc acetate dihydrate (180 mg, prewashed with THF to remove acetic acid as a slight contaminant, 4 5 mol equiv) To the resulting, clear solution was added *N*-(benzyloxycarbonyloxy)succinimide (45 mg) in small portions during 1 h, and the mixture was kept for a further 1 h at room temperature Addition of ether (8 mL) gave a syrup, which was thoroughly washed with ether, to give a solid (~370 mg) The solid was charged onto a column (13 mL) of Amberlite CG 50 (H^+) resin (100–200 mesh), and the column was washed with water (150 mL), and developed with 0 5→1M aqueous

ammonia The ninhydrin-positive fractions eluted were evaporated (zinc ion remaining in the column) The residue was chromatographed on a column of Diaion HP-20AG resin (high-porous styrene–divinylbenzene copolymer without functional groups Mitsubishi Chemical Ind Ltd Tokyo; 100–200 mesh, 15 mL, pre-treated overnight with 10 l water–ethanol) with water–ethanol 10 l→1 2, gradually changed Kanamycin A (slight) **1**, and bis-*N*-(benzyloxycarbonyl)kanamycin A (slight) were eluted, in that order The fractions containing **1** were evaporated, to give solid **1** as a carbonate (3/2 × 1/4 carbonate best fitted the analytical values), 89 mg (78%). R_F 0 5 (t l c with 4 7 2 7 1-propanol–ethanol–chloroform–17% aqueous ammonia). $[\alpha]_D^{25}$ +105° (*c* 0 5 water) (lit [9] +116°, as base monohydrate)

Anal Calc for $C_{26}H_{42}N_4O_{16}$ 3/8 H_2CO_3 C 49 25 H, 6 71 N, 8 73 Found C, 49 12 H 6 83, N, 8 46

*6'-*N*-(Benzyloxycarbonyl)-1,3 3"-tri-*N*-tosylkanamycin A* (**2**) — A mixture of **1** 3/8 carbonate (1 80 g) anhydrous sodium carbonate (1 10 g), and *p*-toluenesulfonyl chloride (1 99 g) in 1 3 water–1,4-dioxane (50 mL) was stirred overnight at room temperature Concentration followed by addition of water, gave a precipitate, which was successively washed with ether and water, and dried at 60° *in vacuo*, to give a solid, 2 98 g (98%) $[\alpha]_D^{25}$ +10° (*c* 0 4, acetone) ^{1}H-n m r data (pyridine-d_5) δ 2 10, 2 32 and 2 36 (s each, 3 H 3 Me of 3 Ts), 5 21 and 5 27 (s each, main part of AB q of -CO_2CH_2Ph), and 5 40 and 5 52 (d each 1 H, J ~3 Hz, H-1',1")

Anal Calc for $C_{47}H_{60}N_4O_{19}S_3$ C, 52 21, H 5 59, N, 5 18 S, 8 90 Found C 52 10, H, 5 56 N 5 12, S, 8 78

*6'-*N*-(Benzyloxycarbonyl)-4",6'-*O*-cyclohexylidene-1,3 3"-tri-*N*-tosylkanamycin A* (**3**) — A solution of **2** (1 29 g) 1,1-dimethoxycyclohexane (0 86 mL, 5 mol equiv.) and *p*-toluenesulfonic acid (45 mg of monohydrate was dried for 1 h at 100° *in vacuo)* in DMF (6 mL, dried over molecular sieves 4A) was kept for 2–7 h* at room temperature until t l c (6 1 chloroform–ethanol) of the mixture gave a single spot (R_F 0 4) When the reaction time was shorter **2** (R_F 0 06) remained, and, when longer, overcyclohexylidenated products (R_F 0 53, 0 56 and 0 85) appeared to some extent In the latter case, addition of water (7–20 mg), followed by standing at room temperature for several hours, hydrolyzed most of the undesirable by-products to **2** The solution was poured into half-saturated, aqueous sodium hydrogencarbonate solution (600 mL), and the precipitate was isolated by centrifugation, washed thoroughly with water, and dried, to give a solid, 1 35 g (98%), $[\alpha]_D^{25}$ +0° (*c* 0 5, acetone), ν_{max}^{KBr} 1700 and 1530 cm^{-1} ^{1}H-n m r data (pyridine-d_5) δ 2 20 (s 3 H, Ts) and 2 35 (s, 6 H, Ts)

Anal Calc for $C_{53}H_{68}N_4O_{19}S_3$ C 54 81, H 5 90, N 4 82, S, 8 28 Found C, 54 89 H, 6 00, N, 4 63 S 8 04

*6'-*N*,4'-*O*-Carbonyl-4",6"-*O*-cyclohexylidene-1,3,3"-tri-*N*-tosylkanamycin A* (**4**) — To an ice-cold solution of **3** (911 mg, 0 79 mmol) in dry DMF (18 mL) was added

*The degree of dryness of the materials, the solvent, the reagents, and the vessels used seems to determine the reaction period required

50% (in oil) sodium hydride [~170 mg (9 mmol) as net NaH], and the mixture was stirred for 1 h under an atmosphere of nitrogen in the cold, and then overnight at room temperature. T.l.c. (6:1 chloroform–ethanol) of the solution showed a single spot (R_F 0.15, *cf.* **3**, 0.4). Addition of 25% acetic acid (3.5 mL), and evaporation *in vacuo*, with occasional addition of toluene, gave a syrupy residue which, on addition of water, solidified. The solid was washed with ether, and then thoroughly with water, and dried. 745 mg (90%), $[\alpha]_D^{25}$ −38° (*c* 0.5, acetone), ν_{max}^{KBr} 1700 cm^{-1}. ^{1}H-n.m.r. data (pyridine-d_5): δ 2.22, 2.32 and 2.37 (s, each 3 H, Ts).

Anal. Calc. for $C_{46}H_{60}N_4O_{18}S_3$: C, 52.46; H, 5.74; N, 5.32; S, 9.13. Found: C, 52.54; H, 5.88; N, 5.09; S, 8.80.

6′-N,4′-O-Carbonyl-4″,6″-O-cyclohexylidene-2″,3″-O-isopropylidene-1,3,3″-tri-N-tosylkanamycin A (**5**). — A reaction flask containing **4** (2.5 g, 2.37 mmol), 2,2-dimethoxypropane (50 mL), 0.08% HCl in dichloromethane (250 mL), and THF (50 mL, distilled from lithium aluminum hydride) was connected to a Soxhlet type of apparatus[1] fitted with a reflux condenser, the Soxhlet extractor being filled with molecular sieves 5A (120 mL, activated at 220° under a stream of nitrogen). The mixture was refluxed violently for 17 min, with vigorous stirring in an oil bath at 110°. Methanol liberated during the reaction was efficiently removed by the sieves. The resulting, pale-brown solution was instantaneously cooled, and poured into 2% ammonia in 1:15 water–1,4-dioxane (300 mL). Evaporation of the mixture, with occasional addition of 2% ammonia in aqueous 1,4-dioxane, gave a syrupy residue which, on addition of ether, solidified (2.48 g after drying). T.l.c. (5:1 chloroform–ethanol) of the solid showed a major spot (R_F 0.37) and two slight spots, R_F 0.22 (**4**) and 0.60. Column chromatography [100 g of silica gel, eluant, 10:1→7:1 chloroform–ethanol (gradually changed)] of the solid gave a chromatographically homogeneous solid, 1.88 g (73%) (R_F 0.37) which was dissolved in chloroform. The solution (180 mL) was heated, with stirring, whereupon a precipitate gradually appeared; this was filtered off, washed with chloroform, and dried. 717 mg (28%), $[\alpha]_D^{25}$ −26° (*c* 0.5, acetone), ^{1}H-n.m.r. data (pyridine-d_5): δ 1.50 and 1.53 [sharp s, each, $C(CH_3)_2$; the singlets were overlapped with the signals of cyclohexylidene protons, δ 1–1.7], 2.22, 2.30, and 2.37 (s, each, 3 H, Ts), 5.56 (d, 1 H, *J* 3 Hz, H-1″), and 6.35 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{49}H_{64}N_4O_{18}S_3$: C, 53.83; H, 5.90; N, 5.13; S, 8.80. Found: C, 53.93; H, 5.95; N, 4.89; S, 8.88.

6′-N,4′-O-Carbonyl-4″,6″-O-cyclohexylidene-5,2′-O-isopropylidene-1,3,3″-tri-N-tosylkanamycin A (**7**). — The chloroformic filtrate (see previous paragraph) and the chloroform washings were combined, and evaporated, giving a residue which was re-treated with hot chloroform (150 mL) as described for **5**. This time slight precipitation (owing to **5**) was observed. Filtration, followed by evaporation of the filtrate gave solid **7**, 1.02 g (40%), $[\alpha]_D^{25}$ +20° (*c* 0.4, acetone). This product still contained a small proportion of **5**, but was used, without purification, for the next experiments. For obtaining pure **7**, purified diacetyl derivative **8** was treated with 1:1 28% aqueous ammonia–ethanol overnight at room temperature (yield, quantitative), $[\alpha]_D^{25}$ +23°

(*c* 0 4, acetone). ^{1}H-n m r data (pyridine-d_5) δ 1 78 [s 6 H, C(CH$_3$)$_2$], 2 25 (s, 6 H, Ts), 2 40 (s 3 H Ts), and 5 86 and 6 05 (s each, 1 H H-1′,1″)

2″-O-Acetyl-6′-N,4′-O-carbonyl-4′ 6″-O-cyclohexylidene-2′,3′-O-isopropylidene-1,3,3″-tri-N-tosylkanamycin A (**6**) — Compound **5** (74 mg) in pyridine (1 1 mL) was treated with acetic anhydride (0 04 mL) in the usual way, to give a solid, 75 2 mg (98%), $[\alpha]_D^{23}$ 0° (*c* 0 5 acetone), ^{1}H-n m r data (pyridine-d_5) δ 1 50 [s, 6 H, C(CH$_3$)$_2$], 2 27 (s, 6 H), 2 37 (s, 3 H) and 2 40 (s, 3 H, Ac and Ts), 5 55 (dd, 1 H, $J_{1,2}$ ~3 5 $J_{2,3}$ 11 Hz H-2″), 6 15 (d, 1 H, *J* 4 Hz, H-1″?), and 6 31 (d, 1 H, *J* 3 Hz, H-1′?)

Anal Calc for $C_{51}H_{66}N_4O_{19}S_3$ C, 53 95 H 5 86, N 4 94, S, 8 47 Found C 53 75 H, 5 96, N 4 71 S 8 15

3′,2″-Di-O-acetyl-6′-N 4′-O-carbonyl-4″,6″-O-cyclohexylidene-5,2′-O-isopropylidene-1 3,3′-tri-N-tosylkanamycin A (**8**) — A solution of 7 (210 mg) and acetic anhydride (0 11 mL) in pyridine (4 2 mL) was kept overnight at room temperature, and evaporated to a syrup which was washed with water to give a solid Column chromatography (eluant, 10 7→10 8→10 9 chloroform–acetone) gave pure **8** (R_F 0 5 in 5 4 chloroform–acetone, *cf* **6** R_F 0 4), 180 mg (80%), $[\alpha]_D^{25}$ +76° (*c* 0 4, acetone) ^{1}H-n m r data (pyridine-d_5) δ 1 80 and 1 87 [s each, 3 H, C(CH$_3$)$_2$], 2 15 (s 3 H) 2 28 (s 6 H), 2 38 (s, 3 H) and 2 58 (s, 3 H, Ac and Ts), 5 40 (t 1 H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3′) 5 60 (dd, 1 H $J_{1,2}$ 3 5, $J_{2,3}$ 11 Hz, H-2″), 5 87 (d, 1 H, $J_{1,2}$ 3 5 Hz H-1′), and 6 67 (d 1 H *J* 3 5 Hz, H-1″) Irradiation at δ 6 67 caused the quartet of H-2″ to collapse to a doublet (*J* ~10 Hz)

Anal Calc for $C_{53}H_{68}N_4O_{20}S_3$ C 54 07 H 5 82, N 4 76 S, 8 17 Found C, 54 00, H, 5 96 N, 4 56 S, 7 78

3′-O-Acetyl-6′-N 4′-O-carbonyl-4′,6″-O-cyclohexylidene-5 2′-O-isopropylidene-1,3 3″-tri-N-tosylkanamycin A (**9**) — A solution of **8** (54 6 mg) in 1 1 28% aqueous ammonia–ethanol (5 mL) was kept for 35 min at 25°, immediately evaporated *in vacuo*, and the residue washed thoroughly with water, to give a solid, 47 9 mg (92%), $[\alpha]_D^{25}$ +49° (*c* 0 3, acetone) ^{1}H-n m r data (pyridine-d_5) δ 1 69 [s, 6 H, C(CH$_3$)$_2$], 2 11, 2 21, 2 27, and 2 53 (s each 3 H Ac and Ts), 5 41 (t, 1 H, *J* 10 Hz, H-3′), 5 88 (d, 1 H, *J* 3 5 Hz, H-1′), and 6 07 (d, 1 H, *J* 3 5 Hz, H-1″)

Anal Calc for $C_{51}H_{66}N_4O_{19}S_3$ C, 53 95 H 5 86 N 4 94 S, 8 47 Found C 53 66, H, 5 85 N, 4 68, S, 8 80

6′-N,4′-O-Carbonyl-4″ 6″-O-cyclohexylidene-3′,2′-bis-O-(imidazolylthiocarbonyl)-5,2′-O-isopropylidene-1,3,3″-tri-N-tosylkanamycin A (**10**) — A solution of 7 (803 mg, ~0.73 mmol dried for 2 h at 75° *in vacuo* in the presence of CaH$_2$) and thiocarbonylbis(imidazole) (782 mg 4 4 mmol) in THF (12 mL, distilled from lithium aluminum hydride) was placed in a pressure tube and, after dry nitrogen had been bubbled through the solution for a while, the tube was stoppered, covered with aluminum foil and heated for 12 h at 55° Evaporation gave a syrupy residue which was thoroughly washed with ether to afford a solid (955 mg) A solution of the solid in hot, 25 1 chloroform–ethanol (52 mL) was kept in a refrigerator for 2 h, and the resulting precipitate was filtered off washed with chloroform, and dried

(548 mg) The mother liquor was evaporated, and the residue was re-treated, as already described, with chloroform–ethanol (26 mL), to give a second crop (105 mg) (total 68%), R_F 0 3 (t l c with 1 1 chloroform–acetone), $[\alpha]_D^{25}$ +130° (c 0 2, acetone), λ_{max}^{MeOH} 274 (ε_{mM} 17 4) (imidazolylthiocarbonyl[19]), 229 (40 2), and 204 nm (40) (Ts), ^{1}H-n m r data (100 0 7–1 pyridine-d_5–D_2O) δ 1 85 and 1 90 [s each, 3 H, $C(CH_3)_2$], 2 23, 2 26, and 2 62 (s each, 3 H, Ts), 6 01 (d, 1 H, J 3 5 Hz, H-1′), 6 05–6 35 (m, 2 H, H-3′,2″), 6 91 (d, 1 H, J 3 5 Hz, H-1″), 7 02 and 7 12 (s each, 1 H, H-4 of imidazole), and 8 90 (s, 2 H, H-2 of imidazole)

Anal Calc for $C_{57}H_{68}N_8O_{18}S_5$ C, 52 12, H, 5 22, N, 8 53, S, 12 00 Found C, 51 96. H, 5 26, N, 8 37 S, 11 92

6′-N,4′-O-Carbonyl-4″,6″-O-cyclohexylidene-3′-deoxy-2″-O-(imidazolylthiocarbonyl)-5,2′-O-isopropylidene-1,3 3″-tri-N-tosylkanamycin A (**11**) — Dry nitrogen was bubbled for a while into a mixture of **10** (582 mg, 0 45 mmol) and tributylstannane (1 165 g, 6 7 mmol) in dry THF (16 mL) in a pressure tube, the tube then was stoppered, vigorously vibrated (Taiyo mixer S-5F) for a short time and the resulting, almost clear, solution heated for 3 h at 110° Cooling, followed by evaporation of the solution, gave a syrup, which was vigorously shaken with ether (5 × 20 mL), to give a solid that was dissolved in chloroform The solution was washed with water, dried (Na_2SO_4), evaporated, and the residue dissolved in a small volume of acetone, and reprecipitated by addition of cyclohexane, 450 mg (86%), t l c (7 1 chloroform–ethanol) R_F 0 65 (*cf*, **10** R_F 0 3), $[\alpha]_D^{25}$ +64° (c 0 3 acetone), λ_{max}^{MeOH} 266 (ε_{mM} 10 3) 230 (59 3), and 204 nm (56 0) ^{1}H-n.m r data (100 0 7–1 pyridine-d_5–D_2O) δ 1 87 and 2 00 [s each, 3 H, $C(CH_3)_2$], 2 27 (s, 6 H, Ts) 2 52 (s, 3 H, Ts), 5 78 (incomplete d, 1 H, H-1′), 6 27 (dd, 1 H, $J_{1\ 2}$ 3 5, $J_{2\ 3}$ 10 Hz, H-2″), 6 97 (d, 1 H, J 3 5 Hz, H-1″), 7 17 (s, 1 H, H-4 of imidazole), and 8 98 (s, 1 H, H-2 of imidazole)

Anal Calc for $C_{53}H_{66}N_6O_{17}S_4$ C 53 61 H, 5 60 N 7 08 S, 10 80 Found C, 53 81, H, 5 76, N, 6 81, S, 10 91

6′-N,4′-O-Carbonyl-4′,6″-O-cyclohexylidene-3′-deoxy-5,2′-O-isopropylidene-1,3,3″-tri-N-tosylkanamycin A (**12**) — A solution of **11** (285 mg) in a mixture of 28% aqueous ammonia (4 mL), THF (1 mL) and ethanol (2 5 mL) was kept for 5 h at room temperature, and evaporated, and the residue was washed with water to give a solid, 252 mg (97%) T l c analysis (7 1 chloroform–ethanol) indicated that it was mainly composed of **12** (R_F 0 54), with a trace of a by-product (R_F 0 6, the same as that of **11**) Column chromatography (eluant 11 1→6 1 chloroform–ethanol) gave **12** (208 mg, 80%), the by-product* (12 mg), and a mixture of the two (~10 mg) For compound **12** $[\alpha]_D^{25}$ +34° (c 0 5, acetone) ^{1}H-n m r data (pyridine-d_5) δ 1 75 [s, 6 H, $C(CH_3)_2$], 2 22, 2 26, and 2 47 (s each, 3 H, Ts), 5 79 (incomplete d, 1 H, H-1′), and 6 08 (d, 1 H, J 3 5 Hz, H-1″)

Anal Calc for $C_{49}H_{64}N_4O_{17}S_3$ C, 54 63, H, 5 99, N, 5 20, S, 8 93 Found C, 54 37, H, 6 17, N, 5 42, S, 9 03

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*Judging from its ^{1}H-n m r spectrum this product had no imidazolylthiocarbonyl group, and seemed to be the 3′ 2′-dideoxy derivative

3'-Deoxykanamycin A (**13**) — Sephadex LH-20 (2 mL, swollen with acetone) was packed into a column and washed with 0 5% hydrochloric acid in acetone, and then thoroughly with acetone A solution of **12** (150 mg) in acetone was passed through the column, eluted with acetone, and the eluate evaporated To a solution of the residue (148 mg, well dried) in liquid ammonia (~12 mL) at −50° was added sodium metal (~200 mg), and the deep-blue solution was kept for 1 5 h at that temperature Addition of 99 1 THF–water until the solution became colorless, followed by gradual warming to room temperature, and evaporation under diminished pressure, gave a glassy residue An aqueous solution (~5 mL, strongly alkaline) of the residue was heated for 1 h at 85° (to hydrolyze the cyclic carbamate), and cooled, Dowex-50W X2 (H^+) resin (5 mL) was added, and the mixture was poured into a column containing the same resin (5 mL) The column was washed with water, eluted with M aqueous ammonia, and the eluate evaporated, giving a residue (75 mg) which was dissolved in 80% aqueous acetic acid (5 mL), and the solution heated for 1 5 h at 80° (to cleave the isopropylidene and cyclohexylidene groups) Evaporation, with occasional addition of toluene, gave a residue that was chromatographed on a column of CM-Sephadex C-25 (NH_4^+) (18 mL) with aqueous ammonia (0 03→ 0 15M, gradually changed) The fractions containing 3'-deoxykanamycin A (**13**) [checked by descending p p c with 6 4 3 1 1-butanol–pyridine–water–acetic acid ($R_{\text{kanamycin A}}$ 2 0) and t l c with 4 7 2 7 1-butanol–ethanol–chloroform–17% NH_4OH ($R_{\text{kanamycin A}}$ 1 6)] were evaporated, to give **13** as its carbonate, 49 5 mg (67%), $[\alpha]_D^{25}$ +119° (*c* 0 5, water) (lit [4] +146°, as free base), ^{1}H-n m r data (D_2O containing 2 5% of ND_3) δ 1 20 (q, 1 H, *J* 12 Hz, H-2*a*), 1 5–2 3 (m, 3 H, H-2*e*,3'*a*, 3'*e*) [widely separated signals (*J* ~12 Hz) appeared between δ 1 5–2 0, suggesting that H-3'*a* has a shift value of δ ~1 8], 5 05 (d, 1 H, *J* 3 5 Hz, H-1' or 1''), and 5 22 (d, 1 H, *J* 3 5 Hz, H-1'' or 1')

Anal Calc for $C_{18}H_{36}N_4O_{10}$ H_2CO_3 C, 43 01, H, 7 22, N, 10 56 Found C, 43 00, H, 7 49, N, 10 60

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