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Synthesis of 4′-deoxy-4′-fluorokanamycin A and B

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ABSTRACT

4′-Deoxy-4′-fluorokanamycins A (**17**) and B (**25**) have been prepared through fluorinative ring-opening of the D-*galacto*-3′,4′-oxiranes (**8** and **21**) derived from kanamycin A and B with potassium hydrogenfluoride in ethane-1,2-diol. The mechanism of preponderant formation of the 4′-deoxy-4′-fluoro-D-gluco (**9** and **22**) over the 3′-deoxy-3′-fluoro-D-gulo derivatives was discussed. In the synthesis of **25**, the unusual 3′,6′-epimine (**23**) was the main product along with the 4′-deoxy-4′-fluoro derivative. The mechanism of this reaction is also discussed. Both **17** and **25** were active against resistant bacteria producing aminoglycoside-adenylylating enzymes for HO-4′.

INTRODUCTION

In the foregoing papers [1–4] we reported the synthesis of several 3′-deoxy-3′-fluoro derivatives of kanamycins; they were 3′-deoxy-3′-fluorokanamycin A (refs. 1–3), 3′,4′-dideoxy-3′-fluorokanamycin A (ref. 2), 3′-deoxy-3′-fluorokanamycin B (refs. 1, 4), and 3′,4′-dideoxy-3′-fluorokanamycin B (ref. 4). All of them were active against resistant bacteria producing enzymes phosphorylating or adenylylating the 3′-hydroxyl group because of their lack of HO-3′; the 4′-deoxy derivatives were additionally active against bacteria modifying the 4′-hydroxyl group. Here we describe the synthesis of 4′-deoxy-4′-fluorokanamycin A (**17**) and B (**25**), which were expected to be active against resistant bacteria modifying [5] the 4′-hydroxyl group. As in the synthesis [3] of 3′-deoxy-3′-fluorokanamycin A, the fluorine could successfully be introduced through ring-opening of a D-*allo*-2′,3′-oxirane derivative of kanamycin A using potassium hydrogenfluoride (KHF_2), a similar fluorination was applied in the present synthesis.

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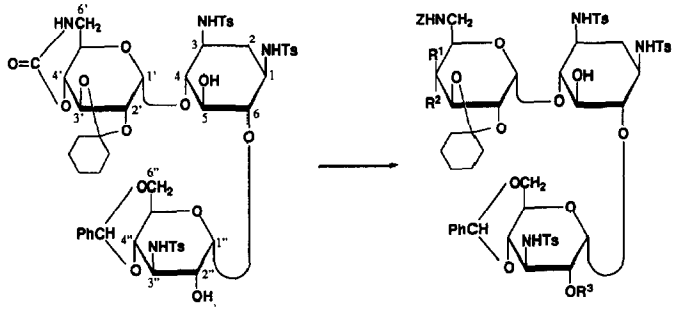
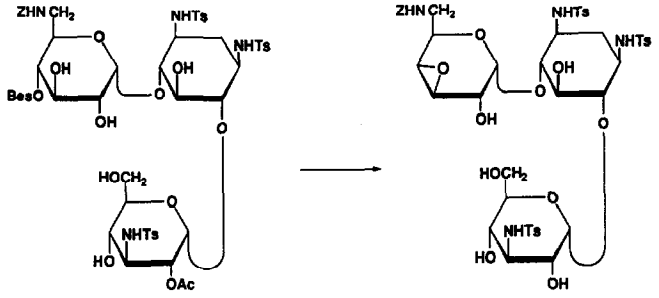


1

	R^1	R^2	R^3
2	H	OH	H
3	H	OH	Ac
4	OH	H	Ac
6	H	OBes	Ac



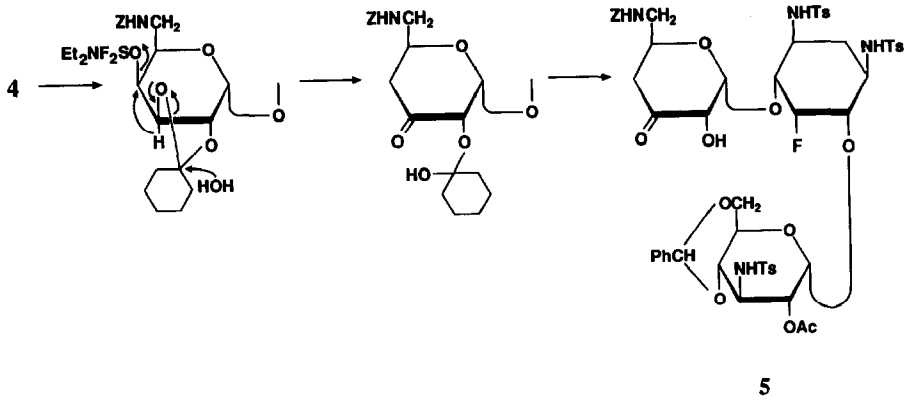
7 8

Z: CO_2CH_2Ph
Bes: SO_2CH_2Ph

RESULTS AND DISCUSSION

4″,6″-*O*-Benzylidene-6′-*N*:4′-*O*-carbonyl-2′,3′-*O*-cyclohexylidene-1,3,3″-tri-*N*-tosylkanamycin A (**1**; ref. 6) chosen as the starting material was converted into the 6′-*N*-(benzyloxycarbonyl) derivative **2** by alkaline cleavage of the cyclic carbamate, followed by 6′-*N*-benzyloxycarbonylation. Acetylation of **2** with *N*-acetylimidazole in 1:9 pyridine–Me_2SO *,[1,7] gave selectively the 2″-*O*-acetyl derivative **3** having HO-4′ free in 91% yield. In our first experiment to prepare **17**, the 4′-triflate of **3**

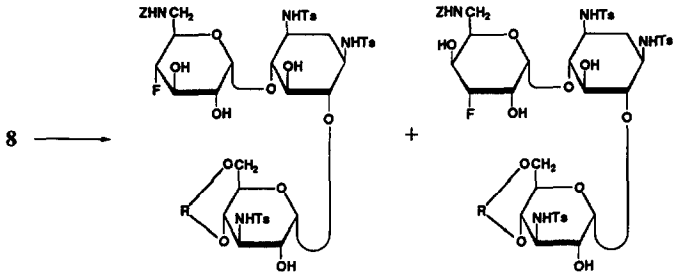
* This selective acetylation method was first discovered by Tomo Nishimura and T. Tsuchiya of our laboratory.



Scheme 1.

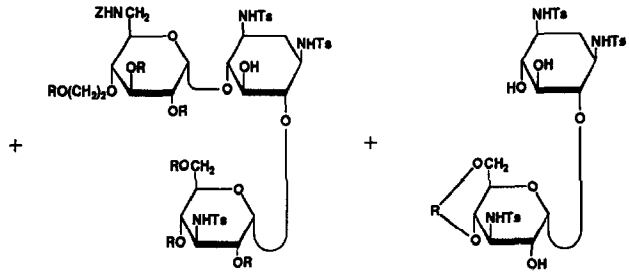
was converted into the 4′-epi derivative **4** by treatment [8] with sodium nitrite in *N*,*N*-dimethylformamide (DMF) (the 4′-epi structure of **4** was confirmed by the small $J_{3',4'}$ and $J_{4',5'}$ values together with the $J_{4',OH}$ coupling) and fluorination of **4** was attempted with diethylaminosulfur trifluoride (DAST). However, no clear 4′-deoxy-4′-fluoro derivative was obtained; instead, compound **5**, having the 4′-deoxy-3′-oxo structure and a 5-epi-fluorine group was formed. Its structure was confirmed by the ^{1}H-, ^{19}F-, and ^{13}C-NMR (C-3′: δ 204.6) spectra, as well as by the resemblance of the *J* values with those for methyl 2-*O*-benzoyl-4-deoxy-6-*O*-trityl-α-D-*erythro*-hexopyranosid-3-ulose [9]. A proposed mechanism involving a hydride shift is shown in Scheme 1. We therefore changed the route to one involving 3′,4′-oxirane ring-opening. 4′-*O*-Benzylsulfonylation of **3** (to give **6**) followed by acid-catalyzed deacetalation gave the pentol **7**, which was treated with sodium methoxide in methanol to give the D-*galacto*-3′,4′-oxirane **8**. The structure was confirmed by its ^{1}H-NMR spectrum; the ground-state conformation of the sugar unit having the oxirane-ring was assumed to be $^0H_1$ by comparison of the *J* values with those of similar compounds reported [10,11], as well as by the long-range H-1′–H-3′ coupling.

Treatment of **8** with KHF_2 in ethane-1,2-diol at 150° (the conditions being similar to those reported [3] for the preparation of 3′-deoxy-3′-fluorokanamycin A from the corresponding D-*allo*-2′,3′-oxirane) gave a 5:1 mixture of products (**9** and **10**) having the 4′-deoxy-4′-fluoro-α-D-glucopyranosyl and 3′-deoxy-3′-fluoro-α-D-gulopyranosyl structures, respectively. Also formed were the 2-deoxy-6-*O*-(3-deoxy-3-tosylamido-α-D-glucopyranosyl)-1,3-di-*N*-tosylstreptamine [7] (**12**) and a 4′-*O*-(2-hydroxyethyl) derivative **11**, formed by incorporation of a solvent molecule. The structure of **11** was confirmed by the lowfield resonance of C-4′ in the ^{13}C-NMR spectrum, as well as by the physical data for the corresponding hexa-*O*-acetyl derivative **15**. The presence of 4′- and 3′-fluorine substituents in the main (**9**) and minor products **10** was proved, respectively, by sequential analysis of signals in the ^{1}H-NMR spectrum, starting from the H-1′ signal, aided by the shift-correlated 2D spectrum. Also the equatorial and axial orientations of fluorine of **9** and **10**,



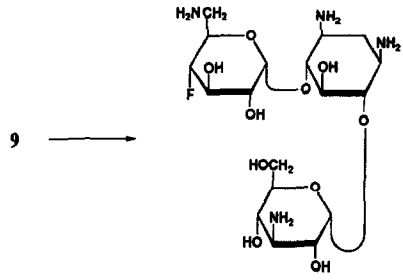
9 R = H, H
13 R = PhCH

10 R = H, H
14 R = PhCH



11 R = H
15 R = Ac

12 R = H, H
16 R = PhCH



17

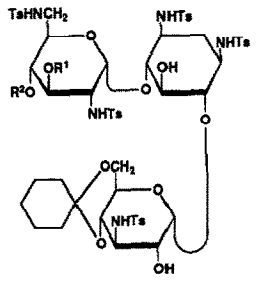
respectively, were determined by the signal-splitting patterns in their ^{1}H- and ^{19}F-NMR spectra (**9**: $J_{4',F}$ 51 and $J_{3',F}$ 16 Hz; **10**: $J_{2',F}$ 34, $J_{3',F}$ 49, and $J_{4',F}$ 7 Hz).

Deprotection of the mixture (**9** and **10**) with sodium in liquid ammonia followed by chromatographic separation of the products gave only the desired 4′-deoxy-4′-fluorokanamycin A (**17**). In advance of this reaction, we tried to separate **9** and **10**; thus, after 4″,6″-*O*-benzylidenation of the mixture (to give **13** and **14**), the mixture was treated [12] with sodium hydride in DMF, expecting that only the benzylidene derivative **14** of **10** could be converted into its 6′-*N*:4′-*O*-carbonate, with **13** remaining intact, facilitating the separation of the resulting products because of differences in mobility. However, this treatment gave only a mixture of **13** and the

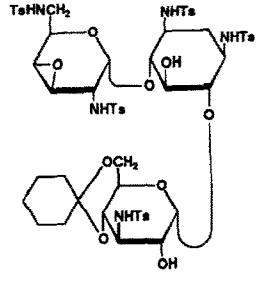
4″,6″-*O*-benzylidene derivative (**16**) of **12**. The mechanism of this unexpected reaction was not pursued, but probably the presence of *trans*-diaxial F-3′ and OH-4′ in **14** destabilizes the structure, resulting in cleaving the fluoro sugar component by the alkaline treatment. However, the 4-deoxy-4-fluoro-D-glucose component of **9** was also partially cleaved by this alkaline treatment (see yields in the Experimental section). This kind of cleavage might also occur in the treatment of **10** with sodium in liquid ammonia.

4′-Deoxy-4′-fluorokanamycin B (**25**) was also prepared through a similar synthetic route. Treatment of 4″,6″-*O*-cyclohexylidene-1,3,2′,6′,3″-penta-*N*-tosylkanamycin B (**18**; ref. 13) with a limited amount of phenylmethanesulfonyl chloride gave the 4′-*O*- (major, **20**) and 3′-*O*-benzylsulfonyl derivatives **19**. Treatment of **20** with sodium methoxide in methanol as described for **8** gave the corresponding D-*galacto*-3′,4′-oxirane (**21**), whose structure was confirmed by the ^{1}H- and ^{13}C-NMR spectra. Treatment of **21** with KHF_2 as described for **9**, however, gave the desired 4′-deoxy-4′-fluoro-penta-*N*-tosylkanamycin B (**22**) in only 24% yield, along with a small amount of the 3′-deoxy-4′-epi-3′-fluoro derivative **24** and a non-fluorinated major product **23**. Changes in the solvent, using such solvents as propane-1,3-diol, diethylene glycol, methyl Cellosolve, diglyme, DMF, *N*,*N*-dimethylacetamide, or sulfolane, aiming to raise the yield of **22**, gave no **22** and gave only **23** or its 4″,6″-*O*-cyclohexylidene derivative. The fact that **23** was one of the final products was confirmed by further treatment of **23** with KHF_2, whereupon **23** was recovered unchanged.

The structures of **22** and **24** were confirmed by their ^{1}H- and ^{19}F-NMR spectra (see Experimental section), and **23** was determined to be a 3′,6′-epimine by its ^{1}H- and ^{13}C-NMR spectra; the relatively small vicinal couplings of all of the ring-protons of the diamino-D-glycosyl unit (the signals concerned could be discriminated from those of the other units by the shift-correlated 2D spectrum) suggests that the residue adopts the $^1C_4$ conformation. Furthermore, two deuterium-exchangeable protons, assignable to TsN*H*-2′ and *H*O-4′, were shown to couple to C*H*-2′ and C*H*-4′, respectively. The C-2′ and -4′ atoms, in turn, resonated within the expected range for *C*-NHTs (δ 57.6) and *C*-OH (δ 69.9), respectively. The absence of an exchangeable proton coupled to C*H*-3′ also confirmed the structure. These results indicate that the initial 3′,4′-anhydro structure (I) of **21** (see Scheme 2), existing in a rapid equilibrium between the $^1H_0$ and $^0H_1$ (preponderant) forms through a rather low energy-barrier for interconversion, is transformed into the 2′,3′-(*N*-tosylepimine) (II) by participation of the neighboring 2′-tosylamido group. The 6′-tosylamido group in II or the approaching fluoride ion then attacks C-3′ (as shown by *a* and *b*, respectively) to give the *N*-tosylpyrrolidine **23** or 3′-deoxy-3′-fluoro-4′-epi derivative **24**. An analogous reaction for II → **23** was also observed [4] in the treatment of a D-*allo*-2,3-(*N*-tosylepimine) with KHF_2. Formation of the 3′-deoxy-3′-fluoro-D-galacto derivative **24** without producing the 2′-deoxy-2′-fluoro-D-ido derivative suggests that one of the conformations of II that reacts with the fluoride ion (at C-3′) is $^5H_0$; in the alternative $^0H_5$ form, the approach of the



18 $R^1 = H, R^2 = H$
19 $R^1 = Bes, R^2 = H$
20 $R^1 = H, R^2 = Bes$



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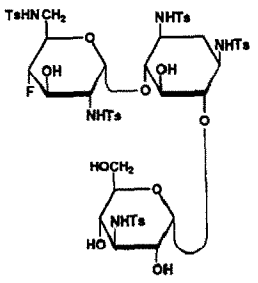

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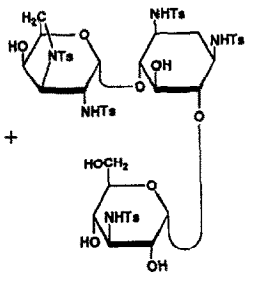
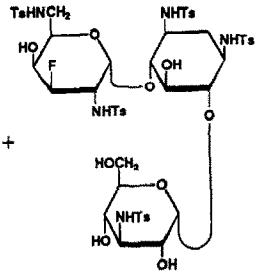
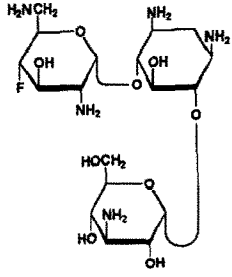


23



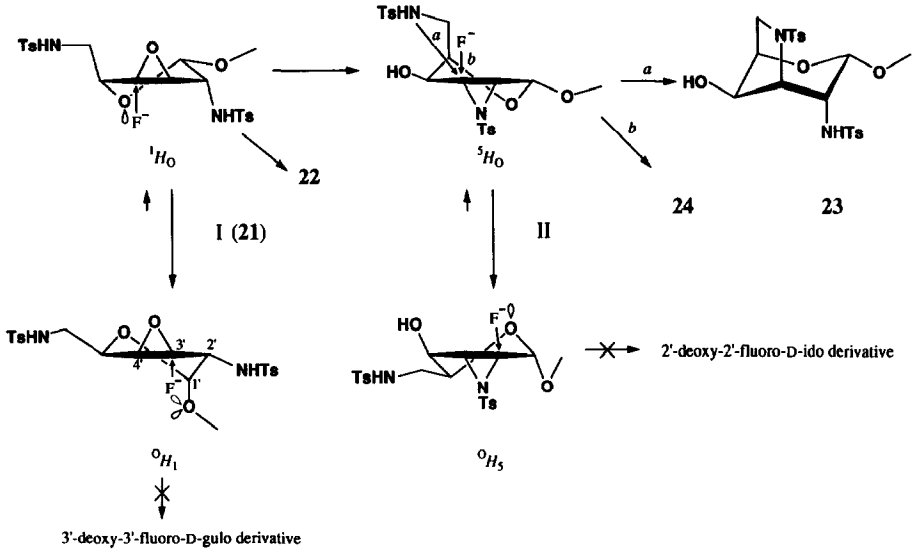
24

22 ⟶



25

fluoride ion at C-2′ (according to the Fürst–Plattner rule) will be hindered by electrostatic repulsion between the axial lone-pair electrons on the pyranoid-ring oxygen [3] and the fluoride ion, as well as partly by the repulsion between the lone-pair electrons on the quasiaxial oxygen atom at C-4′ and the fluoride ion. However, the degree of the latter effect is not clear; if the hydrogen of HO-4′ is oriented to the *endo* position for the pyranoid ring, the two lone-pairs on oxygen



Scheme 2.

will orient to the *exo* position, giving little hindrance for the fluoride ion to approach C-2′. However, if the hydrogen of HO-4′ is *exo*-oriented with respect to the pyranoid ring, the effect will be reversed. Finally, detosylation of **22** with sodium in liquid ammonia gave 4′-deoxy-4′-fluorokanamycin B (**25**).

It should be stressed here that, in this fluorination, both the D-*galacto*-3′,4′-oxiranes **8** and **21** gave the 4′-deoxy-4′-fluoro-D-gluco derivative (**9** and **22**) in preponderance over the 3′-deoxy-3′-fluoro-D-gulo derivative (**10**; no corresponding product was observed for **21**). This may be explained as follows: in the $^{0}H_{1}$ form of I (see Scheme 2), which is the ground-state conformation, approach of the fluoride ion at C-3′ (according to the Fürst–Plattner rule) is hindered by the electrostatic repulsion created between the lone-pair electrons on the axial oxygen atom at C-1′ (one of the lone-pairs on the oxygen is always oriented to the *endo* position [3]) and the fluoride ion; in the $^{1}H_{0}$ conformation, however, the fluoride ion can approach C-4′ to react, although repulsion [3] between the axial lone-pair electrons on the pyranoid-ring oxygen and the fluoride ion is present (the C-2′ substituent is quasiaxial and will give only a minor effect). This suggests that the repulsion between the electron-pairs on O-1′ and the fluoride ion approaching at C-3′ is much larger than the repulsion between the lone-pair electrons on pyranoid-ring oxygen and the fluoride ion approaching at C-4′.

The antibacterial spectra of 4′-deoxy-4′-fluorokanamycins A (**17**) and B (**25**) were compared with those of kanamycin A and B (see Experimental section). Both **17** and **25** showed potent activity against resistant bacteria producing aminoglycoside-adenylylating enzymes for HO-4′ [AAD (4′)] on account of replacement of HO-4′ by fluorine. However, they were inactive against resistant bacteria producing aminoglycoside-phosphorylating enzymes for HO-3′ [APH (3′)]. Furthermore,

17 and **25** were slightly less active than the corresponding parent compounds (kanamycin A and B), respectively, against common bacteria. These results suggest that, even by introduction of the strongly electron-withdrawing fluorine at C-4′, the enzymic action by resistant bacteria modifying the neighboring HO-3′ group is not influenced.

EXPERIMENTAL

General methods.—Melting points were determined on a Kofler block and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. NMR spectra (^{1}H at 250, ^{13}C at 62.9, and ^{19}F at 235.3 MHz) were recorded with a Bruker WM 250 spectrometer unless stated otherwise. Chemical shifts (δ) of ^{1}H, ^{13}C, and ^{19}F spectra were measured downfield from internal Me_4Si (for ^{1}H), internal 1,4-dioxane (for ^{13}C, $\delta = \delta^{dioxane} + 67.4$; in 20% ND_3 in D_2O) or internal Me_4Si (for ^{13}C in pyridine-d_5), or internal Freon 11 (for ^{19}F), unless stated otherwise, and confirmed, in most cases, by shift-correlated 2D spectra. TLC was performed on Kieselgel 60 F_{254} (Merck), and column chromatography on Wakogel C-200, unless stated otherwise.

*4″,6″-O-Benzylidene-6′-*N*-(benzyloxycarbonyl)-2′,3′-O-cyclohexylidene-1,3,3″-tri-*N*-tosylkanamycin A* (**2**).—A mixture of **1** (6.00 g) and NaOH (1.44 g) in 2:1 1,4-dioxane–water (180 mL) was heated for 6 h at 50° (cleavage of the cyclic carbamate). After cooling, benzyl chloroformate (1.1 mL) was added and the solution was kept for 30 min at room temperature. Neutralization with aq AcOH followed by concentration gave a residue, that was thoroughly washed with water. The product was purified by column chromatography with 15:1 $CHCl_3$–MeOH to give **2** as a solid, 5.07 g (77%), $[\alpha]_D^{21}$ −9° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 2.09, 2.24, and 2.33 [each s, 3 H, Ts(*Me*) × 3], 3.67 (dd, $J_{1',2'}$ 3 and $J_{2',3'}$ 9.5 Hz, 1 H, H-2′), 4.72 (t, $J_{3',4'}$ 9.5 Hz, 1 H, H-3′), ABq centered at 5.25 (2 H, $J_{A,B}$ 12.5 Hz, PhCH_2OCO), 5.46 (s, 1 H, PhC*H*), 5.52 (d, $J_{1'',2''}$ 3.8 Hz, 1 H, H-1″), and 6.04 (d, 1 H, H-1′).

Anal. Calcd for $C_{60}H_{72}N_4O_{19}S_3$: C, 57.68; H, 5.81; N, 4.48; S, 7.70. Found: C, 57.43; H, 5.78; N, 4.45; S, 7.61.

*2″-O-Acetyl-4″,6″-O-benzylidene-6′-*N*-(benzyloxycarbonyl)-2′,3′-O-cyclohexylidene-1,3,3″-tri-*N*-tosylkanamycin A* (**3**).—A solution of **2** (500 mg) and *N*-acetylimidazole (135 mg) in 1:9 pyridine–Me_2SO (2.5 mL) was kept for 24 h at room temperature. TLC (12:1 $CHCl_3$–MeOH) of the solution showed two spots at R_F 0.39 (**3**) and 0.3 (**2**, slight). Addition of 5% aq $NaHCO_3$ (200 mL) gave a precipitate, that was chromatographed (15:1 $CHCl_3$–MeOH) to give **3** as a solid, 472 mg (91%), $[\alpha]_D^{22}$ −15° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 2.16, 2.25, and 2.32 [each s, 3 H, Ts(*Me*) × 3], 2.55 (s, 3 H, Ac), 3.61 (dd, $J_{1',2'}$ 3 and $J_{2',3'}$ 9.5 Hz, 1 H, H-2′), 4.20 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, 1 H, H-4′), 4.49 (t, 1 H, H-3′), ABq centered at 5.23 (2 H, $J_{A,B}$ 12 Hz, PhCH_2OCO), 5.60 (s, 1 H, PhC*H*), 5.69 (dd, $J_{1'',2''}$ 3.8 and $J_{2'',3''}$ 10.5 Hz, 1 H, H-2″), 6.10 (br s, 1 H, H-1′), and 6.20 (d, 1 H, H-1″).

Anal. Calcd for $C_{62}H_{74}N_4O_{20}S_3$: C, 57.66; H, 5.78; N, 4.34; S, 7.45. Found: C, 57.70; H, 5.75; N, 4.48; S, 7.50.

2″-O-Acetyl-4″,6″-O-benzylidene-6′-N-(benzyloxycarbonyl)-2′,3′-O-cyclohexylidene-4′-epi-1,3,3″-tri-N-tosylkanamycin A (**4**).—To a cold (−20°) solution of **3** (1.20 g) in CH_2Cl_2 (24 mL) were added trifluoromethanesulfonic anhydride (0.20 mL) and pyridine (0.45 mL), and the solution was gradually warmed to room temperature. TLC (1:1 $CHCl_3$–butanone) of the solution showed a single spot at R_F 0.45 (cf. **3**: R_F 0.35). The solution was poured into 5% aq $NaHCO_3$ and the whole mixture was extracted with $CHCl_3$. Concentration of the organic layer gave a pale-yellow solid, that was thoroughly dried. A mixture of the dried solid and sodium nitrite (1.13 g) in DMF (25 mL) was stirred overnight at room temperature. TLC (1:1 $CHCl_3$–butanone) of the solution showed spots of R_F 0.55, 0.35, 0.25 (**4**), and 0.02. Concentration gave a residue, that was thoroughly washed with water, and chromatographed (1:1 $CHCl_3$–EtOAc) to give **4** as a solid, 385 mg (32%), $[\alpha]_D^{24}$ +48° (*c* 0.5, acetone); ^{1}H-NMR (pyridine-d_5): δ 2.17, 2.25, and 2.33 [each s, 3 H, Ts(*Me*) × 3], 2.56 (s, 3 H, Ac), 4.17 (slightly br d, 1 H, H-3′), 4.51 (dd, 1 H, H-2′), 4.51 (br s, 1 H, H-4′), 4.60 (slightly br t, 1 H, H-5′), ABq centered at 5.26 (2 H, J_{AB} 12.5 Hz, PhCH_2OCO), 5.60 (s, 1 H, PhC*H*), 5.70 (dd, 1 H, H-2″), 6.18 (d, 1 H, H-1″), and 6.32 (d, 1 H, H-1′); $J_{1',2'}$ 3, $J_{2',3'}$ 10, $J_{5',6'a} = J_{5',6'b} \sim 6$, $J_{1'',2''}$ 3.8, and $J_{2'',3''}$ 10.5 Hz; ^{1}H-NMR (acetone-d_6): δ 2.13, 2.28, 2.42, and 2.47 [each s, 3 H, Ac and Ts(*Me*) × 3], 3.55 (dt, $J_{4,5} = J_{5,6}$ 9 and $J_{5,OH}$ 3 Hz, 1 H, H-5), 3.65 (t, *J* 10 Hz, 2 H, H-4″ and H-6″a), ~3.83 (H-5′), 3.85 (slightly br dd, $J_{2',3'}$ 10 and $J_{3',4'}$ ~2 Hz, 1 H, H-3′), 3.96 (dd, $J_{1',2'}$ 3 Hz, 1 H, H-2′), 4.00 (q, 1 H, H-3″), 4.11 (dd, $J_{5'',6''b}$ 5 and $J_{6''a,6''b}$ 10 Hz, 1 H, H-6″b), 4.14 (br s, 1 H, H-4′), 4.25 (dt, $J_{4'',5''} = J_{5'',6''a}$ 10 Hz, 1 H, H-5″), 4.32 (d, $J_{4',OH}$ 4 Hz, 1 H, HO-4′), 4.66 (d, 1 H, HO-5), 5.00 (dd, $J_{1'',2''}$ 3.8 and $J_{2'',3''}$ 10.5 Hz, 1 H, H-2″), 5.12 (s, 2 H, PhCH_2OCO), 5.32 (d, 1 H, H-1″), 5.41 (d, 1 H, H-1′), and 5.45 (s, 1 H, PhC*H*).

Anal. Calcd for $C_{62}H_{74}N_4O_{20}S_3 \cdot H_2O$: C, 56.87; H, 5.85; N, 4.28; S, 7.34. Found: C, 56.85; H, 5.69; N, 4.37; S, 7.13.

2″-O-Acetyl-4″,6″-O-benzylidene-6′-N-(benzyloxycarbonyl)-5,3′,4′-trideoxy-5-epi-5-fluoro-3′-oxo-1,3,3″-tri-N-tosylkanamycin A (**5**).—To a solution of **4** (316 mg) in CH_2Cl_2 (6 mL) was added DAST (0.09 mL; 2.8 mol equiv for **4**) and the solution was kept for 5 h at room temperature. TLC (12:1 $CHCl_3$–MeOH) of the solution showed three spots at R_F 0.4 (2′-hemiacetal intermediate?; see Scheme 1), 0.35, and 0.3 (**5**). The solution was poured into 5% aq $NaHCO_3$, and the whole mixture was extracted with $CHCl_3$. The pale-yellow solid obtained was chromatographed (20:1 $CHCl_3$–MeOH) to give **5** as a solid, 145 mg (50%), along with the solids of R_F 0.4 (70 mg), and 0.35 (49 mg). **5**: $[\alpha]_D^{23}$ +14° (*c* 1, acetone); ^{1}H-NMR (pyridine-d_5; at 500 MHz by a Bruker AM-X500 spectrometer): δ 1.90 (q, 1 H, H-2*ax*), 2.14, 2.22, 2.30, and 2.32 [each s, 3 H, Ac and Ts(*Me*) × 3], 2.52 (slightly br d, 1 H, H-4′*eq*), 2.70 (slightly br dt, 1 H, H-2*eq*), 2.84 (slightly br t, 1 H, H-4′*ax*), 3.72 (br s, 2 H, H-6′a, 6′b), 3.84 (t, 1 H, H-6″a), ~3.88 (1 H, H-1), 4.01 (t, 1 H, H-4″), ~4.14 (1 H, H-3), 4.16 (slightly br dd, 1 H, H-6), 4.20 (slightly br dd, 1 H,

H-4), 4.55 (ddd, 1 H, H-5″), 4.59 (d, 1 H, H-2′; a weak crosspeak was recognized between H-2′ and H-4′*ax* in the 2D spectrum), 4.71 (q, 1 H, H-3″), 4.72 (dd, 1 H, H-6″b), 5.00 (apparent dq, 1 H, H-5′), ABq centered at 5.27 (2 H, J_{AB} 13 Hz, PhCH_2OCO), 5.56 (dd, 1 H, H-2″), 5.57 (s, 1 H, PhC*H*), 5.69 (d, 1 H, H-1″), 5.73 (d, 1 H, H-1′), 5.78 (slightly br d, 1 H, H-5), 7.90 (slightly br t, J ~6 Hz, 1 H, NH-6′), 9.32 (d, 1 H, J 8 Hz, NH-3), and 9.86 (d, 1 H, NH-3″); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12.5, $J_{1,2eq} = J_{2eq,3}$ ~4, $J_{1,6} = J_{3,4}$ ~10, $J_{1',2'}$ 4.5, $J_{4'ax,4'eq}$ 13.5, $J_{4'ax,5'}$ 11.5, $J_{4'eq,5'} = J_{5',6'}$ 3 ~ 4, $J_{1'',2''}$ 3.8, $J_{2'',3''} = J_{3'',4''}$ 10, $J_{3'',NH}$ 8, $J_{4'',5''} = J_{5'',6''a} = J_{6''a,6''b}$ 10, $J_{5'',6''b}$ 5, $J_{4,F} = J_{6,F}$ 27, and $J_{5,F}$ 51 Hz; ^{19}F-NMR (pyridine-d_5): δ −212.7 (slightly br dt, J 27, 27, and 51 Hz, F-5); ^{13}C-NMR (pyridine-d_5; at 125.8 MHz by a Bruker AM-X500 spectrometer; confirmed by shift-correlated ^{1}H–^{13}C 2D spectrum coupled with heteronuclear multiple bond correlation spectroscopy): δ 21.0, 21.2, 21.3, and 21.3 [Ac(*Me*) and Ts(*Me*) × 3], 35.3 (C-2), 43.6 (C-4′), 45.0 (C-6′), 50.9 (d, C-3), 51.4 (d, C-1), 55.1 (C-3″), 64.9 (C-5″), 66.5 (PhCH_2OCO), 68.6 (C-6″), 70.4 (C-5′), 72.2 (C-2″), 76.5 (C-2′), 79.5 (C-4″), 79.7 (d, C-4), 81.6 (d, C-6), 91.3 (d, C-5), 99.9 (C-1″), 102.0 (PhCH), 103.0 (C-1′), 157.6 (PhCH_2O*C*O), 171.2 [Ac(*C*O)], and 204.6 (C-3′); $J_{C-1,F} = J_{C-3,F}$ 5, $J_{C-4,F}$ 19, $J_{C-6,F}$ 18, and $J_{C-5,F}$ 183 Hz.

Anal. Calcd for $C_{56}H_{63}FN_4O_{18}S_3 \cdot H_2O$: C, 55.43; H, 5.40; N, 4.62; S, 7.93. Found: C, 55.52; H, 5.29; N, 4.64; S, 7.82.

*2″-*O*-Acetyl-4″,6″-*O*-benzylidene-6′-*N*-(benzyloxycarbonyl)-4′-*O*-(benzylsulfonyl)-2′,3′-*O*-cyclohexylidene-1,3,3″-tri-*N*-tosylkanamycin A* (**6**).—An ice-cold solution of **3** (1.78 g) and phenylmethanesulfonyl chloride (390 mg) in pyridine (36 mL) was kept for 1 h, then for 1 h at room temperature. Addition of water (0.2 mL) followed by concentration gave a residue, that was washed with water and chromatographed (15:1 $CHCl_3$–MeOH) to give **6** as a solid, 1.85 g (93%), $[\alpha]_D^{21}$ −14° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 2.16, 2.25, and 2.34 [each s, 3 H, Ts(*Me*) × 3], 2.54 (s, 3 H, Ac), ABq centered at 5.11 (2 H, $J_{A,B}$ 14 Hz, PhCH_2), 5.23 (s, 2 H, PhCH_2), 5.38 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, 1 H, H-4′), 5.61 (s, 1 H, PhC*H*), 5.71 (dd, 1 H, H-2″), 6.22 (d, 1 H, H-1″), and 6.40 (br s, 1 H, H-1′).

Anal. Calcd for $C_{69}H_{80}N_4O_{22}S_4$: C, 57.33; H, 5.58; N, 3.88; S, 8.87. Found: C, 57.41; H, 5.64; N, 3.81; S, 8.79.

*2″-*O*-Acetyl-6′-*N*-(benzyloxycarbonyl)-4′-*O*-(benzylsulfonyl)-1,3,3″-tri-*N*-tosylkanamycin A* (**7**).—A solution of **6** (2.01 g) in 80% aq AcOH (60 mL) was heated for 3.5 h at 80°. Concentration gave a residue, that was thoroughly washed with water, and dried to give **7** as a solid, 1.53 g (86%), $[\alpha]_D^{17}$ +52° (*c* 1, DMF); ^{1}H-NMR (pyridine-d_5): δ 2.16, 2.28, 2.31, and 2.34 [each s, 3 H, Ac and Ts(*Me*) × 3], 3.99 (dd, 1 H, H-2′), 4.68 (t, 1 H, H-3′), ~5.07 (H-5′), ~5.25 (H-4′), 5.52 (dd, 1 H, H-2″), 5.62 (d, 1 H, H-1′), and 5.82 (d, 1 H, H-1″).

Anal. Calcd for $C_{56}H_{68}N_4O_{22}S_4$: C, 52.65; H, 5.37; N, 4.39; S, 10.04. Found: C, 52.54; H, 5.45; N, 4.37; S, 9.82.

*3′,4′-Anhydro-6′-*N*-(benzyloxycarbonyl)-4′-epi-1,3,3″-tri-*N*-tosylkanamycin A* (**8**).—To a solution of **7** (744 mg) in MeOH (15 mL) was added 0.5 M NaOMe in MeOH (6 mL) and the solution was kept for 30 min at room temperature.

Conventional work-up gave a product, that was purified by chromatography (6:1 $CHCl_3$–MeOH) to give **8** as a solid, 574 mg (91%), $[\alpha]_D^{22}$ +13° (*c* 1, DMF); ^{1}H-NMR (pyridine-d_5): δ 2.06, 2.25, and 2.31 [each s, 3 H, Ts(*Me*) × 3], 3.46 (dd, 1 H, H-4′), 3.54 (d, 1 H, H-3′), ~4.21 (H-2′), ~5.23 (H-5′), 5.41 (d, 1 H, H-1″), and 5.46 (d, 1 H, H-1′; a weak crosspeak was recognized between H-1′ and H-3′ in the 2D spectrum); $J_{1',2'}$ 3.5, $J_{2',3'}$ ~0, $J_{3',4'}$ 4.0, $J_{4',5'}$ 1.5, and $J_{1'',2''}$ 3.8 Hz.

Anal. Calcd for $C_{47}H_{58}N_4O_{18}S_3 \cdot H_2O$: C, 52.21; H, 5.59; N, 5.18; S, 8.90. Found: C, 52.58; H, 5.59; N, 5.06; S, 8.72.

Reaction of **8** *with* KHF_2 *to give 6′-*N*-(benzyloxycarbonyl)-4′-deoxy-4′-fluoro-1,3,3″-tri-*N*-tosylkanamycin A* (**9**), *6′-*N*-(benzyloxycarbonyl)-3′-deoxy-3′,4′-diepi-3′-fluoro-1,3,3″-tri-*N*-tosylkanamycin A* (**10**), *6′-*N*-(benzyloxycarbonyl)-4′-*O*-(2-hydroxyethyl)-1,3,3″-tri-*N*-tosylkanamycin A* (**11**), *and* **12**.—A mixture of **8** (1.82 g) and KHF_2 (950 mg) in ethane-1,2-diol (27 mL) was kept for 6 h at 150°. After cooling, EtOAc (400 mL) was added and the solution was washed with 5% aq $NaHCO_3$, dried ($MgSO_4$), and concentrated. TLC (1:4:1 $CHCl_3$–EtOAc–MeOH) of the residue showed spots of R_F 0.32 (**9** and **10**), 0.17 (**11** and **12**), and 0.07 (slight) (cf. **8**: R_F 0.5). The residue was chromatographed (the same solvent system described above was used) to give a 5:1 mixture (821 mg, 44%) of **9** and **10**. ^{1}H-NMR (pyridine-d_5; at 500 MHz by a Bruker AM-X500 spectrometer): δ 2.06, 2.29, and 2.35 [each s, 3 H, Ts(*Me*) × 3 (**9**)], 3.38 [t, 1 H, H-5 (**10**)], 3.57 [t, 1 H, H-5 (**9**)], 4.00 [dd, 1 H, H-2′ (**9**)], 4.17 [dd, 1 H, H-2″ (**9**)], ~4.61 [H-2′ (**10**)], 4.76 [dt, 1 H, H-3′ (**9**)], 4.92 [dt, 1 H, H-4′ (**9**)], ~5.20 [H-5′ (**9**)], 5.22 [dt, 1 H, H-3′ (**10**)], 5.35 [d, 1 H, H-1″ (**10**)], 5.42 [d, 1 H, H-1″ (**9**)], 5.61 [t, 1 H, H-1′ (**9**)], and 5.69 [d, 1 H, H-1′ (**10**)]; *J* values for **9**: $J_{1',2'}$ 3.5, $J_{2',3'} = J_{3',4'} = J_{4',5'}$ 9, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ 10, $^5J_{1',F}$ 3.5, $^4J_{2',F}$ ~0, $J_{3',F}$ 16, and $J_{4',F}$ 51 Hz; *J* values for **10**: $J_{1',2'}$ 4, $J_{2',3'} = J_{3',4'}$ ~3.5, $J_{1'',2''}$ 3.8, $J_{1',F}$ ~0, and $J_{3',F}$ 49 Hz; ^{19}F-NMR (pyridine-d_5): δ −195.7 (apparent dd, 5/6 F, F-4′ for **9**) and −199.5 (ddd, 1/6 F, F-3′ for **10**).

Anal. Calcd for $C_{47}H_{59}FN_4O_{18}S_3 \cdot H_2O$: C, 51.26; H, 5.58; N, 5.09; S, 8.74. Found: C, 51.60; H, 5.86; N, 5.11; S, 8.77.

The fractions containing the products of R_F 0.17 (560 mg) were further chromatographed (6:1 $CHCl_3$–MeOH) to give solids of **11**, 338 mg (17%) and **12**, 44 mg (3%) along with the mixture of the two products (13.4 mg).

Compound **11** had $[\alpha]_D^{24}$ +20° (*c* 1, acetone); ^{1}H-NMR (20:1 pyridine-d_5–D_2O): δ 2.06, 2.31, and 2.36 [each s, 3 H, Ts(*Me*) × 3], 3.81 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, 1 H, H-4′), 3.96 (dd, 1 H, H-2′), 4.61 (t, $J_{2',3'}$ 9.5 Hz, 1 H, H-3′), ~4.98 (1 H, H-5′), 5.38 (d, $J_{1'',2''}$ 3.8 Hz, 1 H, H-1″), and 5.54 (d, $J_{1',2'}$ 3.8 Hz, 1 H, H-1′); ^{13}C-NMR (pyridine-d_5): δ 33.5 (C-2), 42.5 (C-6′), 52.6 (C-3 or C-1), 54.4 (C-1 or C-3), 61.8 (C-3″), 62.2 (C-6″), 62.6 (OCH_2CH_2OH), 66.3 ($PhCH_2OCO$), 70.0 (C-4″), 71.0 (C-5′), 72.5 (C-2″), 73.9 (C-2′), 74.7 (C-3′), 75.0 (C-5″), 75.2 (OCH_2CH_2OH), 75.6 (C-5), 81.1 (C-4′), 86.6 and 86.8 (C-4 and C-6), 102.5 (C-1′), and 103.2 (C-1″).

Anal. Calcd for $C_{49}H_{64}N_4O_{20}S_3 \cdot 2H_2O$: C, 50.68; H, 5.90; N, 4.82; S, 8.28. Found: C, 50.58; H, 5.81; N, 5.11; S, 8.22.

*4′-*O*-(2-Acetoxyethyl)-2′,3′,2″,4″,6″-penta-*O*-acetyl-6′-*N*-(benzyloxycarbonyl)-*

1,3,3″-tri-N-*tosylkanamycin A* (**15**).—A solution of **11** (143 mg) and Ac_2O (0.22 mL) in pyridine (3 mL) was kept for 2 days at room temperature. TLC (12:1 $CHCl_3$–MeOH) of the solution showed spots of R_F 0.7–0.55, 0.5 (**15**), and 0.3 (cf. **11**: R_F 0). The products obtained by standard work-up were chromatographed (20:1 $CHCl_3$–MeOH) to give **15** as a solid, 90 mg (53%), $[\alpha]_D^{23}$ +22° (*c* 0.5, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 1.90, 1.93, 2.07, and 2.12 (each s, 3 H), 2.18 (s, 6 H), 2.30, 2.31, and 2.38 (each s, 3 H) [Ac × 6 and Ts(*Me*) × 3], 3.80 (t, $J_{3',4'} = J_{4',5'}$ ~ 10 Hz, 1 H, H-4′), 5.17 (dd, 1 H, H-2′), 5.52 (t, 1 H, H-4″), 5.55 (dd, 1 H, H-2″), 6.00 (t, $J_{2',3'}$ ~ 10 Hz, 1 H, H-3′), 6.03 (d, $J_{1',2'}$ ~ 4 Hz, 1 H, H-1′), and 6.07 (d, 1 H, H-1″).

Anal. Calcd for $C_{61}H_{76}N_4O_{26}S_3$: C, 53.19; H, 5.56; N, 4.07; S, 6.98. Found: C, 52.98; H, 5.58; N, 4.05; S, 6.77.

*4″,6″-O-Benzylidene-6′-*N-*(benzyloxycarbonyl)-4′-deoxy-4′-fluoro-1,3,3″-tri*-N-*tosylkanamycin A* (**13**) *and 4″,6″-O-benzylidene-6′-*N-*(benzyloxycarbonyl)-3′-deoxy-3′,4′-diepi-3′-fluoro-1,3,3″-tri*-N-*tosylkanamycin A* (**14**).—To a solution of a 5:1 mixture (470 mg) of **9** and **10** in DMF (2.4 mL) were added benzaldehyde dimethyl acetal (0.12 mL) and *p*-toluenesulfonic acid (25 mg), and the solution was kept overnight at room temperature. TLC (6:1 $CHCl_3$–MeOH) of the solution showed a single spot at R_F 0.5 (**13** and **14**) (cf. **9** and **10**: R_F 0.25). After addition of 5% aq $NaHCO_3$ (1.8 mL), the mixture was concentrated to give a residue, that was thoroughly washed with water and ether to give a mixture of **13** and **14**, 495 mg; ^{19}F-NMR (pyridine-d_5): δ −195.7 (apparent dd, $J_{3',F}$ 16 and $J_{4',F}$ 51 Hz, ~5/6 F, F-4′ for **13**) and −199.4 (ddd, $J_{2',F}$ 32, $J_{3',F}$ 50, and $J_{4',F}$ 5 Hz, ~1/6 F, F-3′ for **14**).

Treatment of a mixture of **13** *and* **14** *with sodium hydride.*—To an ice-cold solution of the mixture (112 mg) of **13** and **14** in DMF (1.5 mL) was added NaH (25 mg), and the mixture was stirred overnight at room temperature. TLC (6:1 $CHCl_3$–MeOH) of the resulting clear solution showed spots of R_F 0.65 (trace), 0.55 (trace), 0.5 (**13**), 0.43 (**16**), 0.3 (trace; this product will be the 6′-*N*:4′-*O*-carbonyl derivative of **14**), and ~0. Neutralization with aq AcOH followed by concentration gave a residue, that was thoroughly washed with water. Chromatography (8:1 $CHCl_3$–MeOH) of the residue gave solids of **13**, 32 mg (33% based on **9**) and **16**, 23 mg (27% based on the mixture of **9** and **10**).

Compound **13** had $[\alpha]_D^{23}$ +2° (*c* 0.5, acetone); ^{1}H-NMR (pyridine-d_5): δ 2.08, 2.27, and 2.33 [each s, 3 H, Ts(*Me*) × 3], 4.05 (slightly br dd, 1 H, H-2′), 4.75 (dt, 1 H, H-3′), 4.92 (dt, 1 H, H-4′), ~5.16 (H-5′), 5.45 (s, 1 H, PhC*H*), 5.48 (d, 1 H, H-1″), and 5.62 (t, 1 H, H-1′); $J_{1',2'}$ 3.5, $J_{2',3'} = J_{3',4'} = J_{4',5'}$ 9, $J_{1'',2''}$ 3.8, $^5J_{1',F}$ 3.5, $J_{3',F}$ 16, and $J_{4',F}$ 51 Hz.

Anal. Calcd for $C_{54}H_{63}FN_4O_{18}S_3 \cdot H_2O$: C, 54.53; H, 5.51; N, 4.71; S, 8.09. Found: C, 54.03; H, 5.37; N, 5.01; S, 8.11.

Compound **16** had $[\alpha]_D^{23}$ +16° (*c* 0.5, acetone); ^{1}H-NMR (pyridine-d_5): δ 2.08, 2.20, and 2.27 [each s, 3 H, Ts(*Me*) × 3], 4.36 (br d, *J* ~ 10 Hz, 1 H, H-2″), 4.60 (q, 1 H, H-3″), 4.78 (dt, 1 H, H-5″), 5.50 (s, 1 H, PhC*H*), and 5.66 (d, $J_{1'',2''}$ 3.8 Hz, 1 H, H-1″).

Anal. Calcd for $C_{40}H_{48}N_3O_{13}S_3 \cdot 0.5\ H_2O$: C, 54.35; H, 5.59; N, 4.75; S, 10.88. Found: C, 54.58; H, 5.63; N, 4.87; S, 10.39.

4'-Deoxy-4'-fluorokanamycin A (**17**).—A mixture of **9** and **10** (506 mg), purified by passing through a column of Sephadex LH-20 with MeOH, was dissolved in liquid NH_3 (~ 100 mL) at −60° and Na (~ 0.75 g) was added. After 2 min, cold MeOH was added until the deep-blue solution became colorless. Warming followed by concentration gave a residue, that was dissolved in water and the solution was neutralized with Amberlite CG-120 resin (H^+ form, 9.5 g). After the resin was thoroughly washed with water, the products were eluted with M aq NH_3. TLC (2:4:7:7 $CHCl_3$–PrOH–EtOH–17% aq NH_3) of the products showed spots of R_F 0.65, 0.34, 0.27 (**17**), 0.2, 0.17, and 0.07 (trace) (cf. kanamycin A: R_F 0.17). Isolation of **17** was performed by column chromatography of CM-Sephadex C-25 with aq NH_3 (0 → 0.15M) to give a solid, 138 mg (65% based on **9** as its carbonate hemihydrate); from another fraction, a mixture (29 mg) of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (major) and a compound considered to be 3'-deoxy-3',4'-diepi-3'-fluorokanamycin A (^{19}F-NMR: ddd, *J* 9, 26, and 50 Hz) was obtained.

Compound **17** had $[\alpha]_D^{21}$ +116° (*c* 0.5, H_2O); ^{1}H-NMR (20% ND_3 in D_2O): δ 1.22 (q, 1 H, H-2*ax*), 1.96 (dt, 1 H, H-2*eq*), 2.80 (dd, 1 H, H-6'a), ~ 2.88 (2 H, H-1 and 3), ~ 2.98 (H-6'b), 3.00 (t, 1 H, H-3"), 3.24 (t, 1 H, H-6), 3.30 (t, 1 H, H-4"), 3.32 (t, 1 H, H-4), 3.47 (dd, 1 H, H-2"), 3.60 (dd, 1 H, H-2'), 3.64 (t, 1 H, H-5), 3.74 (2 H, H-6"), ~ 3.9 (H-5"), 3.93 (ddd, 1 H, H-3'), ~ 3.96 (H-5'), 4.22 (dt, 1 H, H-4'), 5.02 (d, 1 H, H-1"), and 5.31 (t, 1 H, H-1'); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12.5, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{1,6} = J_{3,4} = J_{4,5} = J_{5,6}$ 9.5, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10, $J_{3',4'} = J_{4',5'}$ 9, $J_{5',6'a}$ 7, $J_{6'a,6'b}$ 14, $^5J_{1',F}$ 3.5, $^4J_{2',F}$ ~ 0, $J_{3',F}$ 15, $J_{4',F}$ 51, $J_{1'',2''}$ 3.8, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10 Hz; ^{13}C-NMR (20% ND_3 in D_2O): δ 36.4 (C-2), 42.1 (C-6'), 49.8 (C-3), 51.3 (C-1), 55.1 (C-3"), 61.0 (C-6"), 70.0 (C-4"), 71.3 (d, C-5'), 71.9 (d, C-3'), 72.1 (d, C-2'), 72.5 (C-2"), 73.0 (C-5"), 74.8 (C-5), 88.5 (C-4), 88.7 (C-6), 91.6 (d, C-4'), 100.0 (C-1'), and 100.9 (C-1"); $J_{C-2',F}$ 9, $J_{C-3',F}$ 18, $J_{C-4',F}$ 180, and $J_{C-5',F}$ 24 Hz; ^{19}F-NMR (20% ND_3 in D_2O; Freon 11 as the external reference): δ −197.8 (apparent dd, F-4').

Anal. Calcd for $C_{18}H_{35}FN_4O_{10} \cdot H_2CO_3 \cdot 0.5\ H_2O$: C, 40.93; H, 6.87; F, 3.41; N, 10.05. Found: C, 40.57; H, 7.15; F, 3.66; N, 10.33.

3'-O-Benzylsulfonyl- (**19**) *and 4'-O-benzylsulfonyl-4",6"-O-cyclohexylidene-1,3,2',6',3"-penta-N-tosylkanamycin B* (**20**).—To a cold (−20°) solution of **18** (1.56 g) in pyridine (30 mL) was added phenylmethanesulfonyl chloride (225 mg) and the solution was kept for 21 h at this temperature. TLC (1:1 $CHCl_3$–butanone) of the solution showed three spots at R_F 0.4 (**20**), 0.28 (**19**), and 0.2 (**18**). Addition of water (0.1 mL) followed by concentration gave a residue, that was dissolved in $CHCl_3$ (100 ml) and the solution was washed with 5% aq $KHSO_4$, 5% aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated to give a solid. Column chromatography (1:1 $CHCl_3$–butanone) of the solid gave solids of **20**, 730 mg (42%), **19**, 390 mg (22%), and **18** recovered, 296 mg (19%).

Compound **19** had $[\alpha]_D^{25}$ −2° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 2.09, 2.17, and 2.18 (each s, 3 H), 2.39 (s, 6 H) [Ts(*Me*) × 5], ~4.20 (H-2′), 4.51 (t, $J_{3',4'} = J_{4',5'}$ ~9.5 Hz, 1 H, H-4′), ABq centered at 4.83 (2 H, J_{AB} 14 Hz, PhCH_2), 5.34 (d, $J_{1'',2''}$ 3.8 Hz, 1 H, H-1″), ~5.38 (H-5′), 5.75 (t, $J_{2',3'}$ ~9.5 Hz, 1 H, H-3′), and 5.88 (d, $J_{1',2'}$ 3.8 Hz, 1 H, H-1′).

Anal. Calcd for $C_{66}H_{81}N_5O_{22}S_6$: C, 53.25; H, 5.48; N, 4.70; S, 12.92. Found: C, 53.20; H, 5.51; N, 4.52; S, 12.71.

Compound **20** had $[\alpha]_D^{25}$ −7° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 2.09, 2.14, 2.18, 2.36, and 2.37 [each s, 3 H, Ts(*Me*) × 5], ~4.05 (H-2′), 4.75 (unresolved t, *J* ~9.5 Hz, H-3′), 5.12 (apparent s, 2 H, PhCH_2), 5.29 (d, $J_{1'',2''}$ 3.5 Hz, 1 H, H-1″), ~5.47 (H-4′), ~5.50 (H-5′), and 5.84 (d, $J_{1',2'}$ 3.5 Hz, 1 H, H-1′).

Anal. Calcd for $C_{66}H_{81}N_5O_{22}S_6$: C, 53.25; H, 5.48; N, 4.70; S, 12.92. Found: C, 53.06; H, 5.46; N, 4.54; S, 12.63.

3′,4′-Anhydro-4″,6″-O-cyclohexylidene-4′-epi-1,3,2′,6′,3″-penta-N-*tosylkanamycin B* (**21**).—Compound **20** (6.12 g) was treated as described for **8** to give **21** as a solid, 5.25 g (96%), $[\alpha]_D^{21}$ +20° (*c* 1, DMF); ^{1}H-NMR (pyridine-d_5): δ 2.15, 2.16, 2.19, 2.32, and 2.36 [each s, 3 H, Ts(*Me*) × 5], 3.31 (t, 1 H, H-5), ~3.55 (H-3′ and H-4′), 4.04 (br s, 1 H, H-2′), 5.36 (t, *J* 6.5 Hz, each peak being slightly split; 1 H, H-5′), 5.43 (d, 1 H, H-1″), and 5.48 (d, 1 H, H-1′); $J_{1',2'}$ 3.8 and $J_{1'',2''}$ 3.8 Hz; ^{1}H-NMR (pyridine-d_5 at 55°): δ 3.44 (br d, 1 H, H-4′; sharpened d on irradiation of H-5′), 3.49 (d, 1 H, H-3′), and 3.96 (d, 1 H, H-2′); $J_{2',3'}$ 0 and $J_{3',4'}$ 4 Hz; ^{13}C-NMR (pyridine-d_5): δ 33.4 (C-2), 45.0 (C-6′), 51.0 (C-3′ or C-4′), 51.7 (C-2′), 53.1 (C-3 or C-1), 54.0 (C-1 or C-3), 54.0 (C-4′ or C-3′), 58.2 (C-3″), 61.8 (C-6″), 66.4 (C-5″), 67.1 (C-5′), 72.0 (C-4″), 72.4 (C-2″), 75.3 (C-5), 83.9 (C-4 or C-6), 86.9 (C-6 or C-4), 97.0 (C-1′), 99.9 [$(CH_2)_5CO(O)$], and 103.5 (C-1″).

Anal. Calcd for $C_{59}H_{73}N_5O_{19}S_5 \cdot H_2O$: C, 53.10; H, 5.66; N, 5.25; S, 12.01. Found: C, 53.24; H, 5.57; N, 5.32; S, 12.07.

Reaction of **21** *with* KHF_2 *to give 4′-deoxy-4′-fluoro-1,3,2′,6′,3″-penta*-N-*tosylkanamycin B* (**22**) *and 6′-deamino-3′-deoxy-4′-epi-1,3,2′,3″-tetra*-N-*tosyl-3′,6′-(*N-*tosylepimino)kanamycin B* (**23**).—A mixture of **21** (450 mg) and KHF_2 (210 mg) in ethane-1,2-diol (8 mL) was kept for 4 h at 150°. TLC (6 : 1 $CHCl_3$–EtOH) of the mixture showed three spots at R_F 0.36 (**22**), 0.31, and 0.28 (**23**, major) (cf. **21**: R_F 0.5). EtOAc (100 mL) was added and worked up as described for **9** to give a mixture of products, that was separated by chromatography (10 : 1 $CHCl_3$–EtOH) to give **22** as a solid, 101 mg (24%), **23** as a solid, 223 mg (52%), and a mixture of products 32 mg (R_F 0.31).

Compound **22** had $[\alpha]_D^{17}$ +18° (*c* 1, DMF); ^{1}H-NMR (pyridine-d_5): δ 1.64 (q, 1 H, H-2*ax*), 2.06, 2.10, and 2.19 [each s, 3 H, Ts(*Me*) × 3], 2.37 [s, 6 H, Ts(*Me*) × 2], 2.5–2.7 [2 H, H-2*eq* and H-1 (or H-3)], 3.09 (t, 1 H, H-5), 3.34 (m, 1 H, H-3 or H-1), 3.56 (t, 1 H, H-6 or H-4), 3.72 (t, 1 H, H-4 or H-6), 3.8–4.1 (3 H, H-2′, 6′a, and 6′b), 4.15 (br dd, 1 H, H-2″), 4.25 (t, 1H, H-4″), 4.49 (apparent q, 1 H, H-3″), 4.81 (dt, 1 H, H-3′), 5.05 (dt, 1 H, H-4′), 5.28 (d, 1 H, H-1″), 5.42 (m, 1 H, H-5′), 5.92 (t, 1 H, H-1′), and 9.15 (d, 1 H, NH-3″); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ ~12,

$J_{1,6} = J_{3,4} = J_{4,5} = J_{5,6}$ ~ 9, $J_{3(\text{or } 1),NH}$ 9.5, $J_{1',2'}$ 3, $J_{2',3'} = J_{3',4'} = J_{4',5'}$ ~ 9, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ ~ 10, $J_{3'',4''} = J_{4'',5''}$ 10, $J_{3'',NH}$ 9, $^5J_{1',F}$ 3, $J_{3',F}$ 15, $J_{4',F}$ 50, and $J_{5',F}$ ~ 4 Hz; ^{19}F-NMR (pyridine-d_5): δ −194.5 (slightly br dd, J 15 and 50 Hz, F-4').

Broadband decoupling of ^{19}F collapsed the signals of H-1' (t), H-3' (dt), and H-4' (dt) to a d, t, and t, respectively. Irradiation of δ 3.9 (H-6'a, 6'b) collapsed the m of H-5' to dd (J ~ 4 and ~ 9 Hz) indicating that the $J_{5',F}$ is ~ 4 Hz.

Anal. Calcd for $C_{53}H_{66}FN_5O_{19}S_5$: C, 50.66; H, 5.30; N, 5.57; S, 12.76. Found: C, 50.33; H, 5.49; N, 5.83; S, 12.47.

Compound **23**: needles (from MeOH), mp 220–221°, $[\alpha]_D^{17}$ −1° (*c* 2, DMF); ^{1}H-NMR (pyridine-d_5): δ 1.75 (q, 1 H, H-2*ax*), 2.09, 2.15, 2.21, 2.37, and 2.39 [each s, 3 H, Ts(*Me*) × 5], ~ 2.4 (H-5), 2.70 (dt, 1 H, H-1 or H-3), 2.92 (br m, 1 H, H-3 or H-1), 3.21 (dt, 1 H, H-2*eq*), 3.60 (t, 1 H, H-6 or H-4), 3.72 (t, 1 H, H-4 or H-6), ~ 3.76 (1 H, H-6'a), 3.81 (dd, 1 H, H-6'b), 4.12 (t, 1 H, H-4''), ~ 4.2 (H-2''), 4.38 (ddd, 1 H, H-2'), ~ 4.45 (H-5'), 4.84 (slightly br d, 1 H, H-3'; sharpened d on irradiation of H-5'), 4.92 (slightly br s, 1 H, H-4'; sharpened s on irradiation of H-5'), 5.25 (br s, 1 H, HO-5); disappeared on deuteration), 5.35 (d, 1 H, H-1''), 5.85 (d, 1 H, H-1'), 7.00 (br s, 1 H, HO-4'; disappeared on deuteration), and 7.69 (d, NH-2'; disappeared on deuteration); $J_{1',2'}$ 3.6, $J_{2',3'}$ 5, $J_{2',NH}$ ~ 6, $J_{3',4'}$ ~ 0, $J_{3',5'}$ ~ 1, $J_{5',6'b}$ 3, and $J_{6'a,6'b}$ 11 Hz; ^{13}C-NMR (pyridine-d_5): δ 33.6 (C-2), 50.8 (C-6'), 52.4 (C-3 or C-1), 54.1 (C-1 or C-3), 57.6 (C-2'), 61.7 (C-3''), 62.0 (C-6''), 67.0 (C-3'), 69.9 (C-4' and C-4''), 72.2 (C-2''), 75.5 (C-5), 75.6 (C-5'), 78.4 (C-5''), 81.7 (C-4 or C-6), 88.1 (C-6 or C-4), 95.6 (C-1'), and 103.3 (C-1'').

On irradiation of H-2', the signals of NH-2' (d), H-3' (slightly br d), and H-1' (d) collapsed to a s, slightly br s, and s, respectively. Irradiation of NH-2' collapsed the H-2' (ddd) to a t in appearance.

Anal. Calcd for $C_{53}H_{65}N_5O_{19}S_5 \cdot 2H_2O$: C, 50.03; H, 5.47; N, 5.50; S, 12.60. Found: C, 49.84; H, 5.51; N, 5.55; S, 12.54.

3'-Deoxy-4'-epi-3'-fluoro-1,3,2',6',3''-penta-N-*tosylkanamycin B* (**24**).—The mixture of products (30 mg, R_F 0.31) just described was further separated by column chromatography with 2:8:1 $CHCl_3$–EtOAc–MeOH to give **24** as a solid (R_F 0.35), 8.4 mg (2.1% based on **21**), a mixture of two products with no fluorine, 17.7 mg (R_F 0.2), and a product of R_F 0.1, 3.5 mg.

Compound **24** had $[\alpha]_D^{23}$ +29° (*c* 0.5, DMF); ^{1}H-NMR (pyridine-d_5): δ 1.63 (q, 1 H, H-2*ax*), 2.07, 2.08, 2.15, 2.38, and 2.40 [each s, 3 H, Ts(*Me*) × 5], 3.09 (t, 1 H, H-5), 3.47 and 3.66 (each t, 1 H, H-4 and H-6), 3.71 (dd, 1 H, H-6'a), 3.98 (dd, 1 H, H-6'b), 4.16 (dd, 1 H, H-2''), 4.50 (t, 1 H, H-3''), 4.62 [br, 1 H, H-4'; br d (J ~ 9 Hz) on deuteration], 4.84 (dt, 1 H, H-2'), 5.26 (d, 1 H, H-1''), 5.29 (apparent t, 1 H, H-5'), 5.35 (ddd, 1 H, H-3'), and 6.00 (t, 1 H, H-1'); $J_{1',2'}$ 4, $J_{2',3'}$ 10, $J_{3',4'}$ ~ 3, $J_{5',6'a}$ 7, $J_{5',6'b}$ 6, $J_{6'a,6'b}$ 12, $^4J_{1',F}$ 4, $J_{2',F}$ 10, $J_{3',F}$ 50, and $J_{4',F}$ ~ 9 Hz; ^{19}F-NMR (pyridine-d_5): δ −197.3 [doublet (50 Hz) having unresolved m, the half-height width being ~ 20 Hz, F-3'].

Anal. Calcd for $C_{53}H_{66}FN_5O_{19}S_5 \cdot H_2O$: C, 49.95; H, 5.38; N, 5.50; S, 12.58. Found: C, 50.15; H, 5.61; N, 5.95; S, 12.76.

4'-Deoxy-4'-fluorokanamycin B (**25**).—Compound **22** (310 mg) purified by passing through a column of Sephadex LH-20 with MeOH was treated for 2 min at −60° with Na (~0.6 g) in liquid NH_3 (~60 mL). Isolation of the product as described for **17** gave **25** as a solid, 76 mg (53% as its 3/2 carbonate), $[\alpha]_D^{21}$ +108° (*c* 1, H_2O); ^{1}H-NMR (20% ND_3 in D_2O): δ 1.22 (q, 1 H, H-2*ax*), 1.95 (dt, 1 H, H-2*eq*), 2.82 (dd, 1 H, H-2'), ~2.82 (1 H, H-6'a), ~2.85 (2 H, H-1 and H-3), ~2.98 (br d, 1 H, H-6'b), 3.01 (t, 1 H, H-3"), 3.24 (t, 1 H, H-6), 3.32 (t, 2 H, H-4 and H-4"), 3.49 (dd, 1 H, H-2"), 3.64 (t, 1 H, H-5), ~3.76 (2 H, H-6"), 3.83 (ddd, 1 H, H-3'), 3.91 (1 H, H-5"), 3.97 (m, 1 H, H-5'), 4.23 (dt, 1 H, H-4'), 5.04 (d, 1 H, H-1"), and 5.31 (t, 1 H, H-1'); $J_{1',2'}$ 3.5, $J_{2',3'}$ 10, $J_{3',4'} = J_{4',5'}$ 9, $^5J_{1',F}$ 3.5, $^4J_{2',F}$ ~0, $J_{3',F}$ 15, $J_{4',F}$ 51, and $J_{5',F}$ ~4 Hz; ^{13}C-NMR (20% ND_3 in D_2O): δ 36.4 (C-2), 42.2 (C-6'), 50.1 (C-3), 51.2 (C-1), 55.0 (C-3"), 55.9 (d, C-2'), 60.9 (C-6"), 70.0 (C-4"), 71.5 (d, C-5'), 72.3 (d, C-3'), 72.5 (C-2"), 73.0 (C-5"), 75.1 (C-5), 88.0 (C-4), 88.8 (C-6), 92.1 (d, C-4'), 100.8 (C-1"), and 101.0 (C-1'); $J_{C\text{-}2',F}$ 7, $J_{C\text{-}3',F}$ 16, $J_{C\text{-}4',F}$ 179, and $J_{C\text{-}5',F}$ 24.5 Hz; ^{19}F-NMR (20% ND_3 in D_2O; Freon 11 as external reference): δ −197.2 (apparent dd, F-4').

Anal. Calcd for $C_{18}H_{36}FN_5O_{19} \cdot 3/2\ H_2CO_3$: C, 40.48; H, 6.79; F, 3.28; N, 12.11. Found: C, 40.59; H, 6.98; F, 3.37; N, 12.04.

Minimal inhibitory concentration (μg/mL) of kanamycin A, **17**, *kanamycin B, and* **25**.—Performed on Mueller–Hinton agar for 18 h at 37°. *Staphylococcus aureus* FDA 209P: 1.56, 1.56, 0.78, and 1.56, in the following order; *S. aureus* Ap 01 [AAD (4')]: > 100, 3.12, 50, and 3.12; *S. Epidermidis* 109 [AAD (4')]: 50, 3.12, 100, and 3.12; *Bacillus subtilis* PCI 219: 0.39, 6.25, 0.39, and 0.78; *Escherichia coli* K-12: 1.56, 3.12, 0.78, and 0.78; *E. coli*. K-12 ML 1629 [APH (3')-I]: > 100, > 100, > 100, and 100; *E. coli* W 677: 0.78, 1.56, 0.78, and 1.56; *E. coli* JR 66/W 677 [AAD (2") and APH (3')-II]: > 100, > 100, > 100, and > 100; *Klebsiella pneumoniae* PCI 602: 3.12, 3.12, 0.78, and 6.25; *Proteus rettgeri* GN 311: 0.78, 0.78, 0.39, and 1.56; *Serratia marcescens*: 6.25, 6.25, 12.5, and 25; *Pseudomonas aeruginosa* A3: 6.25, 0.78, 3.12, and 6.25; *P. aeruginosa* H9 [APH (3')-II]: > 100, 50, > 100, and > 100.

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