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Synthesis of 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of kanamycin B and its analogs. Study on structure–toxicity relationships

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ABSTRACT

5-Deoxy-5-fluoro- (**1**), 5,3′-dideoxy-5-fluoro- (**2**), and 5,3′,4′-trideoxy-5-fluoro-kanamycin B (**3**) have been prepared by treatment of 5-epihydroxyl precursors (prepared by the Mitsunobu reaction) with DAST as the key step. 5,3′-Dideoxy-5,5-difluoro- (**26**) and 5,3′,4′-trideoxy-5,5-difluoro-kanamycin B (**27**) were also prepared by treatment of the corresponding 5-oxo derivatives with DAST. These 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives showed markedly decreased toxicity as compared with the parent compounds.

INTRODUCTION

Recently we reported the synthesis of several fluorinated kanamycin derivatives such as 3′-deoxy-3′-fluorokanamycin A (refs. 1–3) and B (refs. 1, 4), 3′,4′-dideoxy-3′-fluorokanamycin A (ref. 2) and B (ref. 4), and 4′-deoxy-4′-fluorokanamycin A (ref. 5) and B (ref. 5); they were all active against resistant bacteria producing the enzymes phosphorylating or adenylylating the 3′- or 4′-hydroxyl group, as a consequence of substitution of hydroxyl groups by a fluorine atom. Inherent in fluorination is the fact that introduction of a fluorine atom alters the electron density in its vicinity. As fluorine is the most strongly electron-withdrawing atom of all, and has a small atomic size (between that of H and O), attachment of a fluorine atom in a molecule of an aminoglycoside antibiotic decreases the basicity of the amino groups situated nearby through the inductive effect of fluorine, without causing marked steric changes in the vicinity of the fluorine. This decreased basicity of specific amino group(s) should influence the toxicity of the parent antibiotics.

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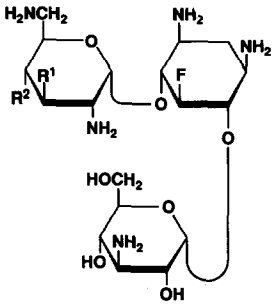
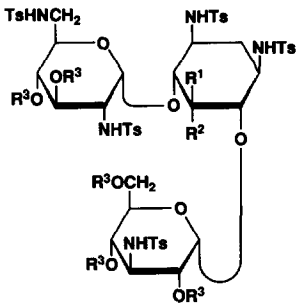
The previously reported [6,7] 3-demethoxy-3-fluorosporaricin A showed enhanced antibacterial activity and decreased toxicity as compared to sporaricin A. 3′-Deoxy-3′-fluorokanamycin B (refs. 1, 4) also showed [8] approximately one-half of the toxicity of kanamycin B, with retention of antibacterial activity. These results strongly suggest that the decreased basicity of NH_2-1 in sporaricin A, and NH_2-2′ in kanamycin B should decrease the toxicity of these antibiotics. The present study develops this conception and describes the synthesis and some biological activities of 5-deoxy-5-fluorokanamycin B, and the 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of 3′-deoxykanamycin B and 3′,4′-dideoxykanamycin B. These compounds are expected to show decreased basicity of the 1- and 3-amino groups in the 2-deoxystreptamine unit.

RESULTS AND DISCUSSION

The synthesis of 5-deoxy-5-fluorokanamycin B (**1**) is described first. The five amino groups of kanamycin B were protected by tosylation and the pentatosyl derivative **4** was acetylated with acetyl chloride in pyridine to give the pentaacetyl derivative **5** having HO-5 free. Inversion of the HO-5 group of **5** was accomplished by applying the Mitsunobu reaction using triphenylphosphine, diethyl azodicarboxylate (DEAD), and benzoic acid (as the acid) to give the 5-*O*-benzoyl-5-epi derivative **6** in good yield. The structure was confirmed by its ^{1}H-NMR spectrum (Table I). Application of the Mitsunobu reaction to some structurally related kanamycin A derivatives (having a 2″-*O*-acetyl group) to invert directly at C-5 was, however, unsuccessful, and similar treatment of 2″-*O*-acetyl-4″,6″-*O*-cyclohexylidene-3′-deoxy-1,3,2′,6′,3″-penta-*N*-tosylkanamycin B (**12**) having the free HO-5 and -4′ groups gave the corresponding 5-*O*-benzoyl-5-epi derivative **14** with the HO-4′ group remaining intact. These results suggest that the reaction at C-5 would be facilitated by the NHTs-2′ group, but the mechanism was not further pursued.

Zemplén deacylation of **6** gave the hexaol **7**, and reacetylation of **7** gave the pentaacetyl derivative **8** having the axial HO-5 group free. Treatment of **8** with diethylaminosulfur trifluoride (DAST) in dichloromethane in the presence of pyridine gave the 5-deoxy-5-fluoro derivative **9** in high yield. Finally, detosylation of **9** with sodium in liquid ammonia followed by deacetylation gave the desired 5-deoxy-5-fluorokanamycin B (**1**), but in low yield. As the product **1** was stable toward sodium in liquid ammonia, with quantitative recovery of **1**, the presence of fluorine at C-5 is not the reason for this low yield. Attempts to raise the yield by varying the reaction conditions were unsuccessful. The structure of **1** was confirmed by its ^{1}H-, ^{19}F-, and ^{13}C-NMR spectra (see Tables I and II). The ^{1}H-NMR spectra showed H-5 resonating as a pair of triplets ($J_{4,5} = J_{5,6} \sim 9$ and $J_{5,F} \sim 50$ Hz), and in the ^{13}C-NMR spectrum, C-5 resonated as a large doublet ($J_{C\text{-}5,F} \sim 180$ Hz) and C-4 and C-6 as small doublets, respectively. These results indicate that the fluorine was introduced at C-5 in the equatorial orientation.





	R^1	R^2
1	OH	OH
2	H	OH
3	H	H

	R^1	R^2	R^3
4	OH	H	H
5	OH	H	Ac
6	H	OBz	Ac
7	H	OH	H
8	H	OH	Ac
9	F	H	Ac

5,3′-Dideoxy-5-fluorokanamycin B (**2**) was next prepared from 3′-deoxykanamycin B, utilizing a similar reaction sequence as described for **1**. 3′-Deoxypenta-*N*-tosylkanamycin B (**10**) was converted into the 4″,6″-*O*-cyclohexylidene derivative **11** by treatment with 1,1-dimethoxycyclohexane. Protection by cyclohexylidenation instead of the peracetylation used for **5** was tried in the hope of obtaining a better yield in the final detosylation step by sodium. Acetylation of **11** gave the diacetyl derivative **13** with the HO-5 group free. Application of the Mitsunobu reaction to this compound gave the 5-*O*-benzoyl-5-epi derivative **15** in good yield. Removal of the acyl groups (to give **16**) and reacetylation gave the 4′,2″-diacetyl-5-epihydroxyl derivative **17**. Treatment of it with DAST gave the 5-deoxy-5-fluoro derivative **18** in high yield. Detosylation of **18** with sodium in liquid ammonia followed by deblocking as described for **1** gave **2** in much improved yield in comparison to that for **1**.

5,3′,4′-Trideoxy-5-fluorokanamycin B (**3**) was prepared similarly. Tosylation of dibekacin (to give **19**) followed by cyclohexylidenation (to give **20**) and benzoylation gave the 2″-*O*-benzoyl-4″,6″-*O*-cyclohexylidene derivative **21**, which, on Mitsunobu reaction, afforded the 5-*O*-benzoyl-5-epi derivative **22**. After debenzoylation (to give **23**) and rebenzoylation, the resulting **24** having a free epi-HO-5 group was treated with DAST to give the 5-deoxy-5-fluoro derivative **25**. Similar deprotection gave 5,3′,4′-trideoxy-5-fluorokanamycin B (**3**) in 49% yield.

5,3′-Dideoxy-5,5-difluorokanamycin B (**26**) and 5,3′,4′-trideoxy-5,5-difluorokanamycin B (**27**) were also prepared. Acetylation of per(*N*-benzyloxycarbonyl)-3′-deoxykanamycin B (**28**) gave the 4′,2″,4″,6″-tetra-*O*-acetyl derivative **29** quantitatively, and this was oxidized with pyridinium chlorochromate to

TABLE I

Selected ^{1}H-NMR chemical shifts (δ, ppm) and coupling constants (Hz) of **1–3** and **5–27**

Compound	Solvent [a] Temp. [b]	H-4 or 6	H-5	H-6 or 4	H-1′	H-2′	H-3′	H-4′	H-1″	H-2″	H-3″	H-4″	$J_{4,5} \approx J_{5,6}$	$J_{4,F} \approx J_{6,F}$	$J_{5,F}$
1 [c]	D	3.50 dt	4.58 dt	3.62 dt	5.25 d	2.71 dd	3.54 dd	3.28 t	5.01 d	3.46 dd	3.02 t	3.30 t	9.5	13	51.5
1	D′	4.24 br q	4.99 dt	4.49 q	5.78 d	~ 3.51	4.07 t	~ 3.54	5.16 d	3.96 dd	~ 3.5	~ 3.7	9	~ 10	50
2 [c]	D	~ 3.5	4.57 dt	3.64 dt	5.08 d	~ 2.9	1.60 q(*ax*) 2.00 dt(*eq*)	~ 3.5	5.01 d	3.46 dd	3.01 t	3.30 t	9	12	51.5
2	D′	4.24 q	4.96 dt	4.49 q	5.63	~ 3.7		~ 3.8	5.16 bt t [d]	~ 4.0	3.54 dt [d]	~ 3.7	9	10.5	50.5
3 [c]	D	3.50 dt	4.55 dt	3.62 dt	5.10 d	2.79 dt			5.01 d	3.46 dd	3.02 t	3.31 t	9	12	51.5
5 [c]	C	3.38 t	3.46 dt	3.58 t	5.15 d	3.50 dt	5.10 dd	4.77 t	5.06 d	5.02 dd	4.02 q	4.87 t	9		
5	C′	~ 3.4	~ 3.5	~ 3.6	5.20 d	3.50 dd	5.11 dd	4.77 t	5.10 d	5.03 dd	4.00 t	4.90 t			
6	C′	3.78 dd	6.01 br t	~ 4.2	5.28 d	3.60 dd	4.89 dd	4.73 t	5.11 d	4.92 dd	3.84 t	4.82 t	~ 2		
7	P	~ 3.8	~ 5.2 (?)	3.97 dd	5.61 d	~ 3.7	~ 4.5	~ 4.1	5.46 d	~ 4.1	~ 4.5	4.35 t	~ 2		
8	C′	~ 3.4	~ 4.5	~ 3.6	5.11 d	3.43 dd	5.16 dd	4.72 t	5.04 d	4.95 dd	4.05 t	4.89 t			
9	C	3.54 q	4.29 dt	3.75 br q	5.19 d	3.44 dt	5.11 t	4.75 t	4.97 d	5.03 dd	~ 4.0	4.87 t	9	10	50
10	P′ 60°	3.40 t	3.16 t	~ 3.7	5.39 d	~ 3.6		~ 3.8	5.14 d	4.10 dd	4.25 t	4.00 t	9		
11	P′ 70°	3.50 t	3.19 t	~ 3.7	5.45 d	~ 3.7		~ 3.9	5.22 br s	~ 4.2			~ 9		
12	P	~ 3.8	~ 3.5	~ 3.8	5.54 d	~ 3.8		4.09 dt	5.82 d	5.57 dd	4.46 t	~ 3.9			
13	P′ 60°	~ 3.7	3.38 t	~ 3.7	5.51 d	~ 3.8		5.15 dt	5.59 d	5.46 dd	4.37 dd	3.91 t	9		

14	P	~ 4.2	6.42 br s	~ 4.4	5.40 d	~ 4.5		~ 4.4	5.59 d	5.47 dd	4.21 t	~ 3.8	2		
15	P′ 80°	~ 4.1	6.24 br t	4.22 dd	5.22 d	~ 3.8		5.01 dt	~ 5.3	~ 5.3	~ 4.1	~ 3.8	~ 2		
16	P	~ 3.9	5.05 br s	~ 3.9	5.30 d	~ 3.8		~ 4.0	5.43 d	4.17 dd	4.28 t	~ 3.8	2		
17	P	~ 3.9	5.07 br s	~ 4.0	5.36 d	~ 3.8		~ 5.3	~ 5.4	~ 5.5	4.46 t	~ 3.9	2		
18	P′ 60°	~ 3.9		~ 4.0	5.22 d	~ 3.8		5.11 dt	5.54 d	5.44 dd	4.39 t	~ 4.0			
18	C′	~ 3.5	4.12 dt	~ 3.7	4.92 d	3.39 dt		4.46 dt	5.01 d	5.06 dd	~ 3.8		~ 9		51
19	P	3.58 t	3.04 t	3.67 t	5.55 d	~ 3.6			5.29 d	4.18 dd	4.56 q	4.19 t	9		
20	P	3.60 t	3.10 t	3.70 t	5.59 d	~ 3.7			5.33 d	4.19 dd	4.29 q	3.86 t	9		
21	P 70°	3.72 t	3.20 m	3.73 t	5.44 d	~ 3.6			5.86 d	5.61 dd	4.58 q	3.94 t	8.5		
22	P′	4.21 br d	6.42 br s	4.21 br d	5.29 d	~ 3.7			5.57 d	5.62 dd	3.78 t	3.95 t	~ 2		
23	P	~ 3.9	5.03 br s	~ 3.9	5.28 br s	~ 3.5			5.45 d	~ 4.2	~ 4.3	~ 3.9	~ 2		
24	P	3.78 br	5.08 br s	3.89 br d	5.29 d	~ 3.6			5.54 d	5.68 dd	4.72 q	4.08	2		
25	C′	3.48 dt	4.26 dt	3.49 dt	4.76 d	~ 3.2			5.22 d	5.14 dd	3.97 t	3.58 t	9	13	51
26 [c]	D	3.65 ddd		~ 3.8	5.11 d	2.94 dt	1.62 q(*ax*) 2.03 dt(*eq*)	~ 3.5	5.02 d	3.49 dd	3.04 t	3.30 t		3 (F*eq*) 21 (F*ax*)	
27 [c]	D	3.70 ddd		~ 3.8	5.18 d	2.88 dt	1.71 dq(*ax*)	1.49 dq(*ax*)	5.08 d	3.55 dd	3.11 t	3.36 t		3 (F*eq*) 21 (F*ax*)	

[a] Abbreviations: C, $CDCl_3$; C′, $CDCl_3$–D_2O; D, 20% ND_3 in D_2O; D′, DCl in D_2O, pD ~ 1; P, pyridine-d_5; P′, 10:1 pyridine-d_5–D_2O. [b] Temperature not cited is room temperature. [c] Measured at 500 MHz. [d] $J_{1'',3''}$ 1.8 Hz.

TABLE II

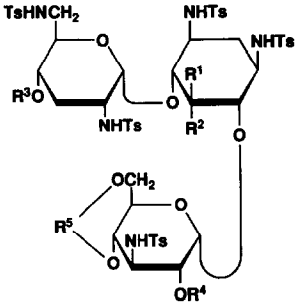
^{13}C-NMR chemical shifts (ppm) and coupling constants ($J_{C,F}$, Hz) of **1–3**, **26**, and **27**

	1 [a]	**1** [b]	**2** [b]	**3** [b]	**26** [b]	**27** [b]
C-1 [c]	48.35d	49.57d	49.68d	49.96d	49.36d	49.41d
	(11.4)	(9.5)	(8.8)	(9.6)	(7.4)	(7.3)
C-2	28.29	36.09	36.16	36.06	35.41	35.44
C-3 [c]	49.75d	50.81d	50.86d	50.82d	50.28d	50.29d
	(11.8)	(9.6)	(9.0)	(9.6)	(7.4)	(7.3)
C-4 [d]	75.79d	83.55d	83.66d	83.70d	81.64t	81.85t
	(17.6)	(15.1)	(16.9)	(15.1)	(17.5)	(17.4)
C-5	95.13d	96.32d	96.47d	96.40d	121.25dd	121.27dd
	(182.2)	(180.8)	(180.6)	(180.8)	(248.5, 252.5)	(248.4, 252.4)
C-6 [d]	81.44d	85.86d	85.88d	85.85d	84.29t	84.33t
	(20.3)	(16.8)	(16.8)	(16.6)	(18.3)	(18.3)
C-1′	95.94d	100.07d	99.57	100.76d	100.29d	101.66d
	(9.7)	(5.1)	(5.0)	(4.9)	(4.9)	(3.6)
C-2′	54.18	56.26	50.05	50.75	50.00	50.78
C-3′	68.86	74.33	35.82	26.67	35.68	26.67
C-4′	71.45	72.03	66.91	28.36	66.72	28.31
C-5′	70.06	74.24	74.76	71.31	74.81	71.57
C-6′	40.96	42.69	42.69	45.96	42.53	45.94
C-1″	101.32	100.23	101.24	101.22	102.05	102.08
C-2″	68.86	72.45	72.50	72.39	72.42	72.42
C-3″	55.77	55.03	55.07	54.99	54.93	54.93
C-4″	66.24	70.04	70.06	69.93	70.00	69.99
C-5″	73.59	72.92	72.96	72.89d (1.9) [e]	73.20 (v.sl.d) [e]	73.22
C-6″	60.78	61.09	61.10	60.99	61.09	61.07

[a] Measured in DCl in D_2O (pD ~1). [b] In 20% ND_3 in D_2O. [c,d] Values are interconvertible. [e] This means the presence of interspace coupling between F-5*eq* and H-5″.

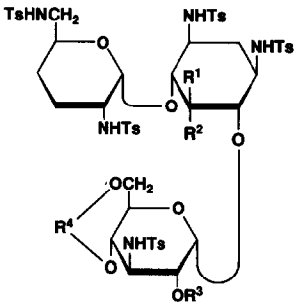
give the 5-oxo derivative **30** in good yield. The structure of **30** was confirmed by the ^{1}H–^{1}H shift-correlated 2D spectrum, in which H-4 and H-6 resonated at lowfield ($\delta \sim 5$), respectively. Treatment of **30** with DAST in dichloromethane gave the 5-deoxy-5,5-difluoro derivative **31** in good yield. Deacetylation (to give **32**) followed by de(benzyloxycarbonyl)ation gave the final product **26**. The 5,5-difluoro structure of **26** was confirmed by the ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra, which showed the presence of two fluorine atoms at C-5 vicinal to the C-4 and C-6 atoms bearing glycosyloxy residues. Applying a similar synthetic route, 5,3′,4′-trideoxy-5,5-difluorokanamycin B (**27**) was prepared from pentakis(*N*-benzyloxycarbonyl)-3′,4′-dideoxykanamycin B (**33**). Acetylation of **33** (to give **34**) followed by oxidation with dimethyl sulfoxide gave the 5-oxo derivative **35**, which was fluorinated with DAST to give the 5-deoxy-5,5-difluoro derivative **36**. Deprotection of **36** gave **27** through **37** in good overall yield. The structures were confirmed as described for **26**.

The antibacterial activities of the compounds prepared (**1**, **2**, **3**, **26**, and **27**) are shown in Table III. The data show that all compounds exhibit similar antibacterial activities against common and some resistant bacteria, but against specific bacteria

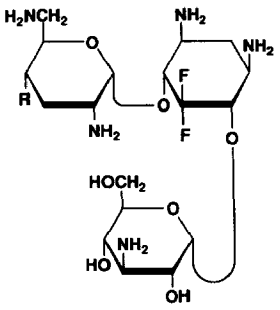


	R^1	R^2	R^3	R^4	R^5
10	OH	H	H	H	H, H
11	OH	H	H	H	$C(CH_2)_5$
12	OH	H	H	Ac	$C(CH_2)_5$
13	OH	H	Ac	Ac	$C(CH_2)_5$
14	H	OBz	H	Ac	$C(CH_2)_5$
15	H	OBz	Ac	Ac	$C(CH_2)_5$
16	H	OH	H	H	$C(CH_2)_5$
17	H	OH	Ac	Ac	$C(CH_2)_5$
18	F	H	Ac	Ac	$C(CH_2)_5$

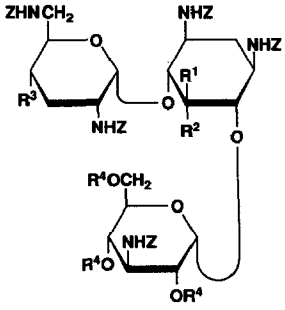
they differ from each other. 5-Deoxy-5-fluorokanamycin B (**1**) was inactive against resistant bacteria (Nos. 9, 10, 14, 17, and 25) producing 3′-phosphotransferases [9] APH(3′)-I and -II, because of the presence of the phosphorylated HO-3′ group. The other 5-fluoro derivatives (**2**, **3**, **26**, and **27**) showed significantly enhanced activities, as compared to the parent compounds, against bacteria producing



	R^1	R^2	R^3	R^4
19	OH	H	H	H, H
20	OH	H	H	$C(CH_2)_5$
21	OH	H	Bz	$C(CH_2)_5$
22	H	OBz	Bz	$C(CH_2)_5$
23	H	OH	H	$C(CH_2)_5$
24	H	OH	Bz	$C(CH_2)_5$
25	F	H	Bz	$C(CH_2)_5$



26 R = OH
27 R = H



	R^1	R^2	R^3	R^4
28	OH	H	OH	H
29	OH	H	OAc	Ac
30	=O		OAc	Ac
31	F	F	OAc	Ac
32	F	F	OH	H
33	OH	H	H	H
34	OH	H	H	Ac
35	=O		H	Ac
36	F	F	H	Ac
37	F	F	H	H

Z = $CO_2CH_2C_6H_5$

2″-adenylyltransferase [9] ANT(2″) (Nos. 11, 14, and 17), 3-acetyltransferase [9] AAC(3) (No. 15; **1** was also active), and in part against 2′-acetyltransferase [9] AAC(2′) (Nos. 22 and 23; **1** was also active). The second fact may be explained by assuming that the decreased basicity of the NH_2-3 group (induced by the 5-fluoro group(s)) lower the susceptibility of the NH_2-3 group to the active site of ANT(2″). However, the first fact was unexpected. This may be partly ascribed to the difference in electrostatic repulsion between F-5*eq*–O-6 or F-5*eq*–ring-oxygen atom (of the 3-amino-3-deoxy-D-glucose unit) and that between HO-5–O-6 or HO-5–ring-oxygen atom; this may somewhat alter the glycosidic bond-angles between the 2-deoxystreptamine and 3-amino-3-deoxy-D-glucose units from the original ones, and impair the fit of the HO-2″ group to the active site of the ANT(2″). The 5-fluoro and 5,5-difluoro derivatives showded much decreased toxicity (Table III) as compared with the parent compounds. This suggests that the fluorination at C-5 decreases the basicity of the NH_2-1 and -3 groups and lowers the intrinsic toxicity. Details on the decrease of the basicity of the amino groups (based on the ^{13}C chemical shifts) will be reported in the near future [10].

EXPERIMENTAL

General methods.—Optical rotations were determined with a Perkin–Elmer 241 polarimeter. ^1H-NMR (at 250 MHz) and ^{19}F-NMR (at 235.3 MHz) spectra were

TABLE III

Minimal inhibitory concentration [a] ($\mu g\ mL^{-1}$) [b] and acute toxicity (LD_{50}) [c] of 3′-deoxykanamycin B (tobramycin, TOB), 3′,4′-dideoxykanamycin B (dibekacin, DKB), **1–3**, **26**, and **27**

No.	Test organism [d]	**1**	TOB	**2**	**26**	DKB	**3**	**27**
1	*St. a.* FDA 209P	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.8
2	*St. a.* Smith	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.4
3	*St. a.* Ap01 [e]	1.6	3.1	0.8	6.2	1.6	1.6	0.8
4	*Micr. l.* FDA16	0.8	6.2	1.6	3.1	12.5	3.1	6.2
5	*Micr. l.* PCI1001	0.8	12.5	1.6	6.2	12.5	3.1	25
6	*Coryn. b.* 1810	0.2	3.1	0.8	3.1	3.1	1.6	0.8
7	*E. c.* NIHJ	< 0.2	0.8	0.2	0.8	0.4	0.4	1.6
8	*E. c.* K-12 R5 [f]	25	A	25	A	50	25	A
9	*E. c.* K-12 ML1629 [g]	A	0.8	0.8	0.8	0.8	0.8	1.6
10	*E. c.* K-12 ML1410 R81 [g]	A	0.8	0.8	0.8	0.8	0.4	0.8
11	*E. c.* K-12 LA290 R55 [h]	3.12	12.5	1.6	0.4	25	6.2	1.6
12	*E. c.* K-12 LA290 R64	< 0.2	1.6	0.4	0.4	6.2	0.4	0.4
13	*E. c.* W677	< 0.2	< 0.2	0.4	< 0.2	0.4	0.2	0.4
14	*E. c.* JR66/W677 [h,i]	A	25	1.6	3.1	50	3.1	6.2
15	*E. c* JR225 [j]	0.4	25	0.8	0.4	A	1.6	3.1
16	*Kl. p.* PCI602	0.4	0.8	0.8	1.6	1.6	1.6	1.6
17	*Kl. p.* 22#3038 [h,i]	A	12.5	3.1	0.8	50	3.1	1.6
18	*Sh. s.* JS11746	0.4	0.4	0.4	0.8	0.8	0.8	0.8
19	*Sal. e.* 1891	1.6	1.6	1.6	3.1	1.6	3.1	6.2
20	*Serr. marc.*	6.2	6.2	3.1	6.2	12.5	12.5	25
21	*Prot. r.* GN311	< 0.2	0.8	0.4	0.2	0.4	0.4	0.8
22	*Prov.* sp. Pv16 [k]	0.8	6.2	3.1	12.5	25	3.1	6.2
23	*Prov.* sp. 2991 [k]	3.1	50	12.5	50	50	25	A
24	*Ps. aerug.* A3	1.6	0.2	0.2	0.2	0.4	0.4	0.8
25	*Ps. aerug.* H9 [i]	A	0.8	0.8	1.6	1.6	1.6	3.1
26	*Ps. aerug.* GN315 [f]	A	50	50	A	A	A	A
	LD_{50} ($mg\ kg^{-1}$)		70	130	270	70	130	250

[a] Judged by agar dilution streak method (Mueller–Hinton agar, 17 h 37°). [b] A: 100 or > 100. [c] Administered by intravenous injection in saline (pH ~ 7), mice, one shot. [d] Abbreviations: *St. a.*, *Staphylococcus aureus; Micr. l., Micrococcus luteus; Coryn. b., Corynebacterium bovis; E. c., Escherichia coli; Kl. p., Klebsiella pneumoniae; Sh. s., Shigella sonnei; Sal. e., Salmonella enteritidis; Serr. mar., Serratia marcescens; Prot. r., Proteus rettgeri; Prov., Providencia; Ps. aerug., Pseudomonas aeruginosa.* [e] Resistant strain producing AAD(4′), [f] AAC(6′), [g] APH(3′)-I, [h] ANT(2″), [i] APH(3′)-II, [j] AAC(3), and [k] AAC(2′).

recorded with Bruker WM 250 and AC 250P spectrometers, unless otherwise stated. ^{1}H-NMR (at 500 MHz) and ^{13}C-NMR (at 125.8 MHz) spectra were recorded with a Bruker AMX 500 spectrometer. Chemical shifts (δ) for ^{1}H, ^{13}C, and ^{19}F were measured, respectively, downfield from internal Me_4Si, Me_4Si with the aid of 1,4-dioxane ($\delta = \delta_{1,4\text{-dioxane}} + 67.4$), and Freon 11 ($CFCl_3$). Chemical shifts in the ^{1}H-NMR spectra were confirmed, if necessary, by the ^{1}H–^{1}H shift-correlated 2D spectra. TLC was performed on Kieselgel 60 F_{254} (Merck), and column chromatography on Wakogel C-200.

3',4',2'',4'',6''-Penta-O-*acetyl-1,3,2',6',3''-penta*-N-*tosylkanamycin B* (**5**).—To an ice-cold solution of **4** (ref. 11, 7.76 g, 6.2 mmol) in dry pyridine (155 mL) was added acetyl chloride [4.4 mL (62 mmol) and 2.2 mL after 1.5 h] and the temperature was kept at 0°. After 3 h, water (8.4 mL) was added and the solution was concentrated. The residue didsolved in $CHCl_3$ was wahsed with aq $NaHCO_3$ (satd), dried (Na_2SO_4), and concentrated to give **5** as a solid; 9.21 g (quantitative), $[\alpha]_D^{24}$ + 19° (*c* 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.59, 1.84, 1.91, 2.02, and 2.03 (each s, 3 H, Ac × 5); 2.40 (9 H), 2.47 (3 H), and 2.50 (3 H) [each s, Ts(*Me*) × 5].

Anal. Calcd for $C_{63}H_{77}N_5O_{25}S_5 \cdot H_2O$: C, 51.03; H, 5.37; N, 4.72; S, 10.81. Found: C, 51.13; H, 5.35; N, 4.56; S, 10.67.

3',4',2'',4'',6''-Penta-O-*acetyl-5*-O-*benzoyl-5-epi-1,3,2',6',3''-penta*-N-*tosylkanamycin B* (**6**).—To a solution of **5** (4.23 g, 2.9 mmol) in dry oxolane (65 mL) were added Ph_3P (2.27 g, 8.7 mmol), diethyl azodicarboxylate (1.34 mL, 8.7 mmol), and benzoic acid (1.06 g, 8.7 mmol), and the solution was kept for 2 h at room temperature. The same amount each of the three reagents were added and the solution was kept for a further 18 h. In TLC (20:1 $CHCl_3$–EtOH, double developments), the solution showed a single spot at R_F 0.33 (cf. **5**: R_F 0.4). Concentration gave a residue, that was dissolved in $CHCl_3$ and the solution was washed with aq $NaHCO_3$ (satd). Concentration gave a residue, that was chromatographed (20:0 → 20:1 $CHCl_3$–EtOH) to give **6** as a solid; 2.38 g (53%), $[\alpha]_D^{23}$ +20° (c 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$–D_2O): δ 1.48 (q, 1 H, *J* 13 Hz, H-2*ax*), 1.54, 1.64, 1.84, 1.95, and 1.97 (each s, 3 H, Ac × 5), 2.17 (dt, 1 H, $J_{1,2eq} = J_{2eq,3} \sim 4$ Hz, H-2*eq*), 2.35, 2.38, 2.40, 2.48, and 2.51 [each s, 3 H, Ts(*Me*) × 5], 2.81 (dd, 1 H, H-6'a), 3.13 and 3.37 (each dt, 1 H × 2, H-1, 3).

Anal. Calcd for $C_{70}H_{81}N_5O_{26}S_5 \cdot H_2O$: C, 52.99; H, 5.27; N, 4.41; S, 10.10. Found: C, 52.83; H, 5.31; N, 4.24; S, 9.89.

5-Epi-1,3,2',6',3''-penta-N-*tosylkanamycin B* (**7**).—Zemplén deacylation of **6** (1.20 g) in MeOH gave **7** as a solid; 930 mg (95%), $[\alpha]_D^{23}$ +34° (*c* 2, MeOH).

Anal. Calcd for $C_{53}H_{67}N_5O_{20}S_5 \cdot 2H_2O$: C, 49.33; H, 5.55; N, 5.43; S, 12.42. Found: C, 49.43; H, 5.55; N, 5.45; S, 12.15.

3',4',2'',6''-Penta-O-*acetyl-5-epi-1,3,2',6',3''-penta*-N-*tosylkanamycin B* (**8**).—To a solution of **7** (898 mg, 0.70 mmol) in dry pyridine (18 mL) was added acetyl chloride (0.77 mL, 11 mmol) and the solution was kept for 1 h at 0°. Addition of water (0.9 mL) followed by concentration gave a residue, that was treated as described for **5** to give **8** as a solid; 1.03 g (quantitative), $[\alpha]_D^{25}$ +19° (*c* 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$–D_2O: δ 1.60, 1.69, 1.94, 1.98, and 2.03 (each s, 3 H, Ac × 5), 2.38, 2.41, and 2.49 [each s, 6, 3, and 6 H, respectively, Ts(*Me*) × 5].

Anal. Calcd for $C_{63}H_{77}N_5O_{25}S_5 \cdot H_2O$: C, 51.03; H, 5.37; N, 4.72; S, 10.81. Found: C, 51.21; H, 5.44; N, 4.56; S, 10.68.

3',4',2'',6''-Penta-O-*acetyl-5-deoxy-5-fluoro-1,3,2',6',3''-penta*-N-*tosylkanamycin B* (**9**).—To an ice-cold solution of DAST (0.45 mL, 3.4 mmol) and pyridine (0.9 mL, 11 mmol) in CH_2Cl_2 (17.5 mL) was added dropwise a cold solution of **8** (1.09 g, 0.74 mmol) in CH_2Cl_2 (25 mL), and the solution was kept for 1.5 h at room

temperature. In TLC (25:1 $CHCl_3$–MeOH, double developments), the solution showed a single spot at R_F 0.5 (cf. **8**: R_F 0.55). The solution was poured into ice-cold aq $NaHCO_3$ (satd) and the lower layer separated was washed with water, dried (Na_2SO_4), and concentrated to give **9** as a solid; 1.09 g (quantitative), $[\alpha]_D^{24}$ +24° (*c*, 1, $CHCl_3$). ^{19}F-NMR ($CDCl_3$): δ −188.9 (dt).

Anal. Calcd for $C_{63}H_{76}FN_5O_{24}S_5 \cdot 0.5H_2O$: C, 51.28; H, 5.26; N, 4.75; S, 10.86. Found: C, 51.16; H, 5.36; N, 4.67; S, 10.72.

5-Deoxy-5-fluorokanamycin B (**1**).—To a solution of **9** (1.10 g, 0.75 mmol) in liquid NH_3 (~ 300 mL) at −60° was added excess Na (~ 1 g) and the solution was kept for 5 min at −60° (detosylation). Cold MeOH was added until the blue color of the solution disappeared and the NH_3 was evaporated by gradually raising the temperature, and finally under diminished pressure. An aq solution (110 mL) of the residue was heated for 30 min at 50° (deacetylation) and, after addition of Dowex-50W X-2 resin (H^+ form, 80 mL), the mixture was stirred for a while. The resin was packed in a column containing the same fresh resin (40 mL) and developed with aq M NH_3. The ninhydrin-positive fractions were concentrated, and the residue was subjected to column chromatography on CM Sephadex C-25 (85 mL; 0 → 0.15 M aq NH_3) to give **1** as a solid (0.4 carbonate salt); 98 mg (26%), $[\alpha]_D^{25}$ +141° (*c* 1, H_2O); ^{1}H-NMR (DCl in D_2O, pD ~ 1): δ 2.08 (q, 1 H, H-2*ax*), 2.60 (br dt, 1 H, H-2*eq*), and 3.31 (dd, 1 H, H-6′a); $J_{3,4} = J_{4,5} = J_{5,6} = J_{1,6}$ ~ 10 Hz. ^{1}H-NMR (500 MHz, 20% ND_3 in D_2O): δ 1.28 (H-2*ax*), 1.96 (dt, H-2*eq*), 2.78 (H-6′a), and 2.97 (dd, 1 H, H-6′b). ^{19}F-NMR (DCl in D_2O, pD ~ 1): δ −191.8 (dt).

Anal. Calcd for $C_{18}H_{36}FN_5O_9 \cdot 0.4H_2CO_3$; C, 43.31; H, 7.27; F, 3.72; N, 13.72. Found: C, 43.48; H, 7.44; F, 3.44; N, 13.82.

3′-Deoxy-1,3,2′,6′,3″-penta-N-*tosylkanamycin B* (**10**).—Tobramycin free base (600 mg) was tosylated as described [11] for **4** to give **10** as a solid; 1.64 g (quantitative), $[\alpha]_D^{24}$ +20° (*c* 2, pyridine).

Anal. Calcd for $C_{53}H_{67}N_5O_{19}S_5 \cdot 2H_2O$: C, 49.95; H, 5.62; N, 5.50; S, 12.58. Found: C, 49.75; H, 5.55; N, 5.79; S, 12.52.

4″,6″-O-Cyclohexylidene-3′-deoxy-1,3,2′,6′,3″-penta-N-*tosylkanamycin B* (**11**).—A mixture of **10** (1.72 g, 1.4 mmol), 1,1-dimethoxycyclohexane (1.25 mL, 8.3 mmol), and *p*-toluenesulfonic acid (60 mg, 0.35 mmol) in dry DMF (35 mL) was stirred for 2 h at 50° under slightly diminished pressure (to evaporate some of the volatile material). The solution was poured into aq $NaHCO_3$ (25 mL, satd), and the whole mixture was concentrated. The residue was washed thoroughly with water and diethyl ether, and dried to give **11** as a solid; 1.76 g (98%), $[\alpha]_D^{20}$ −7° (*c* 1, 1:1 $CHCl_3$–MeOH).

Anal. Calcd for $C_{59}H_{75}N_5O_{19}S_5 \cdot H_2O$: C, 53.01; H, 5.80; N, 5.24; S, 12.00. Found: C, 53.00; H, 5.69; N, 5.25; S, 11.96.

2″-O-Acetyl-4″,6″-O-cyclohexylidene-3′-deoxy-1,3,2′,6′,3″-penta-N-*tosylkanamycin B* (**12**).—A mixture of **11** (20.8 mg, 0.016 mmol) and *N*-acetylimidazole (1.74 mg, 0.016 mmol) in 9:1 Me_2SO–pyridine [5] (0.1 mL) was kept overnight at room

temperature. The solution was poured into aq $NaHCO_3$ (2.2 mL, satd) and the precipitate was filtered off, washed with water and diethyl ether, and dried to give **12** as a solid; 19 mg (89%), $[\alpha]_D^{23}$ +8° (*c* 2, $CHCl_3$).

Anal. Calcd for $C_{61}H_{77}N_5O_{20}S_5 \cdot H_2O$: C, 53.14; H, 5.78; N, 5.08; S, 11.63. Found: C, 52.91; H, 5.71; N, 5.05; S, 11.37.

*4',2''-Di-*O*-acetyl-4'',6''-*O*-cyclohexylidene-3'-deoxy-1,3,2',6',3''-penta-*N*-tosylkanamycin B* (**13**).—To an ice-cold solution of **11** (25.27 g, 19 mmol) in pyridine (500 mL) was added dropwise acetyl chloride (8 mL, 110 mmol). After 2 h at 0°, water (10 mL) was added, and the solution was concentrated. The residue was dissolved in $CHCl_3$ and the solution was washed with aq 10% $KHSO_4$, aq $NaHCO_3$ (satd), dried (Na_2SO_4), and concentrated to give **13** as a solid; 27.03 g (quantitative), $[\alpha]_D^{26}$ +7° (*c* 1, $CHCl_3$); ^{1}H-NMR (10:1 pyridine-d_5–D_2O at 60°): δ 1.89, 2.21 (6 H), 2.26, 2.35, and 2.37 (6 H) (each s, Ac × 2 and Ts(*Me*) × 5).

Anal. Calcd for $C_{63}H_{79}N_5O_{21}S_5 \cdot 1.5H_2O$: C, 52.93; H, 5.78; N, 4.90; S, 11.21. Found: 52.91; H, 5.68; N, 5.03; S, 11.18.

*2''-*O*-Acetyl-5-*O*-benzoyl-4'',6''-*O*-cyclohexylidene-3'-deoxy-5-epi-1,3,2',6',3''-penta-*N*-tosylkanamycin B* (**14**).—To a solution of **12** (113 mg, 0.082 mmol) in dry oxolane (1.7 mL) were added Ph_3P (66 mg, 0.25 mmol), diethyl azodicarboxylate (0.039 mL, 0.25 mmol) and benzoic acid (31 mg, 0.25 mmol), and the solution was kept for 1.5 h at room temperature. Concentration gave a residue, that was dissolved in $CHCl_3$ and the solution was washed with aq $NaHCO_3$ (satd), dried (Na_2SO_4), and concentrated. The syrupy residue was chromatographed (15:0 → 15:1 $CHCl_3$–EtOH) to give **14** as a solid; 84.1 mg (69%), $[\alpha]_D^{19}$ −33° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{68}H_{81}N_5O_{21}S_5 \cdot H_2O$: C, 55.08; H, 5.64; N, 4.72; S, 10.81. Found: C, 55.21; H, 5.54; N, 4.70; S, 10.86.

*4',2''-Di-*O*-acetyl-5-*O*-benzoyl-4'',6''-*O*-cyclohexylidene-3'-deoxy-5-epi-1,3,2',6',3''-penta-*N*-tosylkanamycin B* (**15**).—Compound **13** (448 mg, 0.31 mmol) dissolved in oxolane (7 mL) was treated with Ph_3P (500 mg, 1.9 mmol), diethyl azodicarboxylate (0.3 mL, 1.9 mmol) and benzoi acid (240 mg, 2.0 mmol) as described for **6** to give **15** as a solid; 316 mg (67%), $[\alpha]_D^{21}$ −9° (*c* 1, $CHCl_3$); ^{1}H-NMR (10:1 pyridine-d_5–D_2O at 80°): δ 1.78 (3 H), 2.20 (9 H), 2.28 (3 H), 2.33 (3 H), and 2.36 (3 H) [each s, Ac × 2 and Ts(*Me*) × 5].

Anal. Calcd for $C_{70}H_{83}N_5O_{22}S_5$: C, 55.80; H, 5.55; N, 4.65; S, 10.64. Found: C, 55.60; H, 5.62; N, 4.55; S, 10.38.

*4'',6''-*O*-Cyclobexylidene-3'-deoxy-5-epi-1,3,2',6',3''-penta-*N*-tosylkanamycin B* (**16**).—Zemplén deacylation of **15** (146 mg) in MeOH gave **16** as a solid; 130 mg (99%), $[\alpha]_D^{21}$ −8° (*c* 1.2, 1:1 $CHCl_3$–MeOH).

Anal. Calcd for $C_{59}H_{75}N_5O_{19}S_5 \cdot 2H_2O$: C, 52.31; H, 5.88; N, 5.17; S, 11.83. Found: C, 52.35; H, 5.65; N, 5.25; S, 12.06.

*4',2''-Di-*O*-acetyl-4'',6''-*O*-cyclohexylidene-3'-deoxy-5-epi-1,3,2',6',3''-penta-*N*-tosylkanamycin B* (**17**).—To an ice-cold solution of **16** (11.14 g, 8.2 mmol) in pyridine (220 mL) was added dropwise acetyl chloride (3.6 mL, 51 mmol) and the solution was kept for 2 h. Work-up as described for **5** gave **17** as a solid; 10.8 g

(92%), $[\alpha]_D^{23}$ +19° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5): δ 1.78, 2.12, 2.14, 2.22, 2.23, and 2.37 (6 H) (each s, Ac × 2 and Ts(*Me*) × 5).

Anal. Calcd for $C_{63}H_{79}N_5O_{21}S_5 \cdot 1.5H_2O$: C, 52.93; H, 5.78; N, 4.90; S, 11.21. Found: C, 52.77; H, 5.60; N, 4.98; S, 11.22.

4',2''-Di-O-acetyl-4'',6''-O-cyclohexylidene-5,3'-dedeoxy-5-fluoro-1,3,2',6',3''-penta-N-tosylkanamycin B (**18**).—To an ice-cold solution of DAST (0.15 mL, 1.1 mmol) and pyridine (0.3 mL, 3.7 mmol) in dry benzene (5 mL) was added dropwise a solution of **17** (331 mg, 0.23 mmol) in dry benzene (8.2 mL), and the solution was kept for 2 h at room temperature. In TLC (20:1 benzene–MeOH, seven-fold developments), the solution showed a single spot at R_F 0.5 (cf. **17**: R_F 0.47). Purification of the product as described for **9** gave **18** as a solid; 330 mg (quantitative), $[\alpha]_D^{22}$ +21° (*c* 1, $CHCl_3$). ^{19}F-NMR ($CDCl_3$–D_2O): δ −189.3 (dt).

Anal. Calcd for $C_{63}H_{78}FN_5O_{20}S_5 \cdot 1.5H_2O$: C, 52.85; H, 5.70; N, 4.89; S, 11.20. Found: C, 52.68; H, 5.46; N, 5.04; S, 11.30.

5,3'-Dideoxy-5-fluorokanamycin B (**2**).—Compound **18** (5.27 g, 3.7 mmol) dissolved in liquid NH_3 (1 L) was treated with excess Na (~ 5.3 g) as described for **1**, and after deacetylation followed, the product was treated with Dowex-50W X-2 resin (H^+ form, 600 mL). Purification of the product as described for **1** gave **2** as its solid sesquihydrate; 1.17 g (64%), $[\alpha]_D^{24}$ +141° (*c* 1, water). ^{19}F-NMR (DCl in D_2O, pD ~ 1): δ −192.8 (dt).

Anal. Calcd for $C_{18}H_{36}FN_5O_8 \cdot 1.5H_2O$: C, 43.54; H, 7.92; F, 3.83; N, 14.10. Found: C, 43.27; H, 7.71; F, 3.83; N, 13.81.

3',4'-Dideoxy-1,3,2',6',3''-penta-N-tosylkanamycin B (**19**).—Dibekacin sulfate (3.50 g, the potency being 690 μg/mg) in 2:1 1,4-dioxane–water (70 mL) was treated similarly as described for **4** to give **19** as a solid; 6.06 g (91%), $[\alpha]_D^{24}$ +26° (*c* 1, DMF); ^{1}H-NMR (pyridine-d_5): δ 2.07, 2.14, 2.17, 2.36, and 2.38 (each s, 3 H, Ts(*Me*) × 5).

Anal. Calcd for $C_{53}H_{67}N_5O_{18}S_5 \cdot H_2O$: C, 51.32; H, 5.61; N, 5.65; S, 12.92. Found: C, 51.37; H, 5.64; N, 5.67; S, 12.73.

4'',6''-O-Cyclohexylidene-3',4'-dideoxy-1,3,2',6',3''-penta-N-tosylkanamycin B (**20**).—Compound **19** (1.63 g) was treated as described for **11** to give **20** as a solid; 1.74 g (quantitative), $[\alpha]_D^{24}$ +23° (*c* 1, DMF).

Anal. Calcd for $C_{59}H_{75}N_5O_{18}S_5 \cdot H_2O$: C, 53.66; H, 5.88; N, 5.30. Found: C, 53.67; H, 5.71; N, 5.27.

2''-O-Benzoyl-4'',6''-O-cyclohexylidene-3',4'-dideoxy-1,3,2',6',3''-penta-N-tosylkanamycin B (**21**).—Compound **20** (1.75 g, 1.3 mmol) dissolved in pyridine (35 mL) was treated with benzoyl chloride (0.78 mL, 7.0 mmol) similarly as described for **13** to give **21** a solid; 1.88 g (quantitative), $[\alpha]_D^{22}$ +24° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{66}H_{79}N_5O_{19}S_5 \cdot H_2O$: C, 55.64; H, 5.73; N, 4.92; S, 11.25. Found: 55.32; H, 5.59; N, 5.09; S, 10.86.

5,2''-Di-O-benzoyl-4'',6''-O-cyclohexylidene-3',4'-dideoxy-5-epi-1,3,2',6',3''-penta-N-tosylkanamycin B (**22**).—Compound **21** (1.64 g, 1.2 mmol) dissolved in dry oxolane (25 mL) was treated with Ph_3P (4.5 g, 17 mmol), diethyl azodicarboxylate

(2.7 mL, 17 mmol) and benzoic acid (2.1 g, 17 mmol) (one fifth amount each of the three reagents were added at 0, 1.5, 3, 5, and 20 h) and the solution was kept for a total of 40 h at 50°. In HPTLC (4:1 toluene–acetone) the solution showed a main spot at R_F 0.37 (cf. **21**: R_F 0.4). After work-up as described for **6**, the products were separated twice by column chromatography with 20:0 → 20:1 $CHCl_3$–MeOH and 4:1 toluene–acetone to give **22** as a solid; 950 mg (54%), $[\alpha]_D^{24}$ 0° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{73}H_{83}N_5O_{20}S_5 \cdot H_2O$: C, 57.35; H, 5.60; N, 4.58; S, 10.49. Found: C, 57.48; H, 5.79; N, 4.62; S, 10.19.

*4",6"-O-Cyclohexylidene-3',4'-dideoxy-5-epi-1,3,2',6',3"-penta-*N*-tosylkanamycin B* (**23**).—Zemplén debenzoylation of **22** (950 mg) gave **23** as a solid; 830 mg (quantitative), $[\alpha]_D^{24}$ +14° (*c* 1, DMF).

Anal. Calcd for $C_{59}H_{75}N_5O_{18}S_5 \cdot H_2O$: C, 53.66; H, 5.88; N, 5.30; S, 12.14. Found: C, 53.86; H, 6.07; N, 5.26; S, 11.88.

*2"-O-Benzoyl-4",6"-O-cyclohexylidene-3',4'-dideoxy-5-epi-1,3,2',6',3"-penta-*N*-tosylkanamycin B* (**24**).—A mixture of **23** (770 mg, 0.58 mmol) and benzoyl chloride (0.34 mL, 2.9 mmol) in pyridine (15 mL) was kept for 30 min at room temperature. Purification of the product as described for **5** gave **24** as a solid; 840 mg (quantitative), $[\alpha]_D^{24}$ +19° (*c*, 1, $CHCl_3$).

Anal. Calcd for $C_{66}H_{79}N_5O_{19}S_5$: C, 56.35; H, 5.66; N, 4.98. Found: C, 56.20; H, 5.91; N, 5.19.

*2"-O-Benzoyl-4",6"-O-cyclohexylidene-5,3',4'-trideoxy-5-fluoro-1,3,2',6',3"-penta-*N*-tosylkanamycin B* (**25**).—To an ice-cold solution of DAST (0.33 mL, 2.5 mmol) and pyridine (0.66 mL, 8.2 mmol) in dry benzene (15 mL) was added **24** (770 mg, 0.55 mmol) and the solution was kept for 1 h at room temperature. In HPTLC (4:1 toluene–acetone), the solution showed a single spot at R_F 0.32 (cf. **24**: R_F 0.35). Purification of the product as described for **9** gave **25** as a solid; 790 mg (quantitative) $[\alpha]_D^{24}$ +15° (*c* 1, $CHCl_3$). ^{19}F-NMR (pyridine-d_5): δ −186.0 (dt); $J_{4,F} = J_{6,F}$ 14 and $J_{5,F}$ 49.5 Hz.

Anal. Calcd for $C_{66}H_{78}FN_5O_{18}S_5$: C, 56.27; H, 5.58; N, 4.97; S, 11.38. Found: C, 56.29; H, 5.67; N, 4.96; S, 11.09.

5,3',4'-Trideoxy-5-fluorokanamycin B (**3**).—Zemplén debenzoylation of **25** (740 mg) gave a solid (0.59 g), which was treated with aq 80% AcOH (12 mL, 30 min, 80°). The decyclohexylidenated product (0.52 g) was treated with excess Na (~ 500 mg) in liquid NH_3 (~ 100 mL, 5 min, −50°) to give, after purification of the products as described for **1** involving column chromatography with CM Sephadex C-25 (90 mL, 0 → 0.2 M aq (NH_3), **3** as its solid 0.8 carbonate salt; 104 mg (49%), $[\alpha]_D^{24}$ +139° (*c* 1, H_2O). ^{19}F-NMR (20% ND_3 in D_2O): δ −190.7 (dt).

Anal. Calcd for $C_{18}H_{36}FN_5O_7 \cdot 0.8\ H_2CO_3$: C, 44.88; H, 7.53; N, 13.92; F, 3.78. Found: C, 44.61; H, 7.74; N, 13.84; F, 3.89.

*1,3,2',6',3"-Pentakis(*N*-benzyloxycarbonyl)-3'-deoxykanamycin B* (**28**).—To an ice-cold mixture of tobramycin 2.5 sulfate (1.29 g, 1.8 mmol) and anhydrous Na_2CO_3 (1.2 g, 11 mmol) in 1:1 acetone–water (25 mL) was added benzyl

chloroformate (1.13 mL, 8.2 mmol) and the mixture was stirred for 1 h. Another benzyl chloroformate (0.15 mL) and Na_2CO_3 (24 mg) were added and the mixture was stirred for further 3 h. Addition of water (130 mL) gave precipitates, which were washed with water and diethyl ether to give **28** as a solid; 2.00 g (96%), $[\alpha]_D^{24}$ +53° (*c* 2, pyridine) [lit.[12] $[\alpha]_D^{26}$ +53.8° (*c* 0.3, MeOH)].

Anal. Calcd for $C_{58}H_{67}N_5O_{19} \cdot H_2O$: C, 60.25; H, 6.02; N, 6.06. Found: C, 60.15; H, 6.02; N, 6.25.

*4',2'',4'',6''-Tetra-*O*-acetyl-1,3,2',6',3''-pentakis(*N*-benzyloxycarbonyl)-3'-deoxykanamycin B* (**29**).—A solution of **28** (2.09 g, 1.8 mmol) and acetic anhydride (3.47 mL, 37 mmol) in pyridine (42 mL) was kept overnight at room temperature. Addition of water (3.3 mL) followed by standard work-up gave **29** as a solid; 2.36 g (quantitative), $[\alpha]_D^{23}$ +82° (*c* 1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5 at 70°): δ 1.85, 1.95 (6 H), and 2.09 (each s, Ac × 4), 4.81 (q, 1 H, *J* 10 Hz, H-3''), 5.62 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1'), and 5.83 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1'').

Anal. Calcd for $C_{66}H_{75}N_5O_{23}$: C, 60.68; H, 5.79; N, 5.36. Found: C, 60.61; H, 5.74; N, 5.28.

*4',2'',4'',6''-tetra-*O*-acetyl-1,3,2',6',3''-pentakis(*N*-benzyloxycarbonyl)-5,3'-dideoxy-5-oxokanamycin B* (**30**).—A mixture of **29** (2.40 g, 1.8 mmol), pyridinium chlorochromate (4.75 g, 22 mmol), and molecular sieves 3A (5.5 g) in CH_2Cl_2 (300 mL) was stirred for 3 days at room temperature. In TLC (20:1 $CHCl_3$–EtOH, double developments, the solution showed a main spot at R_F 0.35 (cf. **29**: R_F 0.45). After filtration with the aid of Celite, the filtrate was washed with aq 10% $KHSO_4$, aq $NaHCO_3$ (satd), and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (7:2 $CHCl_3$–acetone) to give **30** as a solid; 1.82 g (75%), $[\alpha]_D^{24}$ +65° (*c* 2, $CHCl_3$); ^{1}H-NMR (pyridine-d_5 at 70°): δ 1.88, 1.92, 1.96, and 1.97 (each s, 3 H, Ac × 4), ~2.5 (2 H, H-2*eq*, 3'*eq*), 2.84 (q, 1 H, *J* 12.5 Hz, H-2*ax*), 3.53 (unresolved dt, 1 H, H-6'a), 3.67 (m, 1 H, H-6'b), 3.96 (br, 1 H, H-1 or -3), ~4.1 (2 H, H-3 or -1, and H-2'), ~4.3 (2 H, H-5' and 6''a), 4.58 (dd, 1 H, $J_{5'',6''b}$ 3.8 and $J_{6''a,6''b}$ 12.5 Hz, H-6''b), ~4.95 (2 H, H-4 or 6, and H-3''), ~5.1 (2 H, H-6 or 4, and H-4'), ~5.23 (1 H, H-1'), ~5.38 (1 H, H-2''), ~5.5 (1 H, H-1''), and 5.51 (t, *J* 10 Hz, H-4'').

Anal. Calcd for $C_{66}H_{73}N_5O_{23} \cdot 0.5\ H_2O$: C, 60.36; H, 5.68; N, 5.33. Found: C, 60.23; H, 5.69; N, 5.61.

*4',2'',4'',6''-Tetra-*O*-acetyl-1,3,2',6',3''-pentakis(*N*-benzyloxycarbonyl)-5,3'-dideoxy-5,5-difluorokanamycin B* (**31**).—To an ice-cold solution of **30** (1.68 g, 1.3 mmol) in dry CH_2Cl_2 (50 mL) was added DAST (2.06 mL, 16 mmol) and the solution was kept for 8 h at room temperature. In TLC (20:1 $CHCl_3$–EtOH) the solution showed a main spot at R_F 0.35. The solution was poured into ice-cold aq $NaHCO_3$ (satd, 200 mL) under stirring and, after stirring for 30 min, the organic layer was separated, dried (Na_2SO_4), and concentrated. The residue was chromatographed with 4:1 $CHCl_3$–acetone to give **31** as a solid; 1.13 g (67%), $[\alpha]_D^{24}$ +67° (*c* 2, $CHCl_3$); ^{1}H-NMR (pyridine-d_5 at 70°): δ 1.83, 1.93, 1.98, and 1.99 (each s, 3 H, Ac × 4), 2.11 (q, 1 H, H-3'*ax*), 2.30–2.42 (3 H, H-2*ax*, 2*eq*, 3'*eq*), 3.54 (dt, 1 H,

H-6′a), 3.73 (ddd, 1 H, H-6′b), ~4.05 (1 H, H-1 or 3), 4.10–4.25 (2 H, H-3 or 1, and H-2′), ~4.35 (2 H, H-5′, 6″a), ~4.4 (H-4 or 6), 4.49 (dd, 1 H, H-6″b), 4.57 (1 H, H-6 or 4), 4.68 (1 H, H-5″), 4.83 (q, 1 H, H-3″), 5.08 (dt, 1 H, H-4′), 5.42 (dd, 1 H, H-2″), 5.50 (t, 1 H, H-4″), 5.55 (d, 1 H, H-1′), 5.65 (d, 1 H, H-1″), 7.99 (d, 1 H, NH-1 or 3), and 8.14 (d, 1 H, NH-3″); $J_{1',2'}$ 3.2, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'} = J_{4',5'}$ 11.5, $J_{3'eq,4'}$ 4.7, $J_{5',6'a} = J_{6'a,NH\text{-}6'} = J_{6'b,NH\text{-}6'}$ 4, $J_{5',6'b}$ 7, $J_{6'a,6'b}$ 14, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ 11, $J_{3'',4''} = J_{4'',5''}$ 10, $J_{3'',NH\text{-}3''}$ 9, $J_{5'',6''b}$ 3.5, and $J_{6''a,6''b}$ 12.5 Hz. ^{19}F-NMR (pyridine-d_5): δ −110.3 (d, 1 F, F-5*eq*) and −129.3 (dt, 1 F, F-5*ax*); $J_{H\text{-}4,Fax} = J_{H\text{-}6,Fax}$ 19.5 and $J_{Fax,Feq}$ 250 Hz.

Anal. Calcd for $C_{66}H_{73}F_2N_5O_{22}$: C, 59.77; H, 5.55; F, 2.86; N, 5.28. Found: C, 59.63; H, 5.54; F, 2.91; N, 4.99.

1,3,2′,6′,3″-Pentakis(N-benzyloxycarbonyl)-5,3′-dideoxy-5,5-difluorokanamycin B (**32**).—To a solution of **31** (400 mg, 0.30 mmol) in 1:1 oxolane–MeOH (8 mL) was added M NaOMe in MeOH (0.2 mL). After 40 min at room temperature, the solution was neutralized with aq HCl and concentrated. The residue was thoroughly washed with water and dried to give **32** as a solid; 347 mg (99%), $[\alpha]_D^{23}$ +70° (*c* 2, pyridine); ^{19}F-NMR (pyridine-d_5): δ −111.1 (d, 1 F, F-5*eq*) and −129.0 (dt, 1 F, F-5*ax*); $J_{H\text{-}4,Fax} = J_{H\text{-}6,Fax}$ 19 and $J_{Fax,Feq}$ 250 Hz.

Anal. Calcd for $C_{58}H_{65}F_2N_5O_{18}$: C, 60.15; H, 5.66; F, 3.28; N, 6.05. Found: C, 60.19; H, 5.76; F, 3.63; N, 6.29.

5,3′-Dideoxy-5,5-difluorokanamycin B (**26**).—A solution of **32** (942 mg) in 20:4:5 1,4-dioxane–water–acetic acid (47 mL) was hydrogenated in the presence of Pd black under 1 atm of H_2. The product was purified by passing a column of CM-Sephadex (developed with 0 → 0.15 M aq NH_3) to give **29** as a solid (hydrate) 409 mg (99%), $[\alpha]_D^{22}$ +134° (*c* 1, H_2O); ^{1}H-NMR (500 MHz, 20% ND_3 in D_2O): δ 1.32 (q, 1 H, H-2*ax*), 2.07 (dt, 1 H, H-2*eq*), 2.74 (dd, 1 H, H-6′a), 2.97 (dd, 1 H, H-6′b), 3.00 and 3.03 (each 1 H, H-3, 3), and 3.72–3.79 (2 H, H-6″a, 6″b); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 13, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'}$ 12, $J_{2',3'eq} = J_{3'eq,4'}$ 4, $J_{5',6'a}$ 6.5, $J_{5',6'b}$ 2.5, and $J_{6'a,6'b}$ 13.5 Hz. ^{19}F-NMR (20% ND_3 in D_2O): −110.7 (d, 1 F, F-5*eq*) and −128.6 (dt, 1 F, F-5*ax*); $J_{Fax,Feq}$ 246 Hz.

Anal. Calcd for $C_{18}H_{35}F_2N_5O_8 \cdot H_2O$: C, 42.77; H, 7.38; F, 7.52; N, 13.85. Found: C, 42.59; H, 7.07; F, 7.23; N, 13.62.

1,3,2′,6′,3″-Pentakis(N-benzyloxycarbonyl)-3′,4′-dideoxykanamycin B (**33**).—Dibekacin (free base, 1.01 g) was treated similarly as described for **28** to give **33** as a solid; 2.35 g (92%), $[\alpha]_D^{23}$ +56° (*c* 1, pyridine).

Anal. Calcd for $C_{58}H_{67}N_5O_{18} \cdot H_2O$: C, 61.10; H, 6.10; N, 6.14. Found: C, 61.26; H, 6.04; N, 6.20.

2″,4″,6″-Tri-O-acetyl-1,3,2′,6′,3″-pentakis(N-benzyloxycarbonyl)-3′,4′-dideoxykanamycin B (**34**).—$[\alpha]_D^{23}$ +59° (*c* 2, $CHCl_3$); ^{1}H-NMR (500 MHz, pyridine-d_5 at 70°): δ 1.50 (dq, 1 H, H-4′*ax*), 1.60 (br d, 1 H, H-4′*eq*), 1.85, 1.95, and 2.09 (each s, 3 H, Ac × 3), 1.85–1.93 (3 H, H-2*ax*, 3′*ax*, 3′*eq*), 2.46 (br dt, 1 H, H-2*eq*), 3.38–3.48 (2 H, H-6′a, 6′b), 3.83 (br q, H-5), 3.91 (t, 1 H, *J* 9 Hz, H-4 or 6), 3.9–4.0 (2 H, H-1 or 3, and H-2′), 4.06–4.13 (2 H, H-3 or 1, and H-6 or 4), 4.37 (apparent

s, 2 H, H-6″a, 6″b), 4.81 (q, 1 H, H-3″), 5.38 (dd, 1 H, $J_{1'',2''}$ 3.5 and $J_{2'',3''}$ 11 Hz, H-2″), 5.45 (t, 1 H, H-4″), 5.55 (d, 1 H, $J_{1',2'}$ 2.8 Hz, H-1′), 5.82 (d, 1 H, H-1″), 6.64 (br s, 1 H, OH-5).

Anal. Calcd for $C_{64}H_{73}N_5O_{21} \cdot H_2O$: C, 60.70; H, 5.97; N, 5.53. Found: C, 60.77; H, 5.72; N, 5.57.

*2″,4″,6″-Tri-*O*-acetyl-1,3,2′,6′,3″-pentakis(*N*-benzyloxycarbonyl)-5,3′,4′-trideoxy-5-oxokanamycin B* (**35**).—To a solution of **34** (1.60 g) in dry Me_2SO (4.8 mL) was added acetic anhydride (3.2 mL) and the solution was kept for 3 days at room temperature. The solution was poured, dropwise, into ice-cold aq $NaHCO_3$ (satd, 160 mL) and the mixture was stirred for 2 h. The precipitates were filtered, dissolved in $CHCl_3$, and the solution was concentrated. The residue was chromatographed (30:1 $CHCl_3$–EtOH) to give **35** as a solid; 1.18 g (75%), $[\alpha]_D^{24}$ +71° (*c* 2, $CHCl_3$); ^{1}H-NMR (500 MHz, pyridine-d_5 at 70°): δ 1.43–1.54 (2 H, H-4′*ax*, 4′*eq*), 1.82–1.93 (2 H, H-3′*ax*, 3′*eq*), 1.88, 1.96, and 1.97 (each s, 3 H, Ac × 3), 2.51 (dt, 1 H, H-2*eq*), 2.76 (q, 1 H, H-2*ax*), 3.26–3.38 (m, 2 H, H-6′a, 6′b), 3.9–4.0 (2 H, H-1 or 3, and H-2′), 4.1–4.2 (2 H, H-3 or 1, and H-5′), 4.29 (slightly br d, 1 H, H-6″a), 4.56 (dd, 1 H, H-6″b), 4.86 (br d, *J* 10.8 Hz, H-4 or 6), 4.94 (q, 1 H, H-3″), 5.08 (br, 1 H, H-6 or 4), ~5.2 (H-1′), 5.37 (dd, 1 H, H-2″), 5.48 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.50 (t, 1 H, H-4″), and 8.11 (2 H, NH-1 or 3, and NH-3″).

Anal. Calcd for $C_{64}H_{71}N_5O_{21}$: C, 61.68; H, 5.74; N, 5.62. Found: C, 61.73; H, 5.64; N, 5.52.

*2″,4″,6″-Tri-*O*-acetyl-1,3,2′,6′,3″-pentakis(*N*-benzyloxycarbonyl)-5,3′,4′-trideoxy-5,5-difluorokanamycin B* (**36**).—Compound **35** (898 mg, 0.72 mmol) was treated with DAST (1.24 mL, 9.4 mmol) similarly as described for **31**. The crude product was chromatographed with 9:2 $CHCl_3$–acetone to give **36** as a solid; 596 mg (65%), $[\alpha]_D^{23}$ +69° (*c* 2, $CHCl_3$); ^{1}H-NMR (pyridine-d_5 at 70°): δ 1.84, 1.97 (6 H) (each s, Ac × 3). ^{19}F-NMR (pyridine-d_5): δ −109.8 (d, 1 F, F-5*eq*) and −129.3 (dt, 1 F, F-5*ax*); $J_{H\text{-}4,F\text{-}5ax} = J_{H\text{-}6,F\text{-}5ax}$ 19.5 and $J_{Fax,Feq}$ 250 Hz.

Anal. Calcd for $C_{64}H_{71}F_2N_5O_{20}$: C, 60.61; H, 5.64; F, 3.00; N, 5.52. Found: C, 60.60; H, 5.50; F, 2.89; N, 5.47.

*1,3,2′,6′,3″-Pentakis(*N*-benzyloxycarbonyl)-5,3′,4′-trideoxy-5,5-difluorokanamycin B* (**37**).—$[\alpha]_D^{24}$ +72° (*c* 2, pyridine); ^{19}F-NMR (pyridine-d_5): δ −110.9 (d, 1 F, F-5*eq*) and −129.1 (br dt, 1 F, F-5*ax*); $J_{Fax,Feq}$ 250 Hz.

Anal. Calcd for $C_{58}H_{65}F_2N_5O_{17} \cdot H_2O$: C, 60.04; H, 5.82; F, 3.28; N, 6.04. Found: C, 59.79; H, 5.65; F, 3.55; N, 6.07.

5,3′,4′-Trideoxy-5,5-difluorokanamycin B (**27**).—Compound **37** (2.55 g) was treated similarly as described for **26** to give **27** as the solid 0.4 carbonate salt; 1.00 g (92%), $[\alpha]_D^{21}$ +144° (*c* 2, H_2O). ^{1}H-NMR (500 MHz, 20% ND_3 in D_2O): 1.37 (q, 1 H, H-2*ax*), 1.78–1.84 (2 H, H-3′*eq*, 4′*eq*), 2.13 (dt, 1 H, H-2*eq*), 2.71 (dd, 1 H, H-6′a), 2.73 (dd, 1 H, H-6′b), 3.05 (dt, 1 H, H-1 or 3), ~3.1 (H-3 or 1), 3.81 (dd, 1 H, H-6″a), and 3.83 (dd, 1 H, H-6″b). ^{19}F-NMR (20% ND_3 in D_2O): δ −110.8 (d, 1 F, F-5*eq*) and −128.6 (dt, 1 F, F-5*ax*); $J_{Fax,Feq}$ 246 Hz.

Anal. Calcd for $C_{18}H_{35}F_2N_5O_7 \cdot 0.4H_2CO_3$: C, 44.53; H, 7.27; F, 7.66; N, 14.11. Found: C, 44.78; H, 7.23; F, 7.74; N, 13.71.

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