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Synthesis of 5-deoxy-5-epifluoro derivatives of arbekacin, amikacin, and 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]tobramycin (study on structure — toxicity relationships)

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Abstract

As part of a study on fluorination—toxicity relationships for aminoglycoside antibiotics, 5,3′-dideoxy-5-epifluorokanamycin B (**10**), 5,3′,4′-trideoxy-5-epifluorokanamycin B (**11**), 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-5-deoxy-5-epifluorotobramycin (**19**), 5-deoxy-5-epifluoroarbekacin (**20**), and 5-deoxy-5-epifluoroamikacin (**21**) have been prepared. The acute toxicities of these three 5-deoxy-5-epifluoro compounds showed values almost identical or similar to those for arbekacin (ABK) and amikacin (**15**), making a sharp contrast with the toxicities of the corresponding 5-deoxy-5-fluoro derivatives. This fact is explained on the basis of basicity changes (retention for the 5-epifluoro derivatives and reduction for the 5-fluoro derivatives) at the H_2N-3 groups of the fluorinated compounds compared to the parent compounds; this hypothesis was substantiated by the p*K*a values at the H_3N^+-1, 3 groups (determined by the shift changes depending on pD values at C-2 and C-4, 6 in their ^{13}C NMR spectra) of 2,5-dideoxy-5-epifluorostreptamine (**23**) and 2,5-dideoxy-5-fluorostreptamine (**24**), chosen as model compounds, and 2-deoxystreptamine (DST).

Keywords: Arbekacin; Amikacin; 1-*N*-[(*S*)-4-Amino-2-hydroxybutanoyl]tobramycin

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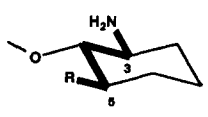
1. Introduction

In the foregoing papers [1,2] we reported the synthesis of 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of kanamycin B, tobramycin (3′-deoxykanamycin B), dibekacin (3′,4′-dideoxykanamycin B) and some of their 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivatives, including arbekacin (1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3′,4′-dideoxykanamycin B). These studies established that displacement of their HO-5 groups with a fluorine or 5,5-difluorination gave rise to kanamycin B analogs whose toxicity was lower than the parent compounds. This biological improvement was attributed to basicity reduction at the H_2N-3 group induced by the strongly electron-withdrawing F-5. 5,5-Difluorination may enhance the tendency. In the 2-deoxystreptamine moiety of these 5-deoxy-5-fluoro derivatives, the H_2N-3 (equatorial) and F-5 (equatorial) substituents are in a W conformation (see formula II), and this provides an electron-inductive pathway from the 3-amino to 5-fluorine, decreasing the basicity of the amino group.

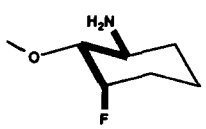
Accordingly, our present interest was to examine the biological effect of converting the equatorial F-5 to the axial orientation. If the foregoing assumption relating fluorination and toxicity is true, inversion of the fluorine at C-5 might lead to a toxicity increase of the resulting 5-epifluoro compounds, in comparison to the 5-fluoro compounds, because the axial 5-fluorine is expected to withdraw electrons less strongly than the equatorial fluorine (see formulas II and IV). Another interest is the difference in electron-withdrawing strength between equatorial HO-5 and axial F-5 (compare formulas I and IV) in terms of toxicity; in other words, which of the HO-5 (parent compounds) or axial F-5 structures will decrease toxicity the most?

2. Results and discussion

Several 5-deoxy-5-epifluorodeoxystreptamine antibiotics have been synthesized: 5-deoxy-5-epifluorosisomicin [3,4], 5-deoxy-5-epifluorokanamycin A [3], 5,6″-dideoxy-5-epifluoro-6″-fluorokanamycin A [5], and 5-deoxy-4″-epi-5-epifluorokanamycin A [5]. Our synthetic route to introduce epimeric fluorine follows straightforwardly by these precedents. As HO-5 is surrounded by two glycosyl residues at C-4 and C-6, and is thus significantly crowded, it does not undergo acetylation with acetic anhydride in pyridine. Thus it can be fluorinated by diethylaminosulfur trifluoride (DAST) [6] after the other

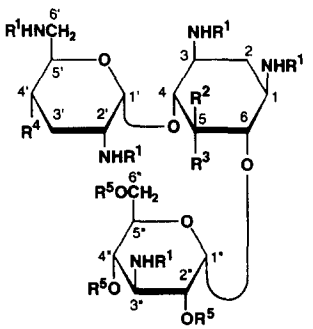


I R= OH
II R= F
III R= H

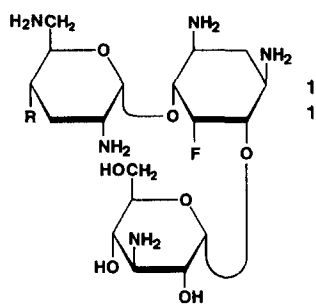


IV

hydroxyl groups have been acetylated. Synthesis of 5,3′-dideoxy-5-epifluorokanamycin B (5-deoxy-5-epifluorotobramycin **10**) is described as the typical example. Protection of the five amino groups of 3′-deoxykanamycin B (**1**) with di-*tert*-butyl dicarbonate (to give **2**) followed by acetylation gave the tetra-*O*-acetyl derivative **3** having a free 5-hydroxyl group, which was then treated with DAST to give the epifluoro product **4** in high yield. Deprotection of **4** afforded **10**. 5,3′,4′-Trideoxy-5-epifluorokanamycin B (5-deoxy-5-epifluorodibekacin **11**) was similarly prepared from dibekacin (**6**) through the three intermediates (**7**, **8**, and **9**). The structures of **10** and **11** were confirmed by their ^{1}H and ^{19}F NMR spectra. Small vicinal H–H couplings relating to H-5 and large $J_{4,F}$ and $J_{6,F}$ values in **10** and **11**, indicate that an axial fluorine had been introduced at C-5.



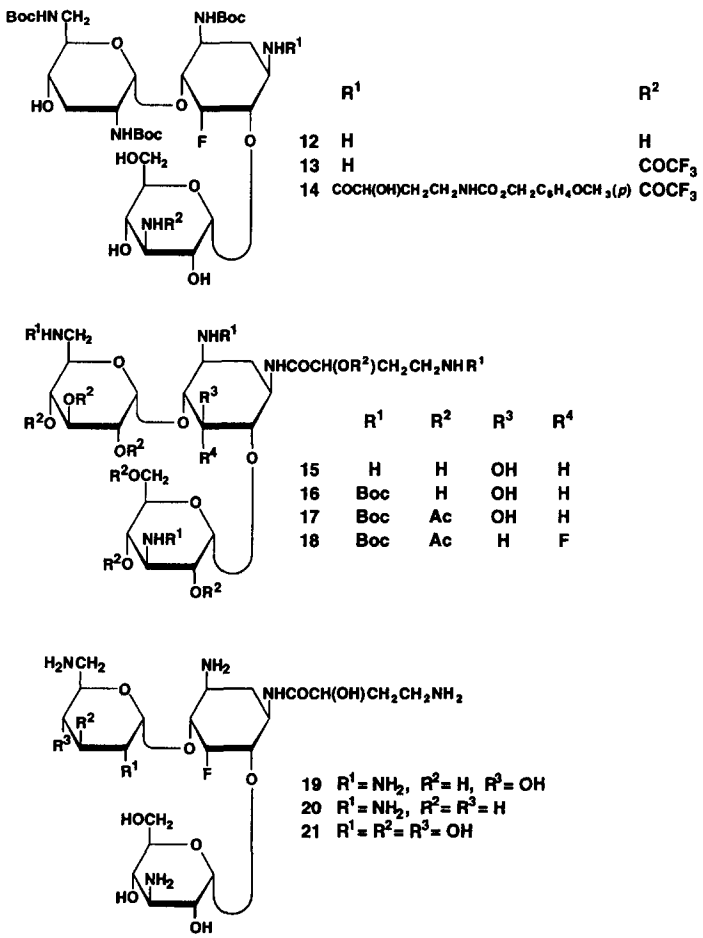
	R^1	R^2	R^3	R^4	R^5
1	H	OH	H	OH	H
2	Boc	OH	H	OH	H
3	Boc	OH	H	OAc	Ac
4	Boc	H	F	OAc	Ac
5	Boc	H	F	OH	H
6	H	OH	H	H	H
7	Boc	OH	H	H	H
8	Boc	OH	H	H	Ac
9	Boc	H	F	H	Ac



10 R= OH
11 R= H

Attachment of a (*S*)-4-amino-2-hydroxybutanoyl residue to **10** and **11** to afford the 1-*N*-acyl derivatives **19** and **20** was performed utilizing the modified $Zn(OAc)_2$–CF_3CO_2Et method [7]. Treatment of **10** with di-*tert*-butyl dicarbonate (instead of benzyl chloroformate as reported [7]) in the presence of $Zn(OAc)_2$ in *N*,*N*-dimethylformamide (DMF; instead of Me_2SO [7]) gave the 3,2′6′-tricarbamate **12** quantitatively. The 1,3″-diamine **12** was then treated with CF_3CO_2Et in DMF, whereupon the 3″-*N*-trifluoroacetyl derivative **13** was obtained. The position trifluoroacetylated was confirmed by the low-field shift of H-3″ (δ 4.17); the corresponding protons of kanamycin analogs

(**10**, **11**, and **19–21**) having a free H_2N-3″ group resonated at δ 3.00–3.04. The selectivity is explained on the basis of formation of an unstable $OCOCF_3$ group at C-2″ or C-4″, which donates the $COCF_3$ group readily to the neighboring H_2N-3″ while the H_2N-1 remains intact. A (*S*)-4-(*p*-methoxybenzyloxycarbonylamino)-2-hydroxybutanoyl group was attached to the free amino group of **13** utilizing its active ester to give **14**. Removal of the protecting groups of **14** gave **19**. Compound **20** was prepared similarly. The structures of **19** and **20** were confirmed by their ^1H, ^{19}F, and ^{13}C NMR spectra (Table 1).



5-Deoxy-5-epifluoroamikacin (**21**) was prepared likewise from amikacin (**15**). Tetrakis(*N*-*tert*-butoxycarbonyl)amikacin (**16**) [8] was acetylated and the hepta-*O*-acetyl derivative **17** was fluorinated to give the 5-epifluoro derivative **18**. Deprotection gave

Table 1
^{13}C-NMR chemical shifts (ppm) and coupling constants ($J_{C,F}$, Hz) of **10, 11, 19, 20**, and **21** measured in 26% ND_3 in D_2O

	10	**11**	**19**	**20**	**21**
C-1	48.22 [a]d	48.20 [c]d	47.50d	47.49d	47.51d
	(3.9)	(4.1)	(4.1)	(4.5)	(4.6)
C-2	36.28	36.30	34.68	34.70	34.69
C-3	47.67 [a]d	47.67 [c]d	47.32d	47.31d	47.12d
	(3.5)	(3.5)	(3.7)	(4.4)	(4.0)
C-4	78.86 [b]d	79.09 [d]d	78.29d	78.53d	77.95d
	(17.0)	(17.0)	(17.0)	(17.0)	(17.0)
C-5	90.23d	90.44d	90.14d	90.35d	89.60d
	(177.3)	(177.0)	(178.3)	(178.0)	(179.4)
C-6	84.98 [b]d	84.96 [d]d	79.74d	79.71d	79.71
	(16.8)	(16.8)	(17.1)	(17.2)	(17.4)
C-1′	95.76	97.15	95.70	97.10	95.41
C-2′	49.58	50.22	49.49	50.14	71.82
C-3′	35.95	26.85	35.89	26.80	73.23
C-4′	66.86	28.27	66.77	28.17	71.76
C-5′	74.52	71.25	74.41	71.19	73.49
C-6′	42.54	45.82	42.44	45.71	42.42
C-1″	101.90	101.85	101.02	100.97	101.05
C-2″	72.46	72.43	72.31	72.30	72.31
C-3″	54.91	54.88	54.93	54.89	54.94
C-4″	70.76	70.74	70.62	70.61	70.41
C-5″	73.41	73.36	73.52	73.46	73.49
C-6″	61.86	61.82	61.79	61.75	61.52
C-1‴			177.56	177.56	177.59
C-2‴			70.50	70.50	70.50
C-3‴			36.83	36.89	36.95
C-4‴			38.04	38.03	38.05

[a,b,c] and [d] Values are interconvertible.

the final product **21**. The structure was confirmed by the ^{1}H, ^{19}F, and ^{13}C NMR spectra (Table 1).

Acute toxicities of **19**, **20**, and **21** were measured alongside arbekacin and amikacin (**15**) (Table 2). The results show that the kanamycin B derivatives (**19** and **20**) gave values almost identical with those for arbekacin, and that the kanamycin A derivative **21** gave a value similar to that for amikacin. This result established that epifluorination at C-5 does not reduce the toxicities of the parent compounds, as had been first expected. This fact makes a sharp contrast with 5-deoxy-5-fluorination [2], whereby the toxicities of the parent compounds were considerably decreased (LD_{50}: 120 ~ 140 mg kg^{-1}, mouse, i.v.). In addition, the result (epifluorination gave products having substantially the same toxicity as the parent compounds) suggests that the electron-withdrawing ability of axial F-5 (formula IV) is similar in degree to that for equatorial HO-5 (formula I). 5-Deoxyarbekacin [9], formula III, showed high acute toxicity (LD_{50} 31 mg kg^{-1}, mouse, i.v. [10]), a result that also supports the foregoing proposal for basicity —

Table 2
Minimal inhibitory concentration [a] ($\mu g\ mL^{-1}$) [b] of **10, 11, 19, 20, 21**, arbekacin (ABK), and amikacin (**15**), for selected strains, and acute toxicity (LD_{50}) [c] of **19, 20, 21**, ABK, and **15**

No.	Test organism [d]	**10**	**11**	**19**	**20**	ABK	**21**	**15**
1	*St. a.* FDA 209*P*	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.39	< 0.2
2	*St. a.* Smith	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
3	*St. a.* Ap01 [e]	50	3.12	6.25	0.39	0.78	6.25	25
4	*St. a.* MS16526 [f]	> 100	> 100	12.5	1.56	12.5	50	50
5	*St. a.* TY-04282 [f]	> 100	0.2	12.5	< 0.2	< 0.2	6.25	3.12
6	*Micr. l.* FDA16	25	12.5	0.78	0.78	0.78	6.25	3.12
7	*Micr. l.* PCI1001	25	12.5	0.78	0.78	0.78	3.12	3.12
8	*Coryn. b.* 1810	3.12	6.25	< 0.2	< 0.2	< 0.2	0.78	1.56
9	*E. c.* NIHJ	0.39	0.39	0.39	0.39	0.39	0.39	< 0.2
10	*E. c.* K-12 R5 [g]	50	A	12.5	12.5	A	50	50
11	*E. c.* K-12 ML1629 [h]	0.39	0.78	0.78	0.78	0.78	0.78	0.78
12	*E. c.* K-12 ML1410 R81 [h]	0.39	0.78	0.39	0.78	0.78	1.56	1.56
13	*E. c.* K-12 LA290 R55 [i]	0.78	3.12	1.56	0.78	0.39	0.78	6.25
14	*E. c.* K-12 LA290 R64	0.39	0.39	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
15	*E. c.* W677	3.12	0.78	< 0.2	< 0.2	< 0.2	< 0.2	0.39
16	*E. c.* JR66/W677 [i,j]	3.12	1.56	0.78	0.78	0.78	1.56	3.12
17	*E. c.* JR225 [k]	6.25	0.2	< 0.2	< 0.2	< 0.2	0.39	0.39
18	*Kl. p.* PCI602	0.39	0.39	0.39	0.39	0.39	0.78	0.78
19	*Kl. p.* 22#3038 [i,j]	1.56	3.12	0.78	1.56	1.56	3.12	3.12
20	*Sh. s.* JS11746	0.78	1.56	0.39	0.39	0.78	0.78	0.78
21	*Sal. e.* 1891	3.12	3.12	1.56	1.56	1.56	3.12	3.12
22	*Serr. marc.*	6.25	12.5	1.56	1.56	3.12	3.12	1.56
23	*Prot. r.* GN311	< 0.2	0.39	0.78	1.56	1.56	0.39	0.39
24	*Prov.* sp. Pv16 [l]	3.12	6.25	0.39	< 0.2	0.78	0.39	0.78
25	*Prov.* sp. 2991 [l]	12.5	12.5	0.78	0.78	3.12	0.78	0.78
26	*Ps. aerug.* A3	0.39	0.39	< 0.2	< 0.2	< 0.2	< 0.2	0.78
27	*Ps. aerug.* H9 [j]	1.56	3.12	0.78	1.56	1.56	6.25	3.12
28	*Ps. aerug.* GN315 [g]	A	A	6.25	6.25	3.12	50	25
	LD_{50} ($mg\ kg^{-1}$) [m]			64	65	68	250	205

[a] Judged by agar dilution streak method (Mueller-Hinton agar, 17 h, 37°C).
[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′), [g] AAC(6′), [h] APH(3′)-I, [i] ANT(2″), [j] APH(3′)-II, [k] AAC(3), and [l] AAC(2′). [f] MRSA. [m] Values of 95% confidence limit.

toxicity relationships. The lack of an electron-withdrawing group, such as HO or F, at C-5 considerably increases the basicity at H_2N-3, thus enhancing the toxicity.

Numerical estimation of the relationship between the basicity at H_2N-3 and orientation of F-5 should provide a firm basis for the foregoing basicity — toxicity discussion; thus 2,5-dideoxy-5-epifluorostreptamine (**23**) and 2,5-dideoxy-5-fluorostreptamine (**24**)

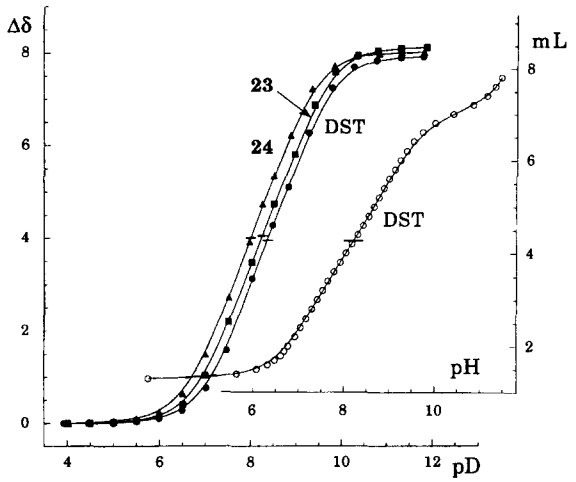
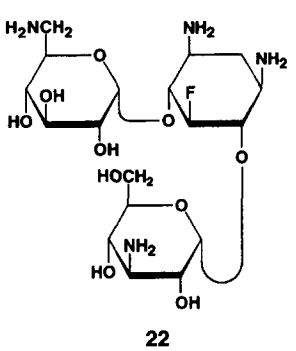
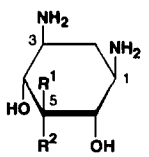


Fig. 1. Titration curves for pD-^{13}C shift values of C-2 (the left vertical axis; the values in strongly acidic region are taken as zero) of **23** (■), **24** (▲), and DST (●), along with a titration curve (○) for pH-aq 0.1 M NaOH volumes (the right vertical axis) for an aq solution of DST·2HCl + HCl. The midpoints between upper and lower plateaus are indicated by a short line on the curves.

have been prepared as model compounds, **23** from **21**, and **24** from 5-deoxy-5-fluoro-kanamycin A[1] (**22**). The pKa values of the H_3N^+-1,3 groups of **23** and **24** and



22



DST R^1 = OH, R^2 = H

23 R^1 = H, R^2 = F

24 R^1 = F, R^2 = H

[1] This compound was prepared as reported in [1] by a similar sequence of reactions: benzyloxycarbonylation of NH_2 groups of kanamycin A, selective OH acetylation (Ac_2O in pyridine) except for HO-5, mesylation of HO-5 (MsCl in CH_2Cl_2 in the presence of dimetylaminopyridine), inversion of MsO-5 by NaOAc in DMF, deacetylation (Na_2CO_3 in 10:1 MeOH–H_2O), selective acetylation except for epi HO-5 (Ac_2O in pyridine), fluorination at C-5 (DAST in CH_2Cl_2), and deprotection (Na_2CO_3 in 10:1 MeOH–H_2O, then Pd–H_2 in 4:1:1 1,4-dioxane–H_2O–AcOH). Details will be reported elsewhere. Physicochemical data for **22** are given in the Experimental section.

that of 2-deoxystreptamine (DST) [11], taken as a reference compound, were determined by measuring the shift values of either C-2 or C-4,6 in their ^{13}C NMR spectra upon varying the pD values, as reported before [12]. As the C-2 and C-4,6 signals are expected to incur an upfield shift of 5–6 ppm [13] (β shift) upon protonation of H_2N-1,3, the pKa values of the protic salt H_3N^+-1,3 can be determined by measuring the midpoint (of the pD values in the D_2O solution) between the lines before and after protonation on the titration curve obtained for the C-2 (or C-4,6) shift – pD value (see Fig. 1). The pKa values obtained for three compounds (**23**, **24**, and DST) based upon C-2 ($\Delta\delta \sim 8$ ppm) and C-4,6 ($\Delta\delta \sim 5$ ppm) were almost identical and were 8.27 (for **23**), 8.01 (for **24**), and 8.36 (for DST). Additionally, as a reference experiment, acidified DST was titrated with aq NaOH to compare the present and classical methods (Fig. 1). Both methods for DST gave similar pKa values, however, the classical one was prone to disturbance by coexistent acids or bases (see the far right curve in Fig. 1), whereas, the ^{13}C method was hardly influenced by coexistents. This was expected from the nature of the ^{13}C method, and therefore the method should be superior to the classical one. The pKa values obtained (see above) showed that the equatorial F-5 in **24** significantly lowered the basicity (by ΔpKa 0.35) of the H_2N-1,3 groups of DST. At the same time it was found that the axial F-5 in **23** lowered the basicity of the same amino groups only slightly (by ΔpKa 0.09). The foregoing pKa outcome could likewise be applied to the basicity change of the H_2N-3 groups of arbekacin analogs having an equatorial fluorine and axial fluorine (or equatorial hydroxyl) at C-5. It is surprising that such a small change as ΔpKa ~ 0.3 (this is an inductive value based on the pKa values between **23** and **24**) brought about a large toxicity change [$\Delta LD_{50} \sim 65$ mg kg^{-1}, mouse, i.v., between **19** (or **20**) and 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-5,3′-dideoxy-5-fluorokanamycin B (or the corresponding 3′,4′-dideoxy analog) [2]]; however, this fact is not so strange if we consider that the pKa change occurred near the pH range of blood or tissue fluid [12].

Antibacterial activities of theses synthetic products (Table 2) show that, in each group of compounds, there is no substantial difference between the epifluorinated and parent compounds. Noteworthy is the fact that both 5-deoxy-5-fluoro [1] and 5-deoxy-5-epifluoro compounds (this paper) showed enhanced activity against resistant bacteria producing AAC(2′) (see nos. 24 and 25; and nos. 22 and 23 in [1]). This result suggests that the introduction of fluorine at C-5 appreciably influences the activity of 2′-acetyltransferases of resistant bacteria, possibly because of the proximity of F-5 and HO-2′. Further, it should be stressed that **20** showed, among the arbekacin analogs synthesized recently (approximately new 15 compounds were prepared) in our two laboratories (data unpublished), the strongest activity against methicillin-resistant *Staphylococcus aureus* (MRSA) of clinical origin.

3. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer 241 polarimeter. ^1H-NMR [at 250 and 500 MHz (for **4**, **10**, **11**, **13**, **19**, **20**, and **21**)], ^{19}F NMR [at 235.3 and 470.6 MHz (for **4**, **10**, **11**, **19**, **20**, and **21**)], and ^{13}C NMR [at 62.9

and 125.8 MHz (for **10, 11, 13, 19, 20**, and **21**)] spectra were recorded with Bruker AC 250P and AMX 500 spectrometers, respectively. Chemical shifts (δ) for ^{1}H, ^{13}C, and ^{19}F spectra 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-shift assignments in the ^{1}H and ^{13}C spectra were confirmed, if necessary, by the shift-correlated 2D spectra. Unless otherwise noted, TLC was performed on Kieselgel 60 F_{254} (Merck), and column chromatography on Kieselgel 60 (230–400 mesh, Merck).

General procedure to determine the pKa values of H_3N^+-1,3 by ^{13}C NMR spectroscopy.—Solutions of **23, 24**, and DST [11] (each as base, 0.1 mmol) in D_2O (0.5 mL) were acidified to pD ~ 1 with DCl in D_2O, bubbled for 5 min with Ar to remove CO_2, and brought to pD ~ 4 by adding 1 M NaOD in D_2O. After measuring the ^{13}C shift value at C-2 and C-4, 6, the solution was treated similarly on each addition of 0.5 M NaOD in D_2O in pD ~ 0.5 intervals until strongly alkaline (pD ~ 12). As another experiment, the pH values of acidified DST (0.31 mmol) in water (13 mL) were measured on each addition of an aliquot of aq 0.1 M NaOH (see Fig. 1).

*3'-Deoxy-1,3,2',6',3''-pentakis(*N-tert-*butoxycarbonyl)kanamycin B* (**2**).—To an aqueous solution (7.5 mL) of 3'-deoxykanamycin B (**1** as base, 1.50 g, 3.21 mmol) and Et_3N (1.0 mL, 7.2 mmol), di-*tert*-butyl dicarbonate (4.31 g, 19.8 mmol) was added and the mixture was stirred for 40 min at 60°C. Addition of aq 28% NH_3 (0.5 mL) followed by concentration gave a residue, which was thoroughly washed with water and dried in vacuo at 40°C to give **2** as a solid (3.08 g, 98%), $[\alpha]_D^{23} + 55°$ (*c* 1.1, 2:1 $CHCl_3$–MeOH); ^{1}H NMR (pyridine-d_5) δ 1.44, 1.46, 1.48, 1.51, and 1.56 (each s of 9 H, 5 Boc), 5.52 (br s, 1 H, H-1''), and 5.59 (br s, 1 H, H-1'). Anal. Calcd for $C_{43}H_{77}N_5O_{19} \cdot 1/2\ H_2O$: C, 52.86; H, 8.05; N, 7.17. Found: C, 52.71; H, 7.71; N 7.35.

*3',4'-Dideoxy-1,3,2',6',3''-pentakis(*N-tert-*butoxycarbonyl)kanamycin B* (**7**).—Prepared in 96% yield from **6** as described for **2**, $[\alpha]_D^{20} + 59°$ (*c* 1.2, MeOH); ^{1}H NMR (pyridine-d_5) δ 1.45, 1.48, 1.50, 1.51, and 1.54, (each s of 9 H, 5 Boc), 2.85 (br d, 1 H, H-2*eq*), 5.52 (br s, 1 H, H-1''), and 5.58 (br s, 1 H, H-1'). Anal. Calcd for $C_{43}H_{77}N_5O_{18} \cdot 1/2\ H_2O$; C, 53.73; H, 8.18, N, 7.29. Found: C, 53.61; H, 8.09; N, 7.68.

*3'-Deoxy-1,3,2',6',3''-pentakis(*N-tert-*butoxycarbonyl)-4',2'',4'',6''-tetra*-O-*acetylkanamycin B* (**3**).—A mixture of **2** (3.11 g, 3.18 mmol) and Ac_2O (11.5 mL, 122 mmol) in pyridine (45 mL) was kept overnight at room temperature (use of less Ac_2O caused insufficient acetylation of **2**). Addition of MeOH (11.5 mL) followed by concentration gave a residue, which was thoroughly washed with water and dried in vacuo to give **3** as a solid (3.62 g, 99%), $[\alpha]_D^{20} + 70°$ (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5) δ 1.48 (s, 27 H, 3 Boc), 1.54 (s, 9 H, Boc), and 1.59 (s, 9 H, Boc); 1.96 (s, 3 H, Ac), 2.09 (s, 6 H, 2 Ac), and 2.19 (s, 3 H, Ac); 5.79 (br s, 1 H, H-1'), and 5.88 (br s, 1 H, H-1''). Anal. Calcd for $C_{51}H_{85}N_5O_{23} \cdot 1/2\ H_2O$: C, 53.49; H, 7.57; N, 6.12. Found: C, 53.52; H, 7.55; N, 6.09.

*3',4'-Dideoxy-1,3,2',6',3''-pentakis(*N-tert-*butoxycarbonyl)-2'',4'',6''-tri*-O-*acetylkanamycin B* (**8**).—Prepared in 97% yield from **7** as descrived for **3**, $[\alpha]_D^{20} + 65°$ (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 45 H, 5 Boc), 2.04, 2.10, and 2.16 (each s of 3 H, 3 Ac). Anal. Calcd for $C_{49}H_{83}N_5O_{21}$: C, 54.57; H, 7.76; N, 6.49. Found: C, 54.25; H, 7.71; N, 6.52.

*5,3'-Dideoxy-5-epifluoro-1,3,2',6',3''-pentakis(*N-tert-*butoxycarbonyl)-4',2'',4'',6''-te-*

tra-O-*acetylkanamycin B* (**4**).—To an ice-cold solution of **3** (3.55 g, 3.10 mmol) in CH_2Cl_2 (60 mL) DAST (0.71 mL, 5.37 mmol) was added and the solution was warmed gradually to room temperature, then maintained for 1 h at the temperature. Aqueous $NaHCO_3$ (saturated, 60 mL) was added under vigorous stirring, and the organic layer isolated, dried (Na_2SO_4) and concentrated. The residue was chromatographed (100:1 $CHCl_3$–MeOH) on a column (52 cm × 5.7 cm^2) to give **4** as a solid (3.45 g, 96%), $[\alpha]_D^{22}$ + 62° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5) δ 1.45, 1.48, 1.51, 1.56, and 1.63 (each s of 9 H, 5 Boc); 2.06 (s, 6 H, 2 Ac), 2.10 (s, 3 H, Ac), and 2.12 (s, 3 H, Ac); 5.56 (br s, 1 H, H-1′), and 5.64 (br s, 1 H, H-1″). ^{19}F NMR (pyridine-d_5) δ −214.27 (dt, *J* 26, 26, and 52 Hz, F-5). Anal. Calcd for $C_{51}H_{84}FN_5O_{22} \cdot H_2O$: C, 52.98; H, 7.50; F, 1.64; N, 6.06. Found: C, 53.10; H, 7.52; F, 1.70; N, 6.17.

*5-Epifluoro-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)-2″,4″,6″-tri*-O-*acetyl-5,3′,4′-trideoxykanamycin B* (**9**).—Prepared in 94% yield from **8** as described for **4**, $[\alpha]_D^{20}$ + 55° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 45 H, 5 Boc), 2.04, 2.07, and 2.12 (each s of 3 H, 3 Ac), 5.06 (br d, 1 H, $J_{5,F}$ 54 Hz, H-5). Anal. Calcd for $C_{49}H_{82}FN_5O_{20} \cdot 1/2\ H_2O$:C, 54.03; H, 7.68; F, 1.74; N, 6.43. Found: C, 53.92; H, 7.65; F, 1.79; N, 6.53.

*5,3′-Dideoxy-5-epifluoro-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)kanamycin B* (**5**).—To a solution of **4** (3.27 g, 2.83 mmol) in MeOH (49 mL) 28% NaOMe in MeOH (0.49 mL) was added and, after 1 h at room temperature, the solution was neutralized with aq HCl and concentrated. The residue was thoroughly washed with water and dried in vacuo at 40°C to give **5** as a solid (2.56 g, 91%), $[\alpha]_D^{22}$ + 64° (*c* 1.1, $CHCl_3$); ^{1}H NMR (pyridine-d_5)δ 1.40 (9 H), 1.48 (18 H), 1.52 (9 H), and 1.60 (9 H) (each s, 5 Boc); 5.46 (d, 1 H, *J* 3.5 Hz, H-1′ or -1″), 5.62 (d, 1 H, *J* 3.0 Hz, H-1″ or -1′), and 5.99 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (pyridine-d_5) (too br to determine exact shifts). Anal. Calcd for $C_{43}H_{76}FN_5O_{18} \cdot 1.5\ H_2O$: C, 51.80; H, 7.99; F, 1.91; N, 7.02. Found: C, 51.49; H, 8.01; F, 1.79; N, 7.35.

5,3′-Dideoxy-5-epifluorokanamycin B (**10**).—To ice-cold CF_3CO_2H (7.5 mL) was added **5** (2.48 g, 2.49 mmol). The solution was warmed to room temperature, and then kept for 2 h at this temperature. Concentration gave a residue, which was washed with diethyl ether. An aq solution of the residue was neutralized with aq NH_3 and subjected to chromatography of CM Sephadex C-25 (NH_4^+ form) with aq 0 → 0.15 M NH_3 to give **10** as the solid hemihydrate (after drying in vacuo at 40°C) 1.17 g (98%), $[\alpha]_D^{22}$ + 153° (*c* 2, H_2O); ^{1}H NMR (26% ND_3 in D_2O) δ 1.19 (ddd, 1 H, H-2*ax*), 2.06 (dt, 1 H, H-2*eq*), 3.46 (ddd, 1 H, H-4 or -6), 3.55 (ddd, 1 H, H-6 or -4), and 5.37 (br d, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{1,6} = J_{3,4} \sim 10$, $J_{4,5} = J_{5,6}$ 1.5, $J_{4,F} = J_{6,F}$ 29, and $J_{5,F}$ 53 Hz; δ 1.63 (ddd, 1 H, H-3′ax), 2.70 (dd, 1 H, H-6′a), 2.92 (dt, 1 H, H-2′), 2.97 (dd, 1 H, H-6′b), and 4.94 (d, 1 H, H-1′). $J_{1',2'} = J_{2',3'eq}$ 4, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4}$ 12, $J_{5',6'a}$ 7, $J_{5',6'b}$ 2, and $J_{6'a,6'b}$ 13.5 Hz; δ 3.04 (t, 1 H, H-3″), 3.18 (t, 1 H, H-4″), 3.45 (dd, 1 H, H-2″), 3.62 (dd, 1 H, H-6″a), 3.83 (ddd, 1 H, H-5″), 3.87 (dd, 1 H, H-6″b), and 5.03 (d, 1 H, H-1″). $J_{1'',2''}$ 4, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10, $J_{5'',6''a}$ 7.5, $J_{5'',6''b}$ 2 and $J_{6''a,6''b}$ 12 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −213.72 (dt, *J* 29, 29, and 53 Hz, F-5). Anal. Calcd for $C_{18}H_{36}FN_5O_8 \cdot 1/2\ H_2O$: C, 45.18; H, 7.79; F, 3.97; N, 14.64. Found: C, 45.45; H, 8.04; F, 4.02; N, 14.43.

5,3′,4′-Trideoxy-5-epifluorokanamycin B (**11**).—After deacetylation of **9** (NaOMe in MeOH), the product was treated as described for **10**, yield 87% based on **9**, $[\alpha]_D^{20}$ + 135°

(*c* 1, H_2O); ^1H NMR (26% ND_3 in D_2O) δ 1.19 (ddd, 1 H, H-2*ax*), 2.06 (dt, 1 H, H-2*eq*), 3.46 (ddd, 1 H, H-4 or -6), 3.54 (ddd, 1 H, H-6 or -4), and 5.35 (br d, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12.5, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{3,4} = J_{1,6}$ 10, $J_{4,5} = J_{5,6}$ 2, $J_{4,F} = J_{6,F}$ 29.5, and $J_{5,F}$ 53 Hz; δ 1.40 (dddd, 1 H, H-4′*ax*), 1.66 (dddd, 1 H, H-3′*ax*); 2.63 (dd, 1 H, H-6′a) and 2.66 (dd, 1 H, H-6′b) show a ABX pattern; 2.80 (dt, 1 H, H-2′), and 4.96 (d, 1 H, H-1′). $J_{1',2'} = J_{2',3'eq} = J_{3'ax,4'eq} = J_{3'eq,4'ax}$ 4, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'ax} = J_{4'ax,4'eq} = J_{4'ax,5'}$ 12.5, $J_{5',6'a}$ 7, $J_{5',6'b}$ 4, and $J_{6'a,6'b}$ 13 Hz; δ 3.04 (t, 1 H, H-3″), 3.19 (t, 1 H, H-4″), 3.46 (dd, 1 H, H-2″), 3.63 (dd, 1 H, H-6″a), 3.83 (ddd, 1 H, H-5″), 3.88 (dd, 1 H, H-6″b), and 5.04 (d, 1 H, H-1″). $J_{1'',2''}$ 4, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10, $J_{5'',6''a}$ 7, $J_{5'',6''b}$ 2.5, and $J_{6''a,6''b}$ 12 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −213.68 (dt, *J* 29.5, 29.5, and 53 Hz, F-5). Anal. Calcd for $C_{18}H_{36}FN_5O_7 \cdot 1/2\ H_2O$: C, 46.74; H, 8.06; F, 4.10; N, 15.14. Found: C, 46.44; H, 8.13; F, 3.87; N, 15.29.

5,3′-Dideoxy-5-epifluoro-3,2′,6′-tris(N-tert-*butoxycarbonyl)kanamycin B* (**12**).—To a slurry of a mixture of **10** (hemihydrate 761 mg, 1.59 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (1.50 g, 6.85 mmol) in water (1.53 mL) DMF (12.3 mL) was added and the mixture was warmed until dissolution. After cooling to room temperature, di-*tert*-butyl dicarbonate (1.25 g, 5.73 mmol) in DMF (12.4 mL) was added and the solution was kept overnight at room temperature. After concentration to low volume in vacuo, THF (100 mL) was added, and the organic solution was washed with aq 28% NH_3 saturated with NaCl (5 × 15 mL). Concentration gave a residue (1.23 g, ~98%), which was used without purification.

5,3′-Dideoxy-5-epifluoro-3″-N-*trifluoroacetyl-3,2′,6′-tris*(N-tert-*butoxycarbonyl)kanamycin B* (**13**).—To a solution of crude **12** (78.8 mg, ~0.1 mmol) in DMF (1.6 mL) CF_3CO_2Et (0.016 mL, 0.13 mmol) was added and the solution was kept for 1 h at room temperature. Concentration in vacuo gave a residue, which was dissolved in small amount of EtOH and brought to pH ~4 with CF_3CO_2H. Addition of large amount of diethyl ether gave a precipitate that was thoroughly washed with the same solvent to give **13** as the solid trifluoroacetate sesquihydrate (79.4 mg, ~77%), TLC (9:2:0.5 $CHCl_3$–MeOH–aq 28% NH_3): R_f 0.35 (cf. **12**: R_f 0.15), $[\alpha]_D^{21}$ + 65° (*c* 1, MeOH); ^1H NMR (CD_3OD) δ 1.44, 1.46, and 1.47 (each s of 9 H, 3 Boc), 1.64 (ddd, 1 H, H-2*ax*), 2.23 (br dt, 1 H, H-2*eq*), ~3.62 (H-1), ~3.69 (H-4), ~3.79 (H-6), 3.98 (br t, 1 H, H-3), and 5.48 (br d, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12, $J_{1,2eq} = J_{2eq,3}$ 4, and $J_{5,F}$ 52 Hz; δ 1.65 (ddd, 1 H, H-3′*ax*), 1.94 (br 1 H, H-3′*eq*), ~3.31 (H-6′a), ~3.43 (H-4′, 6′b), ~3.54 (H-5′), ~3.69 (H-2′), and 4.96 (br d, 1 H, H-1′). $J_{1',2'}$ 3 and $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'}$ 12 Hz; δ 3.52 (t, 1 H, H-4″), 3.63 (dd, 1 H, H-2″), 3.69 (dd, 1 H, H-6″a), 3.83 (dd, 1 H, H-6″b), ~3.86 (H-5″), 4.17 (t, 1 H, H-3″), and 5.07 (d, 1 H, H-1″). $J_{1'',2''}$ 3.5, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10, $J_{5'',6''a}$ 5, $J_{5'',6''b}$ 2, and $J_{6''a,6''b}$ 12 Hz. ^{19}F NMR (CD_3OD) δ −214.81 (dt, 1 F, $J_{4,F} = J_{6,F}$ 26 Hz, F-5), −75.54 (s, 3 F, CF_3CONH or CF_3CO_2H), and −75.28 (s, 3F, CF_3CO_2H or CF_3CONH). ^{13}C NMR (CD_3OD) δ 49.53 (d, C-1; the *J* value could not be correctly measured by overlapping with the solvent peaks), 32.70 (C-2), 47.91 (d, 3 Hz, C-3), 75.30 (d, 16.9 Hz, C-4; determined by HMBC between C-4 and H-1′), 88.91 (d, 182.3 Hz, C-5), 81.63 (d, 17.3 Hz, C-6; determined by HMBC between C-6 and H-1″), 93.72 (C-1′), 50.05 (C-2′), 34.41 (C-3′), 67.03 (C-4′), 73.50 (C-5′), 42.28 (C-6′), 102.85 (C-1″), 71.24 (C-2″), 56.67 (C-3″), 68.30 (C-4″), 74.93 (C-5″), 62.30 (C-6″); 28.83 and 28.89 (Me of Boc's);

117.69 (q, 286.9 Hz × 3) and 118.26 (q, 292.8 Hz × 3) (NHCO*C*F_3 and*C*F_3CO_2H); 157.83 ($NHCO_2$-2′; determined by HMBC between this C and H-2′), 158.14 ($NHCO_2$-3; determined by HMBC between this C and H-3), 159.30 ($NHCO_2$-6′), 160.25 (q, 36.7 Hz, NH*C*O*C*F_3; determined by HMBC between this C and H-3″), and 163.13 (q, 34.6 Hz, CF_3CO_2H). Anal. Calcd for $C_{35}H_{59}F_4N_5O_{15} \cdot CF_3CO_2H \cdot 1.5H_2O$: C, 44.13; H, 6.31; F, 13.21; N, 6.96. Found: C, 44.32; H, 6.65; F, 13.06; N, 7.13.

5,3′-Dideoxy-5-epifluoro-1-N-*[(*S*)-4-(*p*-methoxybenzyloxycarbonylamino)-2-hydroxybutanoyl]-3″*-N-*trifluoroacetyl-3,2′,6′-tris(*N-tert-*butoxycarbonyl)kanamycin B* (**14**).—To a solution of **13** (trifluoroacetate sesquihydrate, 50.5 mg, 50.2 μmol) in pyridine (1.5 mL) was added *N*-[(*S*)-4-(*p*-methoxybenzyloxycarbonylamino)-2-hydroxybutanoyloxy]succinimide in THF [0.99 mL of 0.088 mmol/mL; prepared by coupling of the corresponding *N*-protected acid (54.7 mg) and *N*-hydroxysuccinimide (23.0 mg) in the presence of DCC (41.4 mg) in THF (2.2 mL)] and the solution was kept for 1 h at room temperature. TLC (9:2:0.5 $CHCl_3$–MeOH–aq 28% NH_3) of the solution showed a single spot at R_f 0.4 (cf. **13**: R_f 0.35). Concentration of the solution gave a residue, which was thoroughly washed with water and diethyl ether, and dried in vacuo at 40°C to give **14** as a solid (55.0 mg, 97%), $[\alpha]_D^{21}$ + 46° (*c* 1.2, MeOH); ^{1}H NMR (pyridine-d_5) δ 1.48, 1.53, and 1.60 (each s of 9 H, 3 Boc), 3.65 (s, 3 H, OMe), 4.86 (br q, 1 H, H-3″), 5.56 (br d, 2 H, H-1′, 1″), 5.97 (br d, 1 H, *J* 52.5 Hz, H-5). ^{19}F NMR (CD_3OD) δ −214.97 (dt, 1 F, $J_{4,F} = J_{6,F}$ 28 Hz, F-5) and −75.49 (s, 3 F, CF_3CONH). Anal. Calcd for $C_{48}H_{74}F_4N_6O_{20}$: C, 50.97; H, 6.59; F, 6.72; N, 7.43. Found: C, 51.00; H, 6.91; F, 6.42; N, 7.45.

1-N-*[(*S*)-4-Amino-2-hydroxybutanoyl]-5,3′-dideoxy-5-epifluorokanamycin B* (**19**).—To a solution of **14** (1.58 g, 1.40 mmol) in DMF (18 mL) aq 28% NH_3 (12 mL) was added and the solution was kept overnight at 30°C [de(trifluoroacetyl)ation]. Concentration in vacuo gave a residue, which was dissolved in a mixture of MeOH (12 mL), H_2O (9.5 mL) and aq 36% HCl (9.5 mL), and the solution was kept for 2 h at room temperature. Chloroform (12 mL) was added and, after shaking, the upper aq layer separated was concentrated with intermittent additions of water. An aqueous solution of the residue was neutralized with aq NH_3 and subjected to column chromatography of Amberlite CG-50 (NH_4^+ form) with aq 0 → 0.5 M NH_3. The ninhydrin-positive fractions were collected and concentrated to give **19** as a solid of 0.7 carbonate (482 mg, 56%), $[\alpha]_D^{23}$ + 96° (*c* 3, H_2O); ^{1}H NMR (26% ND_3 in D_2O) δ 1.43 (ddd, 1 H, H-2*ax*), 2.06 (dt, 1 H, H-2*eq*), 3.23 (m, 1 H, H-3), 3.58 (ddd, 1 H, H-4), 3.93 (dd, 1 H, H-6), 4.21 (br dt, 1 H, H-1), and 5.40 (br d, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12, $J_{1,2eq} = J_{2eq,3}$ 4.5, $J_{3,4} \sim 10$, $J_{4,5} = J_{5,6}$ 2, $J_{1,6}$ 11, $J_{4,F} = J_{6,F}$ 29 and $J_{5,F}$ 52 Hz; δ 1.64 (ddd, 1 H, H-3′*ax*), 2.71 (dd, 1 H, H-6′a), 2.93 (dt, 1 H, H-2′), 2.98 (dd, 1 H, H-6′b), and 4.95 (d, 1 H, H-1′). $J_{1',2'} = J_{2',3'eq}$ 4, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'}$ 12, $J_{5',6'a}$ 7, $J_{5',6'b}$ 2, and $J_{6'a,6'b}$ 13 Hz; δ 3.00 (t, 1 H, H-3″), 3.16 (t, 1 H, H-4″), 3.39 (dd, 1 H, H-2″), 3.62 (dd, 1 H, H-6″a), 3.81 (ddd, 1 H, H-5″), 3.89 (dd, 1 H, H-6″b), and 5.02 (d, 1 H, H-1″). $J_{1'',2''}$ 4, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10, $J_{5'',6''a}$ 7.5, $J_{5'',6''b}$ 2, and $J_{6''a,6''b}$ 12 Hz; δ 1.73 (dddd, 1 H, H-3‴a), 1.91 (ddt, 1 H, H-3‴b), and 4.17 (dd, 1 H, H-2‴). $J_{2''',3'''a}$ 9, $J_{2''',3'''b}$ 4, $J_{3'''a,3'''b}$ 14, $J_{3'''a,4'''a}$ 7.5, $J_{3'''a,4'''b}$ 5.5, $J_{3'''b,4'''a} = J_{3'''b,4'''b}$ 8 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −215.15 (dt, *J* 29, 29, and 52 Hz, F-5). Anal. Calcd for $C_{22}H_{43}FN_6O_{10} \cdot 0.7$

H_2CO_3: C, 44.40; H, 7.29; F, 3.09; N, 13.69. Found: C, 44.40; H, 7.28; F, 3.07; N, 13.46.

1-N-[(S)-4-Amino-2-hydroxybutanoyl]-5,3',4'-trideoxy-5-epifluorokanamycin B (**20**).—Prepared in 40% yield from **11** by adopting a reaction sequence described for **10** → **12** → **13** → **14** → **19**, $[\alpha]_D^{20}$ + 104° (*c* 1.3, H_2O); ^{1}H NMR (26% ND_3 in D_2O) δ 1.43 (ddd, 1 H, H-2*ax*), 2.06 (dt,1 H, H-2*eq*), 3.22 (br dt, 1 H, H-3), 3.57 (br dd, 1 H, H-4), 3.92 (br dd, 1 H, H-6), 4.21 (br dt, 1 H, H-1), and 5.38 (br d, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 13, $J_{1,2eq} = J_{2eq,3}$ 4.5, $J_{3,4} = J_{1,6}$ 11, $J_{4,F} = J_{6,F}$ 29, and $J_{5,F}$ 52 Hz; δ 1.40 (dddd, 1 H, H-4'*ax*), 1.67 (dddd, 1 H, H-3'*ax*); 2.64 (dd, 1 H, H-6'a) and 2.67 (dd, 1 H, H-6'b) show a ABX pattern; 2.81 (dt, 1 H, H-2'), and 4.97 (d, 1 H, H-1'). $J_{1',2'} = J_{2',3'eq}$ 3.5, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'ax} = J_{4'ax,4'eq} = J_{4'ax,5'}$ 12, $J_{3'ax,4'eq} = J_{3'eq,4'ax} = J_{5',6'b}$ 4, $J_{5',6'a}$ 7, and $J_{6'a,6'b}$ 13 Hz; δ 3.00 (t, 1 H, H-3''), 3.17 (t, 1 H, H-4''), 3.39 (dd, 1 H, H-2''), 3.62 (dd, 1 H, H-6''a), 3.89 (dd, 1 H, H-6''b), and 5.02 (d, 1 H, H-1''). $J_{1'',2''}$ 4, $J_{2'',3''} = J_{3'',4''} = J_{4'',5''}$ 10, $J_{5'',6''a}$ 7, $J_{5'',6''b}$ 2, and $J_{6''a,6''b}$ 12 Hz; δ ~ 1.73 (H-3'''a), 1.91 (ddt, 1 H, H-3'''b), and 4.17 (dd, 1 H, H-2'''). $J_{2''',3'''a}$ 9, $J_{2''',3'''b}$ 4, $J_{3'''a,3'''b}$ 14, and $J_{3'''b,4'''a} = J_{3'''b,4'''b}$ 8 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −215.07 (dt, *J* 29, 29, and 52 Hz, F-5). Anal. Calcd for $C_{22}H_{43}FN_6O_9 \cdot 1/2\ H_2O$: C, 46.88; H, 7.87; F, 3.37; N, 14.91. Found: C, 46.57; H, 8.22; F, 3.12; N, 14.63.

2',3',4',2'',4'',6'',2'''-Hepta-O-acetyl-3,6',3'',4'''-tetrakis(N-tert-butoxycarbonyl)amikacin (**17**).—Compound **16** [8] (1.43 g, 1.45 mmol) was acetylated with Ac_2O (1.10 mL, 116 mmol) in pyridine (25 mL) as described for **3** to give **17** as a solid (1.73 g, 92% as monohydrate), $[\alpha]_D^{21}$ + 70° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.39 (9 H), 1.43 (9 H) and 1.45 (18 H) (each s, 4 Boc); 2.00 (3 H), 2.04 (6 H), 2.06 (3 H), 2.09 (3 H), 2.10 (3 H), and 2.14 (3 H) (each s, Ac). Anal. Calcd for $C_{56}H_{89}N_5O_{28} \cdot H_2O$: C, 51.80; H, 7.06; N, 5.39. Found: C, 51.95; H, 6.85; N, 5.31.

5-Deoxy-5-epifluoro-2',3',4',2'',4'',6'',2'''-hepta-O-acetyl-3,6',3'',4'''-tetrakis(N-tert-butoxycarbonyl)amikacin (**18**).—Compound **17** (49.0 mg, 37.7 μmol) in CH_2Cl_2 (0.9 mL) was fluorinated with DAST (0.01 mL, 76 μmol) as described for **4** to give a solid; TLC (3:1 $CHCl_3$–acetone): R_f 0.25 (cf. **17**: R_f 0.2). Purification by column chromatography (100:1 → 80:1 → 60:1 $CHCl_3$–MeOH) gave **18** as a solid (31.8 mg, 65%), $[\alpha]_D^{23}$ + 71° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.39, 1.42, 1.44 and 1.46 (each s of 9 H, 4 Boc); 2.00 (3 H), 2.02 (3 H), 2.06 (6 H), 2.08 (6 H) and 2.16 (3 H) (each s, 7 Ac). Anal. Calcd for $C_{56}H_{88}FN_5O_{27} \cdot H_2O$: C, 51.72; H, 6.98; F, 1.46; N, 5.39. Found: C, 51.99; H, 6.78; F, 1.12: N, 5.58.

5-Deoxy-5-epifluoroamikacin (**21**).—Compound **18** (649 mg, 0.499 mmol) was deacetylated as described for **5**, then treated with CF_3CO_2H (1.56 mL) as described for **10**. The crude products was purified by a column of Amberlite CG-50 (NH_4^+ form, 30mL) developed with aq 0 → 0.5 M NH_3 to give **21** as a solid of 0.75 carbonate [after dryness in vacuo at 40°C, 286 mg (90%)], $[\alpha]_D^{24}$ + 101° (*c* 1.7, H_2O); ^{1}H NMR (26% ND_3 in D_2O) δ 1.43 (ddd, 1 H, H-2*ax*), 2.06 (dt, 1 H, H-2*eq*), 3.25 (m, 1 H, H-3), 3.57 (ddd, 1 H, H-4), 3.92 (ddd, 1 H, H-6), 4.22 (br dt, 1 H, H-1), and 5.40 (br d, 1 H, H-5); δ ~ 2.75 (H-6'a), 2.99 (dd, 1 H, H-6'b), 3.31 (t, 1 H, H-4'), 3.57 (dd, 1 H, H-2'), 3.75 (t, 1 H, H-3'), and 5.17 (d, 1 H, H-1'). $J_{1',2'}$ 4, $J_{2',3'} = J_{3',4'} = J_{4',5'}$ 9.5, $J_{5',6'b}$ 2.5 and $J_{6'a,6'b}$ 13.5 Hz; δ 3.01 (t, 1 H, H-3''), 3.20 (t, 1 H, H-4''), 3.39 (dd, 1 H, H-2''), 3.67 (dd, 1 H, H-6''a), 3.80 (ddd, 1 H, H-5''), 3.87 (dd, 1 H, H-6''b), and 5.02 (d, 1 H, H-1'');

δ 1.73 (dddd, 1 H, H-3‴a), 1.91 (ddt, 1 H, H-3‴b), and 4.17 (dd, 1 H, H-2‴); all J values not descried here except for the 6-amino-6-deoxy-D-glucose moiety were almost the same with those for **19** respectively. ^{19}F NMR (26% ND_3 in D_2O) δ −214.92 (dt, J 29, 29, and 52 Hz, F-5). Anal. Calcd for $C_{22}H_{42}FN_5O_{12}0.75\ H_2CO_3$: C, 43.09; H, 6.91; F, 3.00; N, 11.04. Found: C, 43.34; H, 7.02; F, 2.64; N, 11.14.

5-Deoxy-5-fluorokanamycin A (**22**).—$[\alpha]_D^{23}$ + 145° (c 1, H_2O); ^{1}H NMR (DCl in D_2O, pD ~ 1) δ 2.09 (ddd, 1 H, H-2*ax*), 2.67 (br ddd, 1 H, H-2*eq*), 4.26 (ddd, 1 H, H-6 or -4), 4.38 (ddd, 1 H, H-4 or -6), and 5.08 (ddd, 1 H, H-5). $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12.5, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{3,4} = J_{1,6}$ 10, $J_{4,5} = J_{5,6}$ 9, $J_{4\text{ or }6,F}$ 11, $J_{5,F}$ 50, and $J_{6\text{ or }4,F}$ 13 Hz; δ 3.24 (dd, 1 H, H-6′a), 3.45 (t, 1 H, H-4′), 3.52 (dd, 1 H, H-6′b), 3.76 (dd, 1 H, H-2′), 3.86 (t, 1 H, H-3′), and 5.55 (d, 1 H, H-1′). $J_{1',2'}$ 3.5, $J_{2',3'} = J_{3',4'} = J_{4',5'} = J_{5',6'a}$ 9, $J_{5',6'b}$ 3, and $J_{6'a,6'b}$ 13 Hz; δ 3.61 (t, 1 H, H-3″), 4.02 (dd, 1 H, H-2″), and 5.25 (d, 1 H, H-1″). $J_{1'',2''}$ 3.5 and $J_{2'',3''} = J_{3'',4''}$ 10.5 Hz. ^{19}F NMR (DCl in D_2O, pD ~ 1) δ −192.60 (br dt, $J_{5,F}$ 50 Hz, F-5). ^{13}C NMR (DCl in D_2O, pD ~ 1) δ 49.79 (d, 11.5 Hz, C-1 or -3), 28.04 (C-2), 47.38 (d, 10.9 Hz, C-3 or -1), 76.39 (d, 19.3 Hz, C-4 or -6), 92.53 (d, 183.4 Hz, C-5), 81.78 (d, 20 Hz, C-6 or -4), 95.76 (d, 2.8 Hz, H-1′), 71.25 (C-2′), 72.91 (C-3′), 71.68 (C-4′), 69.51 (C-5′), 41.27 (C-6′), 101.02 (C-1″), 68.85 (C-2″), 55.71 (C-3″), 66.38 (C-4″), 73.58 (C-5″), and 60.86 (C-6″). Anal. Calcd for $C_{18}H_{35}FN_4O_{10}$: C, 44.44; H, 7.25; F, 3.91; N, 11.52. Found: C, 44.13; H, 7.41; F, 3.72; N, 11.28.

2,5-Dideoxy-5-epifluorostreptamine (**23**).—A solution of **21** (128 mg, 0.75 carbonate) in aq 4 M HCl (2.5 mL) was heated for 16 h at 100°C. Concentration gave a brown syrup, which was repeatedly concentrated under intermittent additions of water. An aqueous solution of the residue was brought to slight alkaline by addition of Dowex 1 × 2 resin (OH^- form, ~0.3 mL) and the mixture was poured into a column containing the same resin (10 mL). After washing with water (4 mL), the column was allowed to stand for three days at room temperature, during the time the two free aminosugars liberated tightly absorbed in the resin (the reason was unknown; immediate elution with water only gave a mixture of products). Development of the column with water eluted **23** as a crude syrup, which was purified by passing a column of the same resin with water affording **23** as an amorphous powder (30.4 mg, 85%) (attempted recrystallization from MeOH gave a precipitate containing crystalline solids, when checked under microscope; it showed ill-defined mp at above 190°C), TLC with 5:20:6:9 $CHCl_3$–MeOH–aq 28% NH_3–H_2O, R_f 0.52 (cf. 2-deoxystreptamine R_f 0.4), $[\alpha]_D^{21}$ 0° (c 0.6, H_2O); ^{1}H NMR (26% ND_3 in D_2O) δ 1.07 (ddd, 1 H, H-2*ax*), 1.99 (dt, 1 H, H-2*eq*), 2.96 (dddd, 2 H, H-1, 3), 3.38 (ddd, 2 H, H-4, 6), and 4.82 (dt, 1 H, H-5); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 13, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{1,6} = J_{3,4}$ 10, $J_{4,5} = J_{5,6}$ 2, $J_{1,F} = J_{3,F}$ 1.5, $J_{4,F} = J_{6,F}$ 30.4, and $J_{5,F}$ 52.8 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −215.37 (br dt, $J_{4,F} = J_{6,F}$ 30.4 and $J_{5,F}$ 52.8 Hz). ^{13}C NMR (26% ND_3 in D_2O) δ 48.99 (d, J 3.7 Hz, C-1, 3), 36.68 (C-2), 74.75 (d, J 17.5 Hz, C-4, 6), and 95.71 (d, J 174.7 Hz, C-5). Anal. Calcd for $C_6H_{13}FN_2O_2 \cdot 0.2H_2CO_3$: C, 42.17; H, 7.65; F, 10.76; N,15.86. Found: C, 42.51; H, 7.72; F, 10.35; N, 15.82.

2,5-Dideoxy-5-fluorostreptamine (**24**).—5-Deoxy-5-fluorokanamycin A (**22**) (100 mg) was treated similarly as described for **23** to give **24** as an amorphous powder (27.2 mg, 73%) (attempted recrystallization from MeOH gave a precipitate containing crys-

talline solids, when checked under microscope; it showed ill-defined mp at above 210°C), TLC with 5:20:6:9 $CHCl_3$–MeOH–aq 28% NH_3–H_2O, R_f 0.65 $[\alpha]_D^{21}$ 0° (*c* 0.9, H_2O); ^1H NMR (26% ND_3 in D_2O) δ 1.19 (ddd, 1 H, H-2*ax*), 1.96 (dt, 1 H, H-2*eq*), 2.71 (dddd, 2 H, H-1, 3), 3.39 (dt, 2 H, H-4, 6), and 4.16 (dt, 1 H, H-5); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 13, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{1,6} = J_{3,4}$ 10, $J_{4,5} = J_{5,6}$ 9, $J_{1,F} = J_{3,F}$ 1, $J_{4,F} = J_{6,F}$ 12.8 and $J_{5,F}$ 52.3 Hz. ^{19}F NMR (26% ND_3 in D_2O) δ −196.30 (dt, $J_{4,F} = J_{6,F}$ 12.8 and $J_{5,F}$ 52.3 Hz). ^{13}C NMR (26% ND_3 in D_2O) δ 50.51 (d, *J* 3.7 Hz, C-1, 3), 36.54 (d, *J* 1.6 Hz, C-2), 76.46 (d, *J* 16.5Hz, C-4, 6), and 97.52 (d, *J* 176.7 Hz, C-5). Anal. Calcd for $C_6H_{13}FN_2O_2 \cdot 0.5H_2O \cdot 0.15H_2CO_3$: C, 40.48; H, 7.90; F, 10.41; N, 15.35. Found: C, 40.44; H, 8.12; F, 10.11; N, 15.72.

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