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Synthesis of 2″-oxidized derivatives of 5-deoxy-5-epi-5-fluoro-dibekacin and -arbekacin, and study on structure–chemical shift relationships of urethane(or amide)-type NH protons in synthetic intermediates

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Abstract

Three 2″-modified dibekacin-analogs have been prepared as potential compounds active against resistant bacteria producing 2″-*O*-phosphotransferases; one is 5-deoxy-5,2″-diepi-5-fluorodibekacin (**9**) prepared from a suitably protected 2″-*O*-triflyl derivative through the 2″,3″-cyclic carbamate, and the others are 2″-oxo derivatives (**12** and **22**, both as the hydrate) of 5-deoxy-5-epi-5-fluoro-dibekacin and -arbekacin prepared through oxidation at C-2″ of suitably protected derivatives. Relationships between the *t*-butoxycarbonyl(= Boc)-N*H*-shifts of per-*N*-Boc synthetic intermediates and their structures were studied. It was found that the shifts, measured in pyridine-d_5 at 80 °C, which spread over a close range (δ 6–7 ppm), are sensitively influenced by nearby and surrounding groups around the BocNH group in respect of electron-withdrawing character, hydrogen bonding (BocN*H* · · · acceptor), and also solvent effects (BocN*H* · · · NC_5H_5).

Keywords: Dibekacin derivative; Oxo compound; *t*-Butoxycarbonylamino; Amide proton shift; Inductive effect; Hydrogen bond; Pyridine-d_5

1. Introduction

Dibekacin (3′,4′-dideoxykanamycin B), clinically used, is inactivated by resistant bacteria [1] that produce nucleotidyl transferases, giving 2″-*O*-adenylyldibekacin. In contrast, arbekacin, a 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative of dibekacin, is not inactivated by these bacteria and has an improved antibacterial activity; however, recently arbekacin was reported to be inactivated [2] by some methicillin-resistant *Staphylococcus aureus* (MRSA) to give the

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2″-phosphate. Generally, removal of the hydroxyl group phosphorylated by resistant bacteria has been an effective method to recover the activity [3], as demonstrated by dibekacin [4]. However, removal of the HO-2″ group of dibekacin substantially decreased the activity (unpublished data), indicating the importance of this group. Recently 2″-amino-2″-deoxy-dibekacin and -arbekacin have been synthesized [5] and shown to be active against resistant bacteria phosphorylating HO-2″. However, we wished to prepare a more effective derivative lacking the H_2N-2″ group because the presence of excess NH_2 groups in a molecule sometimes increases toxicity or decreases transportation into bacterial cells. Syntheses of the 2″-epimer of dibekacin and the 2″-oxo derivatives of dibekacin and arbekacin have thus been undertaken. Our additional interest in these syntheses was the stability of the oxo compounds, because in natural substances, the coexistence of amino and carbonyl groups has rarely been reported. In our synthesis, the 5-hydroxyl group in dibekacin was replaced with inversion by fluorine, because the hydroxyl group impedes selective oxidation at C-2″, and moreover 5-deoxy-5-epi-5-fluorination is expected to give products of enhanced antibacterial activity [6].

2. Results and discussion

Synthesis.—Pentakis(*N-tert*-butoxycarbonyl)dibekacin (BocDBK, **2**) prepared conventionally [6] from dibekacin (**1**) was converted into the 4″,6″-*O*-cyclohexylidene derivative **3**, which was acetylated to give the 2″-*O*-acetyl derivative **4** having HO-5 free. Lack of acetylation (or benzoylation) here was usually experienced in similar kanamycin derivatives [6–9]. Fluorination of **4** with Et_2NSF_3 (DAST) [10] gave the 5-deoxy-5-epi-5-fluoro derivative **5** in high yield. The axial orientation of fluorine was proved by the ^{19}F NMR spectrum; fluorine resonated as double triplets showing large coupling [6] ($J_{5,F}$ 53 and $J_{4,F} \approx J_{6,F}$ 29 Hz). After deacetylation (to give **6**), the free HO-2″ was triflated, and the resulting **7** was treated with NaOAc in DMF to give the 2″,3″-cyclic carbamate under inversion at C-2″. The structure was confirmed by lack of one Boc group, and by IR and ^1H NMR spectra ($J_{1'',2''} \sim 0$ Hz). Subsequent deprotection gave 5-deoxy-5-epi-5-fluoro-2″-epidibekacin (**9**). The presence of an axial HO-2″ group was confirmed by the shift and coupling constants of H-2″ in its NMR spectrum. The ^{13}C NMR spectrum of **9** (Table 1) also supported the structure.

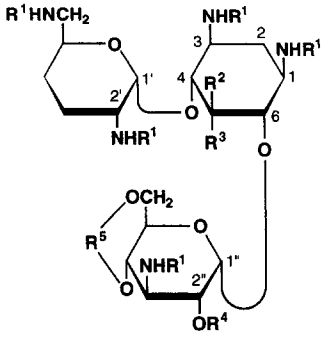
Table 1
^{13}C NMR chemical shifts [a] (δ [b], ppm) and coupling constants ($J_{C,F}$ Hz) for **9**, **12**, **22**, and 5-deoxy-5-epifluoroarbekacin (FABK) in DCl–D_2O (pD 3)

	Compound			
	9	**12**	**22**	FABK
C-1	47.63d	47.69d	46.83d	46.98d
C-2	28.61	28.55	30.41	30.47
C-3	47.88d	47.80d	48.12d	48.07d
C-4	72.24d	72.47d	72.88d	72.90d
C-5	86.49d	86.51d	87.57d	87.79d
C-6	78.73d	79.35d	79.00d	78.80d
C-1′	91.02	91.18	91.12	91.02
C-2′	49.04	48.96	48.98	48.94
C-3′	21.66	21.55	21.58	21.55
C-4′	26.38	26.25	26.30	26.29
C-5′	66.83	66.81	66.73	66.67
C-6′	43.51	43.34	43.37	43.35
C-1″	101.70	102.76	102.79	100.43
C-2″	67.36	92.14	92.09	68.72
C-3″	53.85	58.14	58.34	56.02
C-4″	64.40	66.44	66.50	67.01
C-5″	74.10	73.71	73.71	73.34
C-6″	61.80	61.60	61.59	61.41
C-1‴			176.67	176.62
C-2‴			70.40	70.43
C-3‴			31.62	31.59
C-4‴			37.77	37.73
$J_{1(3),F}$	4.5	4.5	4.5	4.5
$J_{4(6),F}$	17.6	17.5	17.5	17.3
$J_{5,F}$	184.1	184.2	182.4	182.2

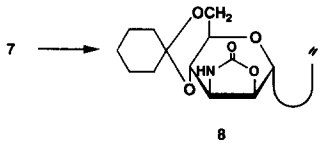
[a] Measured at 125.8 MHz by a Bruker AMX 500 spectrometer and confirmed by the ^1H–^{13}C COSY.
[b] Internal Me_4Si.

5,2″-Dideoxy-5-epi-5-fluoro-2″-oxodibekacin was synthesized from **6**. Treatment of **6** with pyridinium dichromate (PDC) in CH_2Cl_2 gave the 2″-oxo derivative **11** in 80% yield. Presence of the carbonyl group was confirmed by the appearance of H-1″ as a singlet and of C-2″ at low field (δ 196.5) in its ^1H and ^{13}C NMR spectra, respectively. Subsequent deprotection with aqueous 95% CF_3CO_2H (TFA) gave the final product **12**. However, as the penta-TFA salt was hygroscopic and gradually decomposed on storage, it was converted into the tetra(acetic acid) salt by passing through a Dowex resin-column (AcO^- form) to give **12** as a stable salt. Its ^1H, ^{19}F, and ^{13}C NMR spectra (Table 1) confirmed the structure; the resonance of ^{13}C-2″ appeared in the region of anomeric carbons (δ 92.1) indicating the absence of carbonyl and the presence of a hydrate structure, this fact

being further confirmed by its mass spectrum and elemental analysis.



	R^1	R^2	R^3	R^4	R^5
1	H	OH	H	H	H, H
2	Boc	OH	H	H	H, H
3	Boc	OH	H	H	$(CH_2)_5C$
4	Boc	OH	H	Ac	$(CH_2)_5C$
5	Boc	H	F	Ac	$(CH_2)_5C$
6	Boc	H	F	H	$(CH_2)_5C$
7	Boc	H	F	OSO_2CF_3	$(CH_2)_5C$



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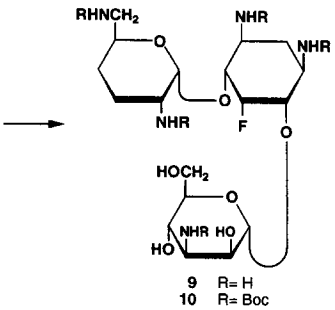
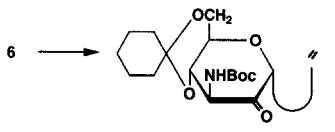


9 R= H
10 R= Boc



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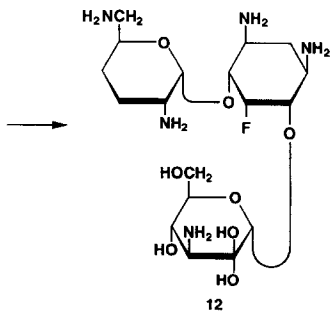
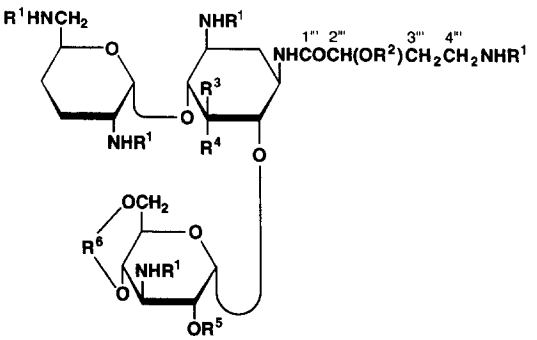
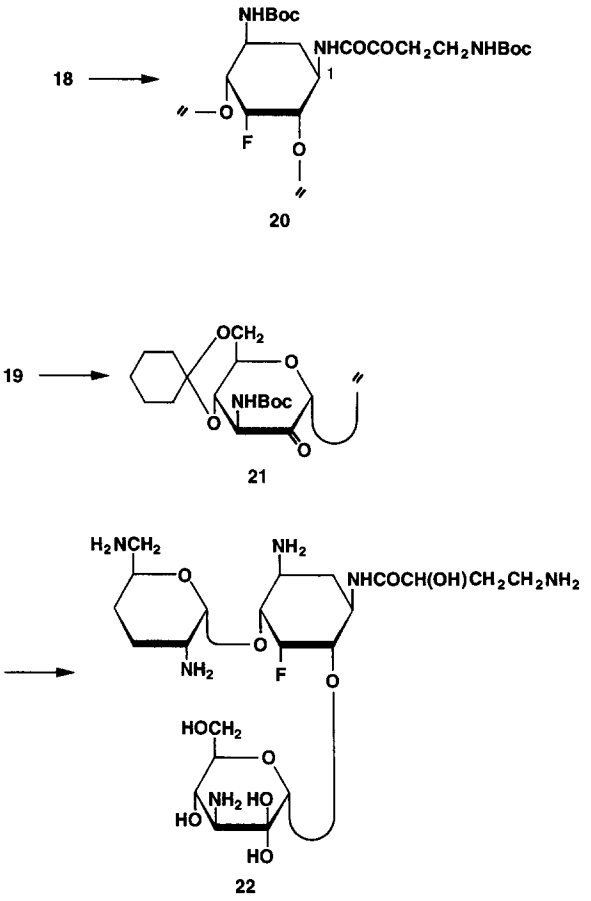


12

The 2″-oxo derivative of 5-deoxy-5-epi-5-fluoro-arbekacin was prepared by applying a similar route as described for **12**. Pentakis(*N-tert*-butoxycarbonyl)arbekacin (**14**), prepared conventionally from arbekacin (**13**) was converted into its 4″,6″-*O*-cyclohexylidene derivative **15**, which was selectively benzoylated to give the 2″,2‴-dibenzoate **16**. Fluorination with DAST afforded the 5-deoxy-5-epi-5-fluoro derivative **17** in good yield. After debenzoylation, the 2″,2‴-diol **18** was oxidized with Dess–Martin periodinane [11], whereupon, however, HO-2‴ was selectively oxidized to give **20**. Therefore, selective protection of the HO-2‴ (or HO-2″) was explored. Treatment of **18** with 3,4-dihydro-2*H*-pyran failed to give any pure positional isomer, but treatment with Ac_2O in pyridine successfully gave the 2‴-acetate **19** in 81% yield after chromatography. The position acetylated was confirmed by its ^1H NMR spectrum. Oxidation of **19** was next tried using PDC, pyridinium chlorochromate, and $(CH_3)_2SO$–Ac_2O; the last was the best. The presence of a 2″-oxo group in product **21** was confirmed by its ^1H and ^{13}C NMR spectra. Deacetylation followed by deprotection with aqueous 95% TFA gave the final product **22** in high yield. Again the 2″-oxo group in **21** was transformed into its hydrate form. Attempts to dehydrate **22** (and **12**) to give the corresponding carbonyl form by heating under diminished pressure (at 50 °C for one week or more) failed, only the starting **22** remained (a carbonyl carbon signal was not observed, as an even trace, in its ^{13}C NMR spectrum). Compounds **12** and **22** were unstable in alkaline solutions.



	R^1	R^2	R^3	R^4	R^5	R^6
13	H	H	OH	H	H	H, H
14	Boc	H	OH	H	H	H, H
15	Boc	H	OH	H	H	$(CH_2)_5C$
16	Boc	Bz	OH	H	Bz	$(CH_2)_5C$
17	Boc	Bz	H	F	Bz	$(CH_2)_5C$
18	Boc	H	H	F	H	$(CH_2)_5C$
19	Boc	Ac	H	F	H	$(CH_2)_5C$



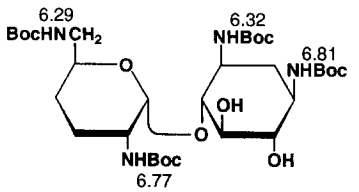
*Structure–chemical shift relationships in BocN*H *(and acyl-N*H*) protons in* N-tert-*butoxycarbonyl compounds.*—Urethane- or amide-type proton-signals have not been utilized positively in structural elucidation of aminoglycoside antibiotics, possibly because they were considered to give inconsistent shift-values with broadened signals, varying according to concentration and temperature in solutions. In the present study, we obtained a number of structurally determined NBoc derivatives as the synthetic intermediates, and therefore we intended to search them for probable regularity between structure and shift-value of BocN*H*-protons in analogous positions (1, 3, 2′, 6′, 3″, and 4‴). However, these BocN*H* protons showed chemical shifts largely influenced by temperature and the solvent used. When measured in dry pyridine-d_5, however, each of these protons gave a comparatively sharp signal with a consistent chemical shift at one temperature, not influenced by the concentration of the compounds (see Table 2), suggesting that no substantial self-association exists. Therefore, we fixed the measuring temperature at 80 °C (signals sharpened as the temperature raised; for example see [12]) and collected the BocN*H*-shift data. Our first questions noted after the start of this study had been (*a*) why the BocN*H*-1 protons always resonate downfield ($\Delta\delta$ 0.35–0.7) as compared with the BocN*H*-3 protons, despite the similarity in structural surroundings in each other and (*b*) why the BocN*H*-2′ protons for the compounds having a F-5*ax* atom always resonate upfield ($\Delta\delta \sim 0.6$) as compared to those having a HO-5*eq* group.

Table 2
^{1}H NMR chemical shifts (δ, ppm) of BocN*H* protons for **2–6**, **8**, **10**, **11**, **14**, and **18–21** in 60 mM solution [a] in pyridine-d_5 at 80 °C

	Functional group at							
	C-5	C-2″	NH-1	NH-3	NH-2′	NH-6′	NH-3″	NH-4‴
2	HO_{eq}	HO_{eq}	6.99	6.32d	6.60d	6.29t	6.95	
3	HO_{eq}	HO_{eq}	6.97d	6.30d	6.72d	6.28t	6.76d	
4	HO_{eq}	AcO_{eq}	6.70d	6.30d	6.76d	6.30t	6.92d	
5	F_{ax}	AcO_{eq}	6.78d	6.42d	5.97d	6.31	6.97d	
6	F_{ax}	HO_{eq}	6.97d	6.41d	6.02d	6.31	6.77d	
8	F_{ax}	$O{=}CO_{ax}$	7.22d	6.52d	6.11d	6.33	9.37s [c]	
10	F_{ax}	HO_{ax}	6.97d	6.32d	6.00d	6.30	6.44d	
11	F_{ax}	=O	7.09d	6.47d	6.09d	6.32	7.30d	
14	HO_{eq}	HO_{eq}	8.09d [b]	6.28d	6.59d	6.27t	6.97d	6.56
18	F_{ax}	HO_{eq}	8.06d [b]	6.35d	5.98d	6.28t	6.80d	6.50t
19	F_{ax}	HO_{eq}	8.17d [b]	6.35d	5.95d	6.25t	6.73d	6.49t
20	F_{ax}	HO_{eq}	8.61d [b]	6.44d	5.99d	6.25t	6.68d	6.53t
21	F_{ax}	=O	8.35d [b]	6.41d	5.97d	6.23t	7.27d	6.40

[a] The shifts of **2**, **5**, and **14** in 6, 30, 60, and 120 mM solutions were as follows: **2**, NH-1: 6.98, 6.98, 6.98, 6.95 (in the order cited); NH-3: 6.32, 6.32, 6.32, 6.30; NH-2′: 6.60, 6.60, 6.60, 6.59; NH-6′: 6.30, 6.29, 6.29, 6.28; NH-3″: 6.96, 6.96, 6.95, 6.95. **5**, NH-1: 6.79, 6.77, 6.78, 6.77; NH-3: 6.43, 6.41, 6.42, 6.40; NH-2′: 5.98, 5.96, 5.97, 5.96; NH-6′: 6.31, 6.30, 6.31, 6.29; NH-3″: 6.99, 6.96, 6.98, 6.96. **14**, NH-1: 8.10, 8.10, 8.09, 8.07; NH-3: 6.30, 6.29, 6.29, 6.25; NH-2′: 6.60, 6.59, 6.58, 6.56; NH-6′: 6.29, 6.29, 6.28, 6.24; NH-3″: 7.01, 7.00, 6.98, 6.93; NH-4‴: 6.57, 6.55, 6.55, 6.52.
[b] AHB–NH proton.
[c] NH for cyclic carbamate.

As shown in Table 2, BocN*H*-protons attached at a methylene group (C-6′ and -4‴) appeared at $\delta \sim 6.3$ and ~ 6.5 (confirmed by the $^1H-^1H$ COSY) as a triplet (or a triplet-like singlet) respectively with only slight fluctuation in shifts among the compounds tested. On the other hand, the BocN*H*-3 protons attached at a methine group of 2-deoxystreptamine (which has a HO-5*eq* group) or 2,5-dideoxy-5-epi-5-fluorostreptamine (which has a F-5*ax* atom) appeared as a doublet ($J \sim 8$ Hz) in most cases, in the vicinity of δ 6.3–6.4. Discrimination of the BocN*H*-1 and -3 signals is not usually easy because of similar steric situations for the groups (see formulas); however, we have succeeded by determining the sequences of H-1′–C-4–H-4–C-3–H-3–NH-3 and H-1″–C-6–H-6–C-1–H-1–NH-1 for **3** and **6**, chosen as reference compounds, by utilizing HMBC and HMQC methods as well as $^1H-^1H$ COSY. A careful check of the BocN*H*-3 shifts, however, indicated that in the former group of compounds (**2**, **3**, **4**, and **14**) they were located at slightly higher fields ($\delta \sim 6.3$) than the latter ($\delta \sim 6.4$; slight deviations were observed in 2″-modified compounds, **8**, **10**, and **11**). This may be attributed to the difference in substituent at C-5, that is, F-5*ax* is slightly more electron-withdrawing than HO-5*eq* [6]. To examine the structure–shift relationship in this position in more detail, three simple disaccharides were prepared; namely the per-*N*-(*t*-butoxycarbonyl) derivatives of 3′,4′-dideoxyneamine, 5-epi-5-fluoro-5,3′,4′-trideoxyneamine, and 5-epi-5-fluoro-6-*O*-methyl-5,3′,4′-trideoxyneamine (**23**, **25**, and **26** in this order). The BocN*H*-3 shift (δ 6.32) of **23** fell in the expected range for 2-deoxystreptamine derivatives having HO-5*eq*, which was approximately $\Delta\delta$ 0.1 smaller than those for **25** and **26** having a F-5*ax* atom, supporting the foregoing assumption. The BocN*H*-6′ values for **23**, **25**, and **26** also showed good agreement with the expected values described before.



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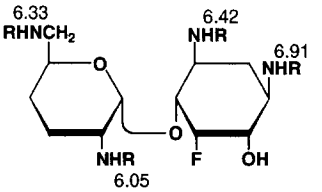
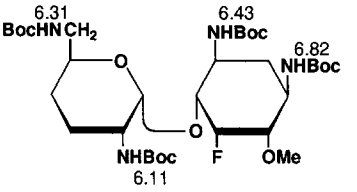


24 R= H

25 R= Boc



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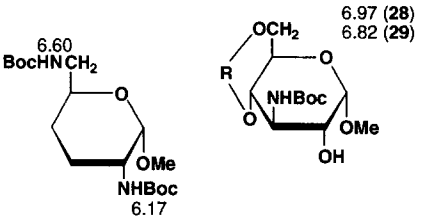
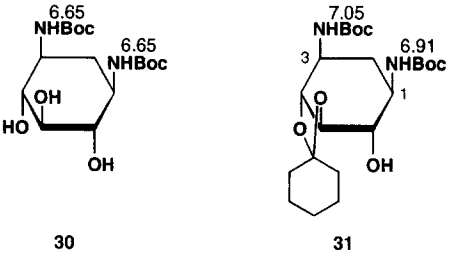


27

28 R= H, H

29 R= $C(CH_2)_5$



30 31

The BocN*H*-2′ signals were roughly divided into two groups resonating at δ 6.6–6.75 (compounds **2**–**4**, **14**, and **23**) and ~ 6.0 (the other compounds including **25** and **26**). The main difference in structure between the two groups was again the substituent at C-5 (HO-5*eq* or F-5*ax*), and this suggests that BocN*H*-2′ $\cdots$ *O*H-5 hydrogen bonding [13] occurs in the former group of compounds, deshielding the NH protons. This was also supported by energy minimum conformations of related compounds calculated by MM2UEC (which show the states of isolated molecules in vacuum at room temperature; see Fig. 1 and Table 3). In these calculations, corrections for

Table 3
Conformational analysis [a] by MM2UEC in some Boc derivatives

Compound	2	3	4	5	6	8	10	11	14
Boc *NH*-1									
H–O-6	3.09 (0.03)	3.15 (0.02)	3.16	3.13 (0.03)	3.06	3.35	3.36	3.24	3.25 (0.05)
N–*H*–O-6	64.5 (−2.3)	63.0 (−2.1)	61.5	62.3 (−1.3)	65.9	53.8	52.9	62.8	58.5 (−3.1)
H–O-2″	2.13 (0.05)	2.13 (0.05)	2.17	2.17 (0.01)	2.14	4.17	5.48	2.01	2.32 (0.09)
N–*H*–O-2″	119.1 (−2.4)	123.3 (−4.2)	118.2	119.5 (−1.6)	128.8	86.6	84.6	124.7	106.4 (−2.5)
Boc *NH*-3									
H–O-4	3.40 (−0.11)	3.42 (−0.07)	3.40	3.36 (−0.06)	3.33	3.28	3.32	3.36	3.37 (−0.05)
N–*H*–O-4	57.2 (4.0)	55.9 (2.9)	56.8	60.3 (2.0)	62.0	66.0	61.9	61.0	57.7 (2.1)
H–O-5′	3.60 (−0.24)	3.65 (−0.17)	3.59	3.41 (−0.09)	3.30	3.14	3.30	3.37	3.49 (−0.11)
N–O-5′	3.24 (−0.13)	3.27 (−0.10)	3.24	3.09 (−0.04)	3.01	2.88	3.05	3.05	3.18 (−0.24)
N–*H*–O-5′	61.3 (6.0)	60.6 (3.6)	62.0	63.3 (2.9)	64.7	66.2	66.9	63.4	63.8 (−4.0)
Boc *NH*-2′									
H–O-4	2.40 (0.06)	2.42 (0.05)	2.52	2.69 (−0.05)	2.64	2.73	2.63	2.65	2.45 (0.02)
N–*H*–O-4	92.5 (−2.7)	92.3 (−2.3)	87.0	80.0 (2.8)	82.1	79.7	83.0	81.7	90.0 (−0.8)
H–O-5(F-5)	2.16 (0.04)	2.13 (0.05)	2.18	4.25 (−0.10)	4.27	4.48	4.14	4.28	2.18 (0.03)
N–*H*–O-5(F-5)	152.1 (−7.1)	153.4 (−6.4)	141.1	116.0 (4.6)	118.1	113.4	121.6	117.3	145.4 (−1.7)
Boc *NH*-6′									
H–O-5′	2.36 (0.04)	2.36 (0.03)	2.37	2.39 (0)	2.39	2.42	2.42	2.39	2.39 (0.04)
N–*H*–O-5′	98.1 (−1.8)	98.3 (−1.2)	98.0	97.6 (−0.7)	97.4	96.6	96.2	97.6	96.8 (−1.6)
H–Boc-3(*t*-Bu*O*)	3.32 (−0.29)	3.38 (−0.16)	3.30	3.27 (−0.13)	3.20	3.15	3.08	3.29	3.13 (−0.14)
N–*H*–Boc-3(*t*-Bu*O*)	149.7 (2.9)	147.0 (2.5)	148.2	144.6 (3.3)	144.1	144.4	148.1	142.5	153.3 (4.4)
H–Boc-3(C=*O*)	2.01 (0.04)	2.01 (0.03)	2.01	2.01 (0.03)	2.01	2.01	2.02	2.01	2.01 (0.03)
N–*H*–Boc-3(C=*O*)	159.4 (−5.3)	162.1 (−2.3)	160.4	159.4 (−1.5)	157.0	154.9	154.3	158.9	155.4 (−4.2)
Boc *NH*-3″									
H–O-2″	2.51 (0)	3.10 (−0.20)	2.83	2.84 (−0.04)	4.00	3.19	3.59	3.12	2.64 (0)
N–*H*–O-2″	88.3 (0.6)	62.9 (9.4)	74.8	74.4 (1.8)	22.2	18.7	42.6	60.6	84.9 (0.2)
H–O-4″	3.92 (0)	3.53 (0.14)	3.72	3.71 (0.02)	2.46	3.03	3.73	3.62	3.87 (−0.02)
N–O-4″	3.04 (−0.01)	2.94 (0.01)	2.97	2.97 (−0.01)	2.70	3.02	2.91	2.96	3.00 (−0.02)
N–*H*–O-4″	26.0 (−0.4)	47.7 (−8.5)	37.1	37.7 (−2.1)	92.1	79.8	31.2	43.0	27.8 (−0.4)
H–Boc-1(*t*-Bu*O*)	2.25 (0.06)	2.25 (0.03)	2.25	2.26 (0.03)	3.64	6.59	6.31	2.23	6.87 (−0.03)
N–*H*–Boc-1(*t*-Bu*O*)	143.3 (−5.8)	136.8 (2.0)	140.1	139.8 (−0.7)	79.7	20.6	101.4	133.0	121.0 (−2.2)
HO-5									
H–O-6″	3.55 (0.19)								3.49 (0.22)
O–*H*–O-6″	171.3 (−11.2)								169.9 (−10.5)
HO-6″									
H–O-5	3.82 (0.08)								3.74 (0.11)
O–*H*–O-5	130.6 (7.0)								131.5 (7.4)

[a] Atom–atom distances (Å) are shown by single bar connecting atoms, and angles (degree) for three atoms are shown by two bars connecting three atoms. The values in parentheses are for those in a medium of dielectric constant 12.3 at room temperature (see text).

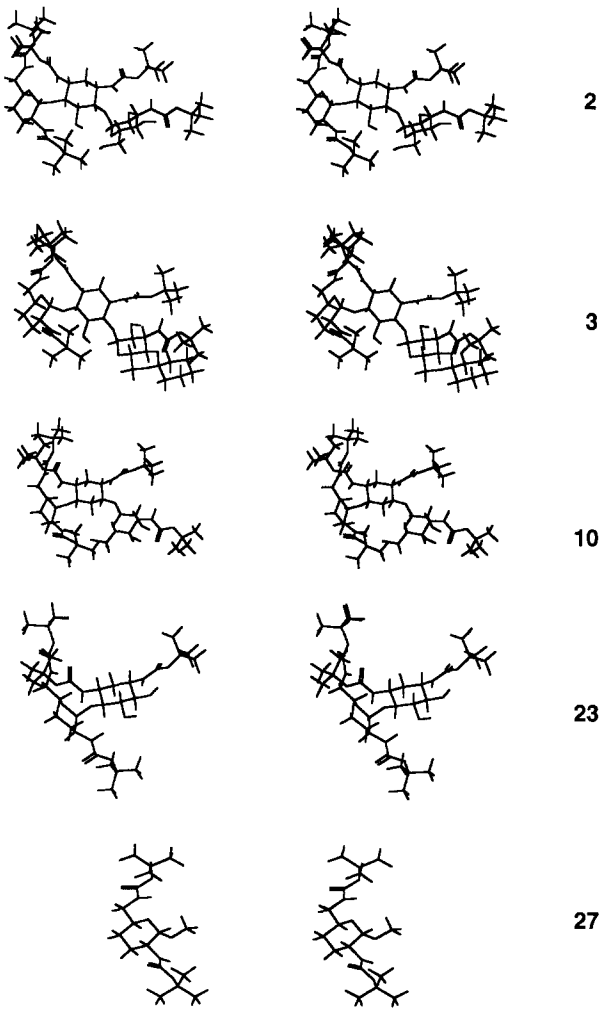


Fig. 1. Stereoviews of **2**, **3**, **10**, **23**, and **27** in the minimum-energy obtained by MM2UEC.

solvent [14,15] by specifying the dielectric constant of pyridine (12.3) were not performed, because the conformations obtained by both calculations showed no substantial difference (see Table 3). In **2**, **3**, and **4**, the N*H*-2′–*O*-5 distances were in the typical range (~ 2.15 Å) for hydrogen-bonding [16–18], and the large N-2′–H-2′–O-5 angles (140–155°) supported this [16,18]. A hydrogen-bonding for N*H*-2′ · · · *O*-4 expected from the location of vicinal NH-2′,O-4 groups, however, should be much weaker, because the distances (2.4–2.5 Å) are relatively large and the N-2′–H-2′–O-4 angles are small (~ 90°) (Table 3). A careful check indicated, however, that there was a small shift-difference ($\Delta\delta$ ~ 0.2) between the group **2** and **14** (both have 4″,6″-diols; δ ~ 6.6) and the group of **3** and **4** (both have a 4″,6″-*O*-cyclohexylidene group; δ ~ 6.75). This observation suggests that the free HO-6″ influences the BocN*H*-2′ shift. A conformational check of **2**, however, indicated that the BocN*H*-2′–*O*H-6″ distance was large, precluding the possibility of a close relationship of these atoms. Instead, a comparatively short distance was observed for *H*O-5–*O*-5″(ring O) (2.66 Å) with a large O-5–H-5–O-5″ angle (136°), the distance being shorter than the corresponding one for **3** (2.85 Å); however, this finding gave no clue for the problem. To examine the BocN*H*-2′ shift-value further, the 2,6-di-*N*-Boc derivative **27** of methyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranoside was prepared. Its BocN*H*-2 shift (δ 6.17) was slightly outside the expected value (δ 6.0), and this difference ($\Delta\delta$ ~ 0.17) is ascribed to the effect of solvent as described later.

For BocN*H*-3″, the shifts were mainly influenced by the vicinal 2″-substituent although a slight disturbance by the neamine moiety was observed. In the 4″,6″-*O*-cyclohexylidene derivatives, the downfield shifts ($\Delta\delta$ ~ 0.2) in **4** and **5** (δ ~ 6.95) from the standard values for **3**, **6**, **18–20** (δ ~ 6.75) may be explained by influence of the electron-withdrawing 2″-*O*-acetyl group. In these compounds, no appreciable N*H*-3″ · · · O-2″(or 4″) hydrogen-bonding is expected, considering the large distances (2.5–4 Å) and small N–H-3″–O-2″ angles (Table 3). In **2** and **14**, which lack the 4″,6″-*O*-cyclohexylidene group, however, the shift-values were slightly larger ($\Delta\delta$ ~ 0.2) than the standard value (δ 6.75). This difference could be ascribed to the free HO-4″ group, which may form a hydrogen-bond with the Boc(C=*O*)-3″ atom {see **2** in Fig. 1; the distance for Boc(C=*O*) · · · *H*O-4″ is 2.10 Å; ∠O-4″–O*H*-4″ · · · *O*=C(O′Bu-3″) 132°, and ∠O*H*-4″ · · · *O*(=C)–*C*=O(O′Bu-3″) 103°, the last value being suitable for hydrogen-bonding [16,19]}. A similar situation was also seen in the BocN*H*-1 shifts for the pair **25** and **26**, the latter compound being unable to form the hydrogen bond in question. The upfield shift of N*H*-3″ ($\Delta\delta$ ~ 0.45) by 2″-epimerization (**2** → **10**) may be attributed in part to the HO-2″*ax* group, which should withdraw bonding electrons much less than the corresponding HO-2″*eq* group. It should be noted that the expected hydrogen-bonding for BocN*H*-3″ · · · O-2″*ax* in **10** is not feasible because of the large distance (3.59 Å) in the energy-minimum conformation of **10**. The large downfield shifts (δ ~ 7.3; $\Delta\delta$ 0.55) of N*H*-3″ in the 2″-oxo compounds (**11** and **21**) may be attributed to the strong electron-withdrawing effect of the carbonyl group. To clarify the BocN*H*-3″ shifts further, the *N*-*t*-butoxycarbonyl derivative [20] (**28**) of methyl 3-amino-3-deoxy-α-D-

glucopyranoside and its 4,6-*O*-cyclohexylidene derivative (**29**) were prepared. The respective shift-differences between **2** and **28**, and between **3** (or **6**) and **29** were only small ($\Delta\delta$ 0.02–0.06), indicating that substitution of the neamine moiety (at C-1″) with a MeO group gave no substantial change. On the other hand, 4,6-*O*-cyclohexylidenation (**28** → **29**) gave an expected upfield-shift ($\Delta\delta$ 0.15) as described before.

In the case of NH-1, the situation is somewhat complex. At first, changing the 1-amino-protecting group from Boc to (*S*)-4-amino-2-hydroxybutanoyl (AHB) (**2** → **14**, **6** → **18**, for example) markedly lowered the shifts ($\Delta\delta \sim 1$). This may be attributed to the stronger electron-withdrawing effect of the latter group. Downfield shifts of the AHB-N*H*-1 protons accompanied by conversions of the functional group at C-2‴ from OH (**14** and **18**; $\delta \sim 8.1$) to OAc (**19** and **21**; δ 8.2–8.35) and to =O (**20**, δ 8.6) are consistent with the order of electron-withdrawing ability (OH < OAc < =O). In regard to the BocN*H*-1 shifts, conversion of HO-2″ to AcO-2″ (**2** or **3** → **4**; **6** → **5**) brought about upfield shifts ($\Delta\delta$ 0.2–0.3). This may be explained in that the hydrogen bonding between BocN*H*-1 · · · *O*H-2″ is weakened by the electron-withdrawing *O*-acetylation; the change in length of N*H*-1–O-2″ (2.13 → 2.17 Å) (Table 3) also supports the prediction. A slight downfield shift of BocN*H*-1 in the 2″-oxo compound **11** (δ 7.09) relative to that for the HO-2″ compound **6** (δ 6.97) may be attributed to the strengthened hydrogen-bonding between N*H*-1 · · · O=C-2″, consequent on the shortening of length (Δ 0.13 Å). However, the N*H*-1 shift, together with those of the other compounds, is considered to be affected by pyridine to a certain extent as described later.

Next we discuss the BocN*H*-1 shifts in disaccharides **23**, **25**, and **26**. A small upfield shift ($\Delta\delta \sim 0.15$) in **23** relative to the structurally related dibekacin derivatives **2** and **3** is attributed to the removal of 3-amino-3-deoxy-D-glucose moiety. The shift-changes [δ 6.81 (**23**) → 6.91 (**25**) → 6.82 (**26**)] accompanied by replacement of HO-5*eq* of **23** to F-5*ax* ($\Delta\delta$ +0.1), and HO-6 to MeO-6 ($\Delta\delta$ −0.1) are attributed, respectively, to 5-epi-5-fluorination and 6-*O*-methylation, as described before. However, in contrast to the foregoing rather understandable shift-changes, the spatial dispositions of the BocNH-1 groups in **25** and **26**, relative to their neighboring groups, as determined by MM2UEC, differ considerably from each other (see Fig. 2). Such conformational changes based on computation do not, however, seem to reflect the actual BocN*H*-1 shift-values in pyridine-d_5. The origin of the shift changes in these compounds may be ascribed to the axial fluorine at C-5. In **23** having a HO-5*eq* group, the C=O in the BocNH-1 group is located in the lower face of the average plane defined by the 2-deoxystreptamine-ring (C=*O* · · · *H*O-6, 2.13 Å; C=*O* · · · *H*-6, 4.07 Å), whereas in **25**, the C=O group is rotated to the upper face to be situated in a position close to both H-6 (2.32 Å) and *H*O-6 (2.12 Å), possibly by a dipole-pairing with the former (C=$O^{\delta-}$–$^{\delta+}$H-6) and hydrogen bonding with the latter (C=*O* · · · *H*O-6). It was surmised that the positive charge in H-6 in **25** was brought about by the electron-withdrawing F-5*ax* situated in an antiperiplanar position. In **26**, the C=O group is also located in a similar position (C=O · · · H-6, 2.44 Å).

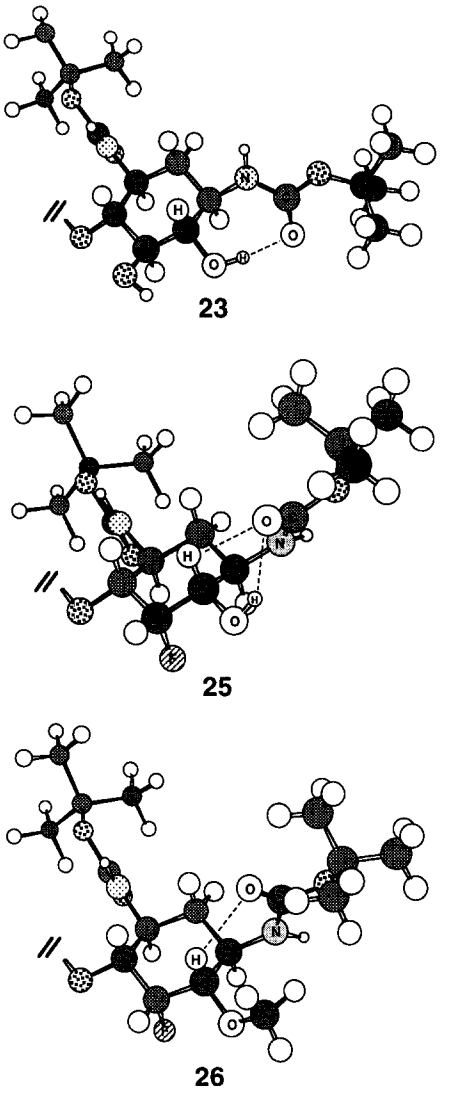


Fig. 2. Conformations of **23**, **25**, and **26** in the minimum-energy obtained by MM2UEC.

Finally, we briefly compare the absolute chemical shifts for different positions. As the shift of BocN*H*-6 in monosaccharide **27** is exceptionally high (δ 6.60), compared to the usual shifts of the same protons ($\delta \sim 6.3$) (a similar tendency is also observed in BocN*H*-2 of **27**, although to a lesser extent, as already described), the BocN*H*-6 proton of **27** is suspected to interact with pyridine [21] more strongly than those of other more complex compounds, by the absence of a bulky BocNH-3 group located nearby (see Fig. 1). The approach of pyridine to form a BocN*H* $\cdots$ $N C_5H_5$ bond will increase the BocN*H*-shift because of an enhanced exchange-rate [22,23] of the proton adjacent to the pyridine nitrogen. To examine the possibility that this kind of proton-exchange enhancement would occur in BocN*H*-1 and -3, a simple derivative of 2-deoxystreptamine, namely 1,3-bis(*N*-*tert*-butoxycarbonyl)-2-deoxystreptamine (**30**) was prepared. The BocN*H* shift (δ 6.65) was considerably different from those for both the BocN*H*-1 (δ 6.81 in **23**, for example) and BocN*H*-3 ($\delta \sim 6.30$) (that is, the shift in **30** was smaller than that for N*H*-1 and larger than that for N*H*-3). Does this mean that the approach of pyridine is enhanced at NH-3, and suppressed at NH-1?

To clarify this question, another reference compound, a 4,5(5,6)-*O*-cyclohexylidene derivative (**31**) of **30** was prepared. This compound showed two BocN*H* shifts (δ 6.91 and 7.05), which were assigned to NH-1 (the position to have HO-6 in the vicinity) and NH-3, respectively, by steric comparison with other compounds (see chemical formula), the NH-1 shift thus determined being larger than that for **30** by $\Delta\delta$ 0.26 but appropriate for an conventional BocN*H*-1 shift. As the steric surroundings near the BocN*H*-1 groups in both **30** and **31** are considered to be almost the same, the shift-difference may be attributed to the difference in mobility (molecular motion) [24] of the compounds in pyridine-d_5, that is, **30** is in a state of more active motion, thus weakening the approach of pyridine. Raising the temperature results in upfield-shift [25] in all BocN*H* protons of all compounds (by weakening the solvation, the rate being different for respective positions), in support of the foregoing conclusion.

Summarizing all discussions thus far, the conclusion may be drawn that BocN*H*-6′ is comparatively protected from the approach of pyridine because of the presence of the bulky BocNH-3 group nearby, and the BocN*H*-3 is likewise protected by the 2,6-diamino-2,3,4,6-tetradeoxy-α-D-glucose moiety bearing a BocN group at C-2 (see Fig. 1). In contrast, it is predicted that both BocN*H*-1 and -3″ hydrogens are exposed to pyridine due to the absence of bulky groups nearby, giving low-field resonances (δ 6.8–7.0). However, a check of minimum-energy conformations (Fig. 1) calculated by MM2UEC suggests that the BocNH-1 and -3″ groups are in close proximity and are shielded from pyridine. This problem requires further discussion; however, we suppose that the energy surface determined by interaction of BocNH-1 and the 3-amino-3-deoxy-D-glucose moiety (bearing the BocNH-3″ group) will be smooth because of the parallel dispositions of the two BocNH groups. As a result, the energy-minimum conformations as shown in Fig. 1 would readily be disrupted at 80 °C in pyridine. In contrast, the energy-minimum conformation for the segment formed by BocN-3 and the 2,6-diamino-2,3,4,6-tetradeoxy-D-glucose moiety (as shown **2** in Fig. 1, for example) may be resistant to the motional changes due to crosswise overlapping of the BocN-3 group onto the D-glucose moiety. However, the consistent (compare the values for **2** and **29**) and relatively low-field shifts ($\delta \sim 6.75$) of BocN*H*-3″ may be explained alternatively by the presence of *two* vicinal, electron-withdrawing hydroxyl groups (or the equivalents) at C-2″ and 4″, which work as a barrier against pyridine (and as a result give consistent values) and give lowfield shifts. Similarly, the BocN*H*-2′ hydrogens (only for those compounds having a F-5*ax* atom in the adjacent moiety, as in **5** and **25**), or the BocN*H*-2′ hydrogen in **27**, are presumed to be protected from pyridine by the axial glycosyloxy or CH_3O group (although the latter is slightly less effective than the former).

In conclusion, the chemical shifts of BocN*H* protons that resonate over a narrow range ($\delta \sim 6.0$–~ 7.0 in pyridine-d_5 at 80 °C) are sensitive reflections (as described in detail in the text) of the stereochemical and electrochemical surroundings of the BocNH groups, in respect of the electronic inductive-effect of nearby functional groups, degree of hydrogen-bonding, and the ease of solvent approach (in this case, pyridine-d_5). Studies aiming to find the chemical and physical factors determining the proton-shifts of BocN*H* hydrogens have not been reported before, although there have been studies in protein chemistry similar in their fundamental approach [26–30]. One possibility drawn from the last conclusive factor is that H_2N-6′ is protected from acetylation by resistant bacteria because of overlap of the BocNH-3 group onto the H_2N-6′ position; indeed this was confirmed by the MM2UEC calculation of 3-*N*-Boc-dibekacin, which showed a conformation similar to that of **2**

(see Fig. 1) in the 3′,4′-dideoxyneamine portion, suggesting the possibility that some weakly bonded 3-*N*-acyl(or urethane-type) derivatives might be useful as prodrugs (this aspect is now under study).

Antibacterial activity.—The 2″-epimer (**9**) of 5-deoxy-5-epi-5-fluorodibekacin [6] showed activity less than one-tenth of that of the parent compound against both normal and resistant bacteria. The corresponding 2″-oxo derivative (as its hydrate form; **12**) showed further decreased activity (see Experimental section). Attachment of an (*S*)-4-amino-2-hydroxybutanoyl group at N-1 of **12** restored the activity somewhat, but it was still far smaller than that for arbekacin. This suggests clearly that the equatorial HO-2″ is essential for activity. This fact is also supported by the weak activity of 2″-deoxykanamycins (unpublished data).

3. Experimental

General methods.—Melting points were determined on a Kofler block and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded with a Jasco A-202 grating spectrophotometer. FAB-mass spectra were recorded with a Jeol SX-102 spectrometer, using glycerol as the matrix. NMR spectra were measured with a Bruker AC 250P (^{1}H at 250, ^{13}C at 62.9, and ^{19}F at 235.4 MHz, all at 27 °C) or AMX 500 (^{1}H at 500, ^{13}C at 125.8, and ^{19}F at 470.5 MHz, all at 30 °C) spectrometers. Chemical shifts (δ) of ^{1}H, ^{13}C, and ^{19}F spectra were recorded downfield from internal Me_4Si (for ^{1}H and ^{13}C) and internal Freon 11 (for ^{19}F), and confirmed, if necessary, by shift-correlated 2D spectra. TLC was performed on Kieselgel 60 F_{254} (Merck) and column chromatography, on Wakogel C-200, unless otherwise stated.

Computation.—All calculations were performed on a Sun SPARC Station 2 with Materia Version 3.1 (Teijin System Technology, Ltd., Japan) using MM2UEC (by Yoshiyuki Hase), a modified version based on MM2(87) by N.L. Allinger, taking account of hydrogen bonding and two lone-pairs on sp^3-hybridized oxygen.

*1,3,2′,6′,3″-Pentakis(*N*-tert-butoxycarbonyl)dibekacin* (**2**).—To an aq solution (70 mL) of **1** (free base, 5.00 g, 11.1 mmol) and Et_3N (15.4 mL, 110 mmol) was added di-*t*-butyl dicarbonate [$(t\text{-BuOCO})_2O$, 14.5 g, 66.4 mmol] in 1,4-dioxane (90 mL), and the mixture was stirred for 1.5 h at 60 °C. After addition of aq 28% NH_3 (6 mL) followed by stirring for 30 min at 60 °C, the mixture was concentrated. Addition of water gave a precipitate, which was thoroughly washed with water and dried (~ 10 Pa, 1 day at 25 °C under P_2O_5) to give **2** as an amorphous powder (9.52 g, 90%), $[\alpha]_D^{22}$ + 66° (*c* 1, DMF) (lit [17] showed no data); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.43 (9 H), 1.48 (18 H), 1.49 (9 H), 1.52 (9 H) (each s, 5 Boc), 3.47 (m, 2 H, H-6′a,b), ~ 3.75 (unresolved m, 5 H, H-1, 3, 4, 5, 6), 3.85 (m, 1 H, H-2′), 4.28 (m, 1 H, H-3″), 5.32 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.38 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′) (H-1′ and -1″ were discriminated by ^{1}H–^{1}H COSY). Anal. Calcd for $C_{43}H_{77}N_5O_{18}$: C, 54.24; H, 8.15; N, 7.36. Found: C, 54.45; H, 8.35; N, 7.43.

*4″,6″-O-Cyclohexylidene-1,3,2′,6′,3″-pentakis(*N*-tert-butoxycarbonyl)dibekacin* (**3**).—To a solution of **2** (9.11 g, 9.57 mmol) in DMF (46 mL) in a distilling flask were added 1,1-dimethoxycyclohexane (2.8 mL, 194 mmol) and anhyd *p*-toluenesulfonic acid (330 mg) and the mixture was stirred for 2 h at 50 °C and ~ 4 kPa, during which time a small amount of solvent (including MeOH) was distilled off. The mixture was poured into aq $NaHCO_3$ (std, 1 L) and the resulting precipitate, after washing with water thoroughly, was dried as described for **2** to give **3** as a solid (10.05 g, quant). An analytical sample was prepared by chromatography with 30:1 $CHCl_3$–MeOH, $[\alpha]_D^{22}$ + 50° (*c* 1, $CHCl_3$) (lit [17], no data reported), IR (KBr) 1700 cm^{-1} [Boc(CO)]; ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.46 (9 H), 1.48 (18 H), 1.49 (9 H), 1.51 (9 H) (each s, 5 Boc), 3.48 (m, 2 H, H-6′a,b), 3.77 (m, 1 H, H-3), 3.78 (m, 1 H, H-4), 3.80 (m, 1 H, H-1), 3.88 (t, 1 H, $J_{1,6} = J_{5,6}$ 10 Hz, H-6), 3.90 (m, 1 H, H-2′), 4.15 (dt, 1 H, $J_{2'',3''} = J_{3'',4''}$ 10, $J_{3'',NH}$ 8 Hz, H-3″), 5.40 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.41 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″). ^{13}C NMR (pyridine-d_5 at 80 °C): δ 25.14 (C-3′), 28.22 (C-4′), 28.66, 28.70, 28.74 [$(CH_3)_3C(O)O$]; 35.59 (C-2), 45.59 (C-6′), 51.23 (C-1), 51.32 (C-3), 51.66 (C-2′), 55.41 (C-3″), 62.36 (C-6″), 66.36 (C-5″), 68.68 (C-5′), 71.31 (C-4″), 71.84 (C-2″), 75.90 (C-5), 83.68 (C-4), 84.43 (C-6), 100.18 (C-1″), 100.73 (C-1′). Anal. Calcd for $C_{49}H_{85}N_5O_{18} \cdot H_2O$: C, 56.04; H, 8.35; N, 6.69. Found: C, 56.36; H, 8.40; N, 6.44.

*2″-O-Acetyl-4″,6″-O-cyclohexylidene-1,3,2′,6′,3″-pentakis(*N*-tert-butoxycarbonyl)dibekacin* (**4**).—A solution of **3** (monohydrate, 8.90 g, 8.47 mmol) and Ac_2O (8.8 mL, 86.2 mmol) in pyridine (180 mL) was kept for 16 h at room temperature. After concentration, conventional work-up gave **4** as an amorphous

powder (8.97 g, 98%), $[\alpha]_D^{21}$ + 64° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.482 (27 H), 1.510 (9 H), 1.515 (9 H) (each s, 5 Boc); 2.18 (s, 3 H, Ac), 3.43–3.54 (m, 2 H, H-6′a,b), 3.71–3.75 (m, 2 H, H-1, 3), 3.91 (br s, 1 H, H-2′), 4.45 (q, 1 H, $J \sim 10$ Hz, H-3″), 5.38 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.44 (dd, 1 H, $J_{1'',2''}$ 3.5, $J_{2'',3''}$ 10.5 Hz, H-2″), 5.61 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″). Anal. Calcd for $C_{51}H_{87}N_5O_{19}$: C, 57.02; H, 8.16; N, 6.52. Found: C, 56.95; H, 8.16; N, 6.75.

*2″-O-Acetyl-4″,6″-O-cyclohexylidene-5-deoxy-5-epi-5-fluoro-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)dibekacin* (**5**).—To a cold (−20 °C) solution of **4** (8.77 g, 8.16 mmol) in CH_2Cl_2 (175 mL), DAST (5.4 mL, 40.9 mmol) was added and the solution was kept for 6 h at the temperature. Aq $NaHCO_3$ (std, 500 mL) was added under vigorous stirring and the product was extracted with CH_2Cl_2. Chromatography (30:1 $CHCl_3$–MeOH) of the crude product gave **5** as an amorphous powder (7.51 g, 86%), HPTLC [Kieselgel 60 F_{254} (E. Merck), 30:1 $CHCl_3$–MeOH]: R_f 0.55 (cf **4**: R_f 0.6), $[\alpha]_D^{22}$ + 65° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.47 (9 H), 1.48 (18 H), 1.50 (9 H), 1.54 (9 H) (each s, 5 Boc), 2.11 (s, 3 H, Ac), 3.34 (t, 2 H, $J_{5',6'a(6'b)} = J_{6'a(6'b),NH}$ 6 Hz, H-6′a,b), 3.91 (m, 1 H, H-2′), 4.05 (m, 1 H, H-1), 4.30 (m, 1 H, H-3), 4.36 (dt, 1 H, $J_{2'',3''} = J_{3'',4''}$ 10, $J_{3'',NH}$ 9 Hz, H-3″), 5.23 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.43 (dd, 1 H, $J_{1'',2''}$ 4, $J_{2'',3''}$ 10 Hz, H-2″), 5.44 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5), 5.47 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.52 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 29 Hz, F-5). Anal. Calcd for $C_{51}H_{86}FN_5O_{18}$: C, 56.91; H, 8.05; F, 1.77; N, 6.51. Found: C, 56.84; H, 7.98; F, 1.51; N, 6.59.

*4″,6″-O-Cyclohexylidene-5-deoxy-5-epi-5-fluoro-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)dibekacin* (**6**).—To a solution of **5** (4.00 g, 3.72 mmol) in MeOH (35 mL), 1 M NaOMe in MeOH (4 mL) was added and the solution was kept for 30 min at room temperature. Conventional post-treatment gave **6** as an amorphous powder (3.85 g, 98%), $[\alpha]_D^{21}$ +64° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.44, 1.46, 1.49, 1.51, 1.55 (each s of 9 H, 5 Boc), 3.36 (*ABX*, 2 H, $J_{5',6'a}$ 5, $J_{5',6'b}$ 6, $J_{6'a,6'b}$ 14 Hz, H-6′a,b), 3.90 (m, 1 H, H-2′), 3.95 (dd, 1 H, $J_{3,4}$ 10, $J_{4,F}$ 27 Hz, H-4), 4.04 (dd, 1 H, $J_{1,6}$ 10, $J_{6,F}$ 27 Hz, H-6), 4.08 (dt, 1 H, $J_{2'',3''} = J_{3'',4''}$ 10, $J_{3'',NH}$ 8 Hz, H-3″), 4.17 (m, 1 H, H-1), 4.34 (m, 1 H, H-3), 5.25 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.29 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), 5.46 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5), 6.28–6.33 (br, 1 H, NH-6′). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.48 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 27 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 80 °C): δ 24.87 (C-3′), 28.64, 28.71 [$(CH_3)_3C(O)O$]; 34.89 (C-2), 45.60 (C-6′), 48.42 (d, $J_{C,F}$ 3.5 Hz, C-3), 49.53 (d, $J_{C,F}$ 5 Hz, C-1), 50.34 (C-2′), 55.79 (C-3″), 62.33 (C-6″), 66.30 (C-5″), 68.57 (C-5′), 70.98 (C-4″), 71.92 (C-2″), 76.75 (d, $J_{C,F}$ 19 Hz, C-4), 81.06 (d, $J_{C,F}$ 18 Hz, C-6), 90.17 (d, $J_{C,F}$ 183 Hz, C-5), 95.46 (C-1′), 102.64 (C-1″). Anal. Calcd for $C_{49}H_{84}FN_5O_{17} \cdot H_2O$: C, 55.93; H, 8.24; F, 1.81; N, 6.66. Found: C, 56.10; H, 8.03; F, 1.52; N, 6.81.

*2″,3″-O,N-Carbonyl-4″,6″-O-cyclohexylidene-5-deoxy-5,2″-diepi-5-fluoro-1,3,2′,6′-tetrakis(*N-tert-*butoxycarbonyl)dibekacin* (**8**).—To an ice-cold solution of **6** (monohydrate, 491 mg, 0.48 mmol) in 3:1 CH_2Cl_2–pyridine (10 mL), $(CF_3SO_2)_2O$ (0.24 mL, 1.43 mmol) was added, and the solution was kept for 30 min at the same temperature. In TLC (20:1 $CHCl_3$–MeOH), a single spot of R_f 0.6 (**7**; cf. **6**: R_f 0.3) appeared. Addition of water (0.13 mL) followed by concentration gave a residue, which was dissolved in $CHCl_3$ and the solution was washed with aq $NaHCO_3$ (std), dried (Na_2SO_4), and concentrated. The crude **7** was dissolved in DMF (10 mL), NaOAc (180 mg) was added, and the mixture was stirred for 4 h at 80 °C. Addition of water (200 mL) gave a precipitate, which was washed thoroughly with water, and dried to give **8** as an amorphous powder (402 mg, 90%), TLC (20:1 $CHCl_3$–MeOH): R_f 0.3, $[\alpha]_D^{21}$ +53° (*c* 1, $CHCl_3$), IR (KBr): 1700 [Boc(CO)], 1770 cm^{-1} (carbamate); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.49, 1.50, 1.52, 1.55 (each s of 9 H, 4 Boc), 3.38 (m, 2 H, H-6′a,b), 3.80 (dd, 1 H, H-3″), 3.92 (m, 1 H, H-2′), 4.27 (m, 1 H, H-1), 4.37 (m, 1 H, H-3), 4.82 (d, 1 H, H-2″), 5.31 (d, 1 H, H-1′), 5.39 (br d, 1 H, H-5), 5.56 (br s, 1 H, H-1″); $J_{1',2'}$ 3.5, $J_{1'',2''} \sim 0$, $J_{2'',3''}$ 6.5, $J_{3'',4''}$ 13, $J_{5,F}$ 53 Hz. ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.84 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 27 Hz, F-5). Anal. Calcd for $C_{45}H_{74}FN_5O_{16}$: C, 56.29; H, 7.77; F, 1.98; N, 7.29. Found: C, 56.10; H, 7.61; F, 1.70; N, 7.10.

5-Deoxy-5,2″-diepi-5-fluorodibekacin (**9**).—An ice-cold solution of **8** (58.8 mg, 0.06 mmol) in aq 95% CF_3CO_2H (1.2 mL) was kept for 2 h. Concentration gave a residue, which was dissolved in aq 1 M NaOH (1.1 mL) and heated for 1.5 h at 80 °C. After neutralization with Dowex 50W-X2 (H^+ form, 200–400 mesh, ~2 mL), the mixture was poured into a column containing the same fresh resin (2 mL). After the column was washed with water, elution with aq 1 M NH_3 gave ninhydrin-positive fractions, which were combined and concentrated to give **9** as a solid (29.4 mg, 93% as monocarbonate), TLC (4:7:2:7 BuOH–

EtOH–$CHCl_3$–aq 17% NH_3): $R_{f,\text{dibekacin}}$ 0.7, $[\alpha]_D^{22}$ +107° (*c* 1, H_2O); ^1H NMR (D_2O–DCl, pD 3): δ 1.65 (dq, 1 H, H-4′*ax*), 1.96 (m, 1 H, H-4′eq), 2.03 (q, 1 H, H-2ax), 2.08–2.18 (m, 2 H, H-3′*ax*, 3′*eq*), 2.64 (dt, 1 H, H-2*eq*), 3.13 (dd, 1 H, H-6′a), 3.31 (dd, 1 H, H-6′b), 3.61–3.67 (m, 1 H, H-2′), 3.64 (dd, 1 H, H-3″), 3.75 (dd, 1 H, H-6″a), 3.80 (t, 1 H, H-4″), 3.82–3.93 (m, 2 H, H-1, 3), 3.97–4.02 (m, 2 H, H-5″, 6″b), 4.15 (m, 1 H, H-5′), 4.27 (dd, 1 H, H-4 or 6), 4.34 (dd, 1 H, H-2″), 4.38 (dd, 1 H, H-6 or 4), 5.24 (d, 1 H, H-1″), 5.51 (d, 1 H, H-1′), 5.80 (br d, 1 H, H-5); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 13, $J_{3,4} = J_{1,6}$ 11, $J_{4,F} = J_{6,F}$ 27, $J_{5,F}$ 51, $J_{1,2'}$ 4, $J_{3'ax,4'ax} = J_{4'ax,4'eq} = J_{4'ax,5'}$ 13, $J_{3'eq,4'ax}$ 5, $J_{5',6'a}$ 8, $J_{5',6'b}$ 3, $J_{6'a,6'b}$ 14, $J_{1'',2''}$ 2, $J_{2'',3''}$ 3, $J_{3'',4''}$ 10, $J_{4'',5''}$ 10, $J_{5'',6''a}$ 8, $J_{6''a,6''b}$ 12 Hz. ^1H NMR (26% ND_3 in D_2O) as expressed by Δδ {(δ's for **9**) – (corresponding δ's for 5-deoxy-5-epi-5-fluorodibekacin [6])}: 0 (H-2*ax*), 0.01 (H-2*eq*), 0.05 (H-5), 0 (H-1′), 0.01 (H-2′), 0.01 (H-4′*ax*), −0.01 (H-6′a), 0.02 (H-6′b), −0.05 (H-1″), 0.53 (H-2″), −0.17 (H-3″), 0.16 (H-4″), −0.01 (H-6″a), 0.04 (H-6″b); $J_{1'',2''}$ 1.5, $J_{2'',3''}$ 3 Hz (cf. 5-deoxy-5-epi-5-fluorodibekacin: $J_{1'',2''}$ 4, $J_{2'',3''}$ 10 Hz). ^{19}F NMR (D_2O–DCl, pD 3): δ −216.89 (dt, $J_{5,F}$ 52, $J_{4,F} = J_{6,F}$ 26 Hz, F-5). Anal. Calcd for $C_{18}H_{36}FN_5O_7 \cdot H_2CO_3$: C, 44.26; H, 7.43; F, 3.69; N, 13.59. Found: C, 44.24; H, 7.73; F, 3.61; N, 13.75.

*5-Deoxy-5,2″-diepi-5-fluoro-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)dibekacin* (**10**).—To an aq solution (0.56 mL) of **9** (monocarbonate, 40 mg, 0.078 mmol) and Et_3N (0.11 mL) was added di-*t*-butyl dicarbonate (102 mg, 0.47 mmol) in 1,4-dioxane (0.72 mL), and the mixture was treated as described for **2** to give **10** as an amorphous powder (73 mg, 99%), $[\alpha]_D^{24}$ +47° (*c* 1, DMF); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.43 (9 H), 1.44 (9 H), 1.49 (9 H), 1.50 (18 H) (each s, 5 Boc); ~ 1.65 (H-4′eq), 1.8–1.95 (m, 2 H, H-3′*ax*, 3′*eq*), 2.03 (q, 1 H, *J* 13 Hz × 3, H-2*ax*), 2.34 (dt, 1 H, *J* 4, 4, 13 Hz, H-2*eq*), 3.31 (t, 2 H, $J_{5'6'a(6'b)} = J_{6'a(6'b),NH}$ 5 Hz, H-6′a,b), 3.8–3.9 (m, 1 H, H-2′), 3.90 (dd, 1 H, *J* 10, 27 Hz, H-4), 4.05–4.30 (3 H, H-1, 5′, 6), 4.3–4.4 (1 H, H-3), 4.4–4.5 (2 H, H-2″, 3″), 5.36 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.42 (s, 1 H, H-1″), 5.82 (br d, 1 H, $J_{5,F}$ 53 Hz). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −214.47 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 27 Hz, F-5). Anal. Calcd for $C_{43}H_{76}FN_5O_{17}$: C, 54.13; H, 8.03; F, 1.99; N, 7.34. Found: C, 53.74; H, 8.20; F, 1.95; N 7.32.

*4″,6″-O-Cyclohexylidene-5,2″-dideoxy-5-epi-5-fluoro-2″-oxo-1,3,2′,6′,3″-pentakis(*N-tert-*butoxycarbonyl)dibekacin* (**11**).—A mixture of **6** (1.00 g, 0.95 mmol), PDC (730 mg, 1.94 mmol), and molecular sieves 3A (1.93 g) in CH_2Cl_2 (20 mL) was stirred for 5 h at room temperature. Ether (100 mL) was added, and the mixture was filtered with the aid of Celite and Silica Gel Merck 60G, and the bed was washed with ether. The combined solutions were concentrated to give, after drying over P_2O_5 in vacuo, **11** as a solid (780 mg, 80%), TLC (30:1 $CHCl_3$–MeOH, doubly developed): $R_{f,\text{compound 6}}$ 1.2, $[\alpha]_D^{22}$ +73° (*c* 1, $CHCl_3$), IR (KBr): 1700 [Boc(CO)], 1750 cm^{-1} (shoulder); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.467, 1.486, 1.489, 1.514, 1.554 (each s of 9 H, 5 Boc), 3.36 (*ABX*, 2 H, $J_{5',6'a}$ 5, $J_{5',6'b}$ 6, $J_{6'a,6'b}$ 14 Hz, H-6′a,b), 3.91 (m, 1 H, H-2′), 4.15 (m, 1 H, H-1), 4.33 (m, 1 H, H-3), 4.89 (dd, 1 H, $J_{3'',4''}$ 10, $J_{3'',NH}$ 8 Hz, H-3″), 5.26 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′), 5.36 (s, 1 H, H-1″), 5.45 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5), 6.29–6.35 (br, 1 H, NH-6′). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.84 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 27 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 27 °C; signals lower than δ 85 are shown): δ 90.43 (d, $J_{C,F}$ 182 Hz, C-5), 95.26 (C-1′), 99.85 [$(CH_2)_5C$], 102.58 (C-1″); 156.03, 156.34 (strong), 156.97 [each Boc(*C*O)], 196.49 (C-2″). Anal. Calcd for $C_{49}H_{82}FN_5O_{17}$: C, 57.02; H, 8.01; F, 1.84; N, 6.79. Found: C, 57.01; H, 7.98; F, 1.65; N, 6.80.

5-Deoxy-5-epi-5-fluoro-2″-hydroxydibekacin (**12**).—An ice-cold solution of **11** (259 mg, 0.25 mmol) in aq 95% CF_3CO_2H (2.5 mL) was kept for 3 h. Addition of ether (12 mL) gave a precipitate, which was filtered, washed with ether, and dried in vacuo to give a solid (~ 250 mg). An aq solution of the TFA salt was slowly passed through a column of Dowex 1-X2 resin (AcO^- form, 40 mL) with water, and the ninhydrin-positive fractions were combined and freeze-dried to give **12** as a solid (149 mg, 84% as tetraacetate of 2-hydrate), $[\alpha]_D^{24}$ +144° (*c* 1, H_2O); MS *m/z* 452.4 (minor) and 470.4 $(M+1)^+$, ^1H NMR (TFA salt in D_2O, pD ~ 3): δ 1.64 (dq, 1 H, H-4′*ax*), 1.93 (narrow m, 1 H, H-4′*eq*), 1.97 (q, 1 H, *J* 13 Hz × 3, H-2*ax*), 2.08 (narrow m, 2 H, H-3′*ax*, 3′*eq*), 2.63 (dt, 1 H, $J_{1,2eq} = J_{2eq,3}$ 5 Hz, H-2*eq*), 3.11 (dd, 1 H, *J* 8, 13 Hz, H-6′a), 3.26 (dd, 1 H, *J* 3, 13 Hz, H-6′b), 3.48 (d, 1 H, $J_{3'',4''}$ 10 Hz, H-3″), 3.61 (m, 1 H, H-2′), 3.72 (t, 1 H, H-4″), 3.74 (1 H, H-6″a), ~ 3.87 (m, 2 H, H-1, 3), 3.96 (1 H, H-6″b), 4.01 (1 H, H-5″), 4.11 (m, 1 H, H-5′), 4.20 (dd, 1 H, *J* 11, 26 Hz, H-4 or 6), 4.27 (dd, 1 H, *J* 11, 26 Hz, H-6 or 4), 5.00 (s, 1 H, H-1″), 5.48 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.78 (d, 1 H, $J_{5,F}$ 51 Hz, H-5). ^{19}F NMR (TFA salt in D_2O, pD ~ 3): δ −216.70 (dt, *J* 26, 26, 52 Hz, F-5). Anal. Calcd for $C_{18}H_{36}FN_5O_8 \cdot$

$4CH_3CO_2H$: C, 44.00; H, 7.39; F, 2.68; N, 9.87. Found: C, 44.22; H, 7.42; F, 2.60; N, 9.56.

*3,2′,6′,3″,4‴-Pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**14**).—To a solution of arbekacin (**13**) sulfate (683 mg base g^{-1}, 5.00 g, 6.18 mmol) in 1:1 1,4-dioxane–water (160 mL), were added $(t\text{-BuOCO})_2O$ (8.10 g, 37.1 mmol) and Et_3N (8.6 mL), and the solution was kept for 3.5 h at 60 °C. Work-up as described for **2** gave **14** as a solid (6.05 g, 91%), $[\alpha]_D^{23}$ +81° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.41 (9 H), 1.46 (9 H), 1.48 (18 H), 1.52 (9 H) (each s, 5 Boc) 3.48 (m, 2 H, H-6′a,b), 3.55 (m, 2 H, H-4‴a,b), 3.76 (m, 1 H, H-3), 3.78 (m, 1 H, H-2′), 4.00 (m, 1 H, H-1), 4.30 (m, 1 H, H-3″), 5.36 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.38 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′). Anal. Calcd for $C_{47}H_{84}N_6O_{20} \cdot H_2O$: C, 52.70; H, 8.09; N, 7.85. Found: C, 52.64; H, 7.96; N, 7.98.

*4″,6″-O-Cyclohexylidene-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**15**).—Compound **14** (monohydrate, 5.75 g, 5.37 mmol) in DMF (30 mL) was treated with 1,1-dimethoxycyclohexane (1.6 mL, 11.1 mmol) as described for **3** to give **15** as a solid (6.05 g, quant), $[\alpha]_D^{23}$ +52° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.45, 1.47, 1.48, 1.50, 1.52 (each s of 9 H, 5 Boc). Anal. Calcd for $C_{53}H_{92}N_6O_{20}$: C, 56.17; H, 8.18; N, 7.42. Found: C, 55.94; H, 8.15; N, 7.41.

*2″,2‴-Di-O-benzoyl-4″,6″-O-cyclohexylidene-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**16**).—Compound **15** (5.28 g, 4.66 mmol) in pyridine (107 mL) was treated with BzCl (2.75 mL, 23.7 mmol) for 2 h at room temperature to give, after standard work-up, **16** as a solid (6.14 g, 97%), $[\alpha]_D^{21}$ +53° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.35, 1.45, 1.47, 1.48, 1.51 (each s of 9 H, 5 Boc), 5.38 (d, 1 H, *J* 3.5 Hz, H-1′), 5.58 (dd, 1 H, *J* 5 and 7.5 Hz, H-2‴), 5.61 (dd, 1 H, *J* 4, 10.5 Hz, H-2″), 6.09 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″). Anal. Calcd for $C_{67}H_{100}N_6O_{22} \cdot H_2O$: C, 59.19; H, 7.56; N, 6.18. Found: C, 59.26; H, 7.42; N, 6.24.

*2″,2‴-Di-O-benzoyl-4″,6″-O-cyclohexylidene-5-deoxy-5-epi-5-fluoro-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**17**).—Compound **16** (monohydrate, 4.02 g, 2.96 mmol) in CH_2Cl_2 (80 mL) was treated with DAST (2.0 mL, 15.1 mmol) for 6 h at −20 °C, then worked up as described for **5** to give, after chromatography (30:1 $CHCl_3$–MeOH), **17** as a solid (2.83 g, 71%), $[\alpha]_D^{23}$ +75° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.37, 1.45, 1.46, 1.49, 1.56 (each s of 9 H, 5 Boc), 5.17 (d, 1 H, *J* 3.5 Hz, H-1′), 5.44 (dd, 1 H, *J* 5.5, 7 Hz, H-2‴), 5.50 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5), 5.55 (dd, 1 H, *J* 4, 10 Hz, H-2″), 5.76 (d, 1 H, *J* 4 Hz, H-1″). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.48 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 27 Hz, F-5). Anal. Calcd for $C_{67}H_{99}FN_6O_{21}$: C, 59.90; H, 7.43; F, 1.41; N, 6.25. Found: C, 59.77; H, 7.44; F, 1.17; N, 6.26.

*4″,6″-O-Cyclohexylidene-5-deoxy-5-epi-5-fluoro-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**18**).—Compound **17** (1.35 g, 1.00 mmol) was debenzoylated in a similar manner as described for **6** to give **18** as a solid (1.03 g, 89%), $[\alpha]_D^{23}$ +53° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.43, 1.47, 1.49, 1.51, 1.54 (each s of 9 H, 5 Boc), 2.04 (q, 1 H, H-2*ax*), 2.18 (m, 1 H, H-3‴a), 2.32 (m, 1 H, H-3‴b), 2.55 (dt, 1 H, H-2*eq*), 3.36 (m, 2 H, H-6′a,b), 3.54 (m, 2 H, H-4‴a,b), 3.90 (m, 1 H, H-2′), 4.06 (m, 1 H, H-3″), 4.19 (dd, 1 H, $J_{1'',2''}$ 4, $J_{2'',3''}$ 10 Hz, H-2″), 4.31 (m, 1 H, H-3), 4.38 (m, 1 H, H-1), 4.44 (dd, 1 H, $J_{2''',3'''a}$ 4.5, $J_{2''',3'''b}$ 8 Hz, H-2‴), 5.24 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.27 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), 5.46 (br d, 1 H, *J* 53 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.97 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 28 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 27 °C; signals lower than δ 85 are shown): δ 89.89 (d, $J_{C,F}$ 183 Hz, C-5), 95.18 (C-1′), 99.66 [$(CH_2)_5C$], 102.08 (C-1″); 156.02, 156.31, 156.98 (strong) [each Boc (*CO*)]; 175.89 (NHCO-1). Anal. Calcd for $C_{53}H_{91}FN_6O_{19} \cdot H_2O$: C, 55.19; H, 8.13; F, 1.65; N, 7.29. Found: C, 55.37; H, 8.10; F, 1.55; N, 7.45.

*4″,6″-O-Cyclohexylidene-5,2‴-dideoxy-5-epi-5-fluoro-2‴-oxo-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**20**).—A mixture of **18** (monohydrate, 195 mg, 0.17 mmol) and Dess–Martin periodinane [11] (95 mg, 0.22 mmol) in CH_2Cl_2 (4 mL) containing *tert*-butanol (0.016 mL) was stirred for 1 h at room temperature. After addition with $CHCl_3$ (20 mL), the solution was successively washed with aq $NaHCO_3$ (std), aq $Na_2S_2O_3$ (half std), and water, dried (Na_2SO_4), and concentrated to give **20** as a solid (195 mg, quant), $[\alpha]_D^{22}$ +56° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.42, 1.45, 1.49, 1.51, 1.54 (each s of 9 H, 5 Boc), 3.2–3.4 (4 H, H-6′a,b, 3‴a,b), 3.55 (q, 2 H, *J* 6 Hz × 3, H-4‴a,b), 3.90 (m, 1 H, H-2′), 4.02 (m, 1 H, H-3″), 4.15 (dd, 1 H, *J* 3.5, 9 Hz, H-2″), 4.32 (m, 1 H, H-3), 4.48 (m, 1 H, H-1), 5.24 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.25 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.46 (br d, 1 H, $J_{5,F}$ 52 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.88 (dt, $J_{5,F}$ 52, $J_{4,F} = J_{6,F}$ 27.5 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 27 °C): δ 22.83, 23.04, 28.00, 28.16, 38.32 [$(CH_2)_5C$]; 24.51 (C-3′), 25.96 (C-4′), 28.57 (Boc), 33.87 (C-2), 36.08 (C-4‴ or C-3‴), 38.19 (C-3‴ or C-4‴), 45.16 (C-6′), 48.01 (C-1 and C-3),

50.16 (C-2′), 55.00 (C-3″), 62.20 (C-6″), 66.03 (C-2″), 68.19 (C-5′), 70.98 (C-4″), 71.40 (C-5″), 76.76 (d, $J_{C,F}$ 18 Hz, C-4); 78.00, 78.22, 78.43, 78.62, 78.71 [5 $C(Me)_3$]; 80.22 (d, $J_{C,F}$ 17 Hz, C-6), 90.26 (d, $J_{C,F}$ 178.5 Hz, C-5), 95.32 (C-1′), 99.63 [$(CH_2)_5C$], 102.74 (C-1″); 156.06, 156.31, 156.62, 156.71, 157.00 [5 Boc(*CO*)]; 162.19 (NHCO-1), 198.22 (C-2‴). Anal. Calcd for $C_{53}H_{89}FN_6O_{19} \cdot H_2O$: C, 55.29; H, 7.97; F, 1.65; N, 7.30. Found: C, 55.40; H, 7.81; F, 1.53; N, 7.50.

*2‴-O-Acetyl-4″,6″-O-cyclohexylidene-5-deoxy-5-epi-5-fluoro-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**19**).—To an ice-cold solution of **18** (702 mg, 0.61 mmol) in pyridine (14 mL), was added Ac_2O (1.4 mL, 13.7 mmol) and the solution was kept at room temperature. After 75 min, water (1.2 mL) was added, and the solution was concentrated. The $CHCl_3$ solution of the residue was washed successively with aq $NaHCO_3$ (std), aq 5% $KHSO_4$, and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed with 50:1 → 20:1 $CHCl_3$–MeOH to give **19** as a solid (585 mg, 81%), $[\alpha]_D^{23}$ +48° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.44, 1.47, 1.48, 1.50, 1.53 (each s of 9 H, 5 Boc), ~1.85 (H-3′), 1.95 (s, 3 H, Ac), 2.04 (q, 1 H, H-2*ax*); 2.26 (br q) and 2.31 (br d) (*ABX* centered at 2.29, 2 H, H-3‴a,b); 2.59 (dt, 1 H, H-2*eq*), 3.34 (m, 2 H, H-6′a,b), 3.41 (m, 2 H, H-4‴a,b), 3.90 (m, 1 H, H-2′), 4.05 (m, 1 H, H-3″), 4.32 (m, 1 H, H-3), 4.35 (m, 1 H, H-1), 5.20 (d, 1 H, *J* 3.5 Hz, H-1′), 5.26 (d, 1 H, *J* 4 Hz, H-1″), 5.38 (t, 1 H, H-2‴), 5.45 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.99 (dt, *J* 27, 27, 53 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 27 °C): δ 20.62 (Ac); 22.84, 23.04, 28.01, 28.24, 38.34 [$(CH_2)_5C$]; 24.56 (C-3′), 25.97 (C-4′), 28.57 (Boc), 32.72 (C-3‴), 34.04 (C-2), 37.06 (C-4‴), 45.12 (C-6′), 47.70 (d, $J_{C,F}$ 4 Hz, C-3), 48.50 (d, $J_{C,F}$ 4.5 Hz, C-1), 50.10 (C-2′), 55.20 (C-3″), 62.23 (C-6″), 65.96 (C-2″), 68.16 (C-5′), 70.89 (C-4″), 71.28 (C-5″), 72.86 (C-2‴), 76.68 (d, $J_{C,F}$ 19 Hz, C-4); 78.02, 78.25, 78.40, 78.55, 78.69 [5 $C(Me)_3$]; 79.80 (d, $J_{C,F}$ 17 Hz, C-6), 89.92 (d, $J_{C,F}$ 181 Hz, C-5), 95.07 (C-1′), 99.60 [$(CH_2)_5C$], 102.13 (C-1″); 155.96, 156.24, 156.76 (strong), 156.93 [each Boc(*CO*)]; 170.50, 171.10 (CH_3CO_2-2‴, NHCO-1). Anal. Calcd for $C_{55}H_{93}FN_6O_{20}$: C, 56.11; H, 7.96; F, 1.61; N, 7.14. Found: C, 56.05; H, 7.93; F, 1.79; N, 7.03.

*2‴-O-Acetyl-4″,6″-O-cyclohexylidene-5,2″-dideoxy-5-epi-5-fluoro-2″-oxo-3,2′,6′,3″,4‴-pentakis(*N-tert-*butoxycarbonyl)arbekacin* (**21**).—To a solution of **19** (426 mg, 0.36 mmol) in dry Me_2SO (6.7 mL), Ac_2O (1.7 mL) was added and the solution was kept for 2 days at room temperature under an N_2 atmosphere. Aq $NaHCO_3$ (std, 200 mL) was added and the resulting precipitate was filtered and washed thoroughly with water. Chromatography of the product with 50:1 → 30:1 $CHCl_3$–MeOH gave **21** as a solid (361 mg, 85%), $[\alpha]_D^{23}$ +46° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.47 (18 H), 1.48 (9 H), 1.51 (9 H), 1.54 (9 H) (each s, 5 Boc), ~1.85 (H-3′), 1.92 (s, 3 H, Ac), 2.15–2.4 (4 H, H-2*ax*,2*eq*,3‴a,b), 3.34 (br t, 2 H, H-6′a,b), 3.42 (m, 2 H, H-4‴a,b), ~3.87 (m, 1 H, H-2′), 4.29 (m, 1 H, H-1), 4.31 (m, 1 H, H-3), 4.84 (dd, 1 H, $J_{3'',4''}$ 10.5, $J_{3'',NH}$ 8.5 Hz, H-3″), 5.21 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′), 5.29 (s, 1 H, H-1″), 5.40 (t, 1 H, H-2‴), 5.44 (br d, 1 H, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −214.49 (dt, *J* 27, 27, 53 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 27 °C; signals lower than δ 85 are shown): δ 90.19 (d, $J_{C,F}$ 184 Hz, C-5), 95.19 (C-1′), 99.87 [$(CH_2)_5C$], 102.27 (C-1″); 155.96, 156.26, 156.54, 156.82, 156.93 [5 Boc(*CO*)]; 170.15, 171.04 (CH_3CO-2‴, NHCO-1), 196.77 (C-2″). Anal. Calcd for $C_{55}H_{91}FN_6O_{20}$: C, 56.20; H, 7.80; F, 1.62; N, 7.15. Found: C, 56.36; H, 8.18; F, 1.65; N, 7.08.

5-Deoxy-5-epi-5-fluoro-2″-hydroxyarbekacin (**22**).—To an ice-cold 0.1% NaOMe in MeOH (0.4 mL), **21** (18.8 mg, 0.016 mmol) was added, and the solution was kept for 0.5 h at the temperature, concentrated, and the residue extracted with $CHCl_3$ (5 mL). After washing the solution with aq 5% $KHSO_4$, it was concentrated, and the residue was dissolved in ice-cold aq 95% CF_3CO_2H (0.2 mL). After 1 h the cold solution was concentrated, and the residue was thoroughly washed with ether to give **22** as an amorphous solid (16.4 mg, 90% as penta(trifluoroacetate) of the 2″-hydrate), $[\alpha]_D^{23}$ +76° (*c* 1, H_2O); MS *m/z* 571.6 $(M+1)^+$, ^{1}H NMR (D_2O, pD 3): δ 1.64 (m, 1 H, H-4′*ax*), 1.85 (q, *J* 13 Hz × 3, 1 H, H-2*ax*), ~1.92 (H-4′*eq*), ~1.97 (m, 1 H, H-3‴a); 2.07 (*ABX*, 2 H, H-3′*ax*, 3′*eq*); 2.19 (ddt, 1 H, *J* 3.5, 7, 7, 14 Hz, 1 H, H-3‴b), 2.37 (dt, 1 H, H-2*eq*), 3.11 (dd, 1 H, *J* 8, 14 Hz, H-6′a), 3.18 (t, 2 H, *J* 7 Hz × 2, H-4‴a,b), 3.26 (dd, 1 H, *J* 3.5, 14 Hz, H-6′b), 3.42 (d, 1 H, *J* 10 Hz, H-3″), 3.60 (m, 1 H, H-2′), 3.69 (t, 1 H, *J* 10 Hz × 2, H-4″), 3.73 (dd, 1 H, H-6″a), 3.82 (dt, 1 H, *J* 4, 11.5, 11.5 Hz, H-3), 3.94–4.01 (m, 2 H, H-5″, 6″b), ~4.1 (1 H, H-5′), 4.11 (1 H, H-6), 4.21 (dd, 1 H, *J* 11, 27 Hz, H-4), 4.31 (dd, 1 H, *J* 4, 9.5 Hz, H-2‴), 4.36 (dt, 1 H, *J* 4, 12, 12 Hz, H-1), 4.90 (s, 1 H, H-1″), 5.48 (d, 1 H, *J* 3.5 Hz, H-1″), 5.62 (sl. br d, 1 H, *J* 52 Hz, H-5). ^{19}F NMR (D_2O, pD 3): δ −216.93 (dt, *J* 27, 27, 52 Hz,

F-5). Anal. Calcd for $C_{22}H_{43}FN_6O_{10} \cdot 5CF_3CO_2H$: C, 33.69; H, 4.24; F, 26.65; N, 7.37. Found: C, 33.91; H, 4.42; F, 26.57; N, 7.33.

*3′,4′-Dideoxy-1,3,2′,6′-tetrakis(*N-tert-*butoxycarbonyl)neamine* (**23**).—To an aq solution (2.7 mL) of 3′,4′-dideoxyneamine [31] (monocarbonate · monohydrate, 265 mg, 0.72 mmol) and Et_3N (0.4 mL, 2.87 mmol) was added di-*t*-butyl dicarbonate (0.69 g, 3.2 mmol) in 1,4-dioxane (2.7 mL), and the mixture was treated as described for **2** to give **23** as an amorphous powder (459 mg, 91%), $[\alpha]_D^{23}$ +56° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.44, 1.45, 1.48, 1.52 (each s of 9 H, 4 Boc); 2.71 (dt, 1 H, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{2ax,2eq}$ 13 Hz, H-2*eq*), 3.4–3.55 (m, 2 H, H-6′a,b), 3.75–3.9 (4 H, H-1,3,4,6), 3.88 (1 H, H-2′), 5.49 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′). Anal. Calcd for $C_{32}H_{58}N_4O_{12} \cdot H_2O$: C, 54.22; H, 8.53; N, 7.90. Found: C, 54.08; H, 8.29; N, 8.15.

5-Epi-5-fluoro-5,3′,4′-trideoxyneamine (**24**).—5-Deoxy-5-epi-5-fluorodibekacin [6] (monocarbonate, 333 mg, 0.65 mmol) in aq 3 M HCl (4 mL) was refluxed at 110 °C for 1 h. The solution was concentrated to its half volume, and neutralized with powdered $NaHCO_3$ (pH 7 ~ 8). The mixture was charged on a column of fresh Amberlite CG-50 (NH_4^+ form, 15 mL). After washing the column with water, the product was eluted with 0.1 → 0.25 M NH_4OH to give **24** as an amorphous powder (186 mg, 89%), $[\alpha]_D^{24}$ +143° (*c* 1, H_2O); ^{1}H NMR (26% ND_3 in D_2O at 27 °C): δ 1.15 (q, 1 H, *J* 13 Hz × 3, H-2*ax*), 1.39 (m, 1 H, H-4′*ax*), 1.55–1.83 (3 H, H-3′*ax*, 3′*eq*, 4′*eq*), 2.03 (dt, 1 H, $J_{1,2eq} = J_{2eq,3}$ 4.5 Hz, H-2*eq*), 2.62 (dd, 1 H, *J* 7, 13.5 Hz, H-6′a), 2.67 (dd, 1 H, *J* 4.5, 13.5 Hz, H-6′b), 2.82 (ddd, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'eq}$ 5, $J_{2',3'ax}$ 11.5 Hz, H-2′), 2.98 (m, 1 H, H-1 or 3), 3.13 (m, 1 H, H-3 or 1), 3.39 (ddd, 1 H, *J* 2, 10, 31 Hz, H-6 or 4), 3.51 (ddd, 1 H, *J* 2, 10, 29 Hz, H-4 or 6), 3.82 (m, 1 H, H-5′), 4.99 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.06 (dt, 1 H, $J_{4,5} = J_{5,6}$ 2, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (26% ND_3 in D_2O at 27 °C): δ −215.44 (dt, *J* 30, 30, 53 Hz, F-5). ^{13}C NMR (26% ND_3 in D_2O at 27 °C): δ 27.02 (C-3′), 28.31 (C-4′), 36.65 (C-2), 45.81 (C-6′), 48.01 (d, $J_{C,F}$ 3.5 Hz, C-3 or 1), 48.92 (d, $J_{C,F}$ 4 Hz, C-1 or 3), 50.24 (C-2′), 71.20 (C-5′), 74.73 (d, $J_{C,F}$ 17 Hz, C-6 or 4), 79.30 (d, $J_{C,F}$ 17 Hz, C-4 or 6), 91.43 (d, $J_{C,F}$ 176 Hz, C-5), 97.13 (C-1′). Anal. Calcd for $C_{12}H_{25}FN_4O_3 \cdot 0.5H_2CO_3$: C, 46.43; H, 8.10; F, 5.88; N, 17.33. Found: C, 46.20; H, 8.36; F, 5.79; N, 17.15.

*5-Epi-5-fluoro-1,3,2′,6′-tetrakis(*N-tert-*butoxycarbonyl)-5,3′,4′-trideoxyneamine* (**25**).—To an aq solution (1 mL) of **24** (hemicarbonate, 100 mg, 0.31 mmol) and Et_3N (0.15 mL, 1.1 mmol) was added di-*t*-butyl dicarbonate (258 mg, 1.18 mmol) in 1,4-dioxane (1 mL), and the mixture was treated as described for **2** to give **25** as an amorphous powder (218 mg, 96%), $[\alpha]_D^{24}$ +74° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.43, 1.468, 1.471, 1.51 (each s of 9 H, 4 Boc); 1.5–2.0 (4 H, H-3′*ax*, 3′*eq*, 4′*ax*, 4′*eq*), 1.75 (q, 1 H, $J_{2ax,2eq} = J_{1,2ax} = J_{2ax,3}$ 13 Hz, H-2*ax*), 2.56 (dt, 1 H, $J_{1,2eq} = J_{2eq,3} = 4.5$ Hz, H-2eq), 3.37 (q, 2 H, $J_{6'a,6'b} = J_{5',6'a} = J_{5',6'b} = 5.5$ Hz, H-6′a,b), 3.82 (ddd, 1 H, $J_{1,6}$ 11, $J_{5,6}$ 2, $J_{6,F}$ 29 Hz, H-6), 3.90 (m, 1 H, H-2′), 3.91 (ddd, 1 H, $J_{3,4}$ 11 $J_{4,5}$ 2, $J_{4,F}$ 28 Hz, H-4), 4.23 (m, 2 H, H-1, 5′), 4.38 (m, 1 H, H-3), 5.31 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.33 (dt, 1 H, $J_{4,5} = J_{5,6} = 2$, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −214.51 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 28 Hz, F-5). ^{13}C NMR (pyridine-d_5 at 80 °C): δ 24.77 (C-3′), 28.38 (C-4′), 28.58, 28.60, 28.705, 28.713 [4(CH_3)$_3$C(O)O]; 35.70 (C-2), 45.64 (C-6′), 48.84 (d, $J_{C,F}$ 3 Hz, C-3), 50.36 (C-2′), 50.84 (d, $J_{C,F}$ 5 Hz, C-1), 68.47 (C-5′), 72.97 (d, $J_{C,F}$ 18 Hz, C-6), 77.36 (d, $J_{C,F}$ 18 Hz, C-4), 91.85 (d, $J_{C,F}$ 180 Hz, C-5), 95.55 (C-1′). Anal. Calcd for $C_{32}H_{57}FN_4O_{11} \cdot H_2O$: C, 54.07; H, 8.37; F, 2.67; N, 7.88. Found: C, 54.16; H, 8.16; F, 2.49; N, 7.95.

*5-Epi-5-fluoro-6-*O*-methyl-1,3,2′,6′-tetrakis(*N-tert-*butoxycarbonyl)-5,3′,4′-trideoxyneamine* (**26**).—To a mixture of **25** (80 mg, 0.11 mmol) and potassium *tert*-butoxide (63 mg, 0.56 mmol) in THF (1.6 mL) was added MeI (35 μL, 0.56 mmol), and the mixture was stirred for 30 min at room temperature. After concentration, the residue was extracted with $CHCl_3$ and the organic solution was washed with aq 5% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. Chromatography of the residue with 30:1 $CHCl_3$–MeOH gave **26** as a solid (80 mg, quant), $[\alpha]_D^{23}$ +52° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5 at 80 °C): δ 1.45, 1.478, 1.482, 1.51, (each s of 9 H, 4 Boc), 1.5–2.0 (5 H, H-2*ax*, 3′*ax*, 3′*eq*, 4′*ax*, 4′*eq*), 2.48 (dt, 1 H, $J_{1,2eq} = J_{2eq,3}$ 5, $J_{2ax,2eq}$ 13 Hz, H-2*eq*), 3.39 (s, 3 H, OMe), 3.3–3.4 (2 H, H-6′a,b), 3.51 (ddd, 1 H, $J_{1,6}$ 11, $J_{5,6}$ 2, $J_{6,F}$ 28 Hz, H-6), 3.81 (ddd, 1 H, $J_{3,4}$ 11, $J_{4,5}$ 2, $J_{4,F}$ 27 Hz, H-4), 3.92 (m, 1 H, H-2′), 4.1–4.3 (m, 2 H, H-1, 5′), 4.34 (m, 1 H, H-3), 5.34 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.36 (dt, 1 H, $J_{4,5} = J_{5,6}$ 2, $J_{5,F}$ 53 Hz, H-5). ^{19}F NMR (pyridine-d_5 at 80 °C): δ −213.68 (dt, $J_{5,F}$ 53, $J_{4,F} = J_{6,F}$ 28 Hz, F-5). Anal. Calcd for $C_{33}H_{59}FN_4O_{11}$: C, 56.07; H, 8.41; F, 2.69; N, 7.93. Found: C, 55.96; H, 8.27; F, 2.57; N, 8.00.

*Methyl 2,6-bis(*tert-*butoxycarbonylamino)-2,3,4,6-tetradeoxy-α-*D-erythro-*hexopyranoside* (**27**).—To an

aq solution (0.9 mL) of methyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranoside [32] (free base, 64 mg, 0.40 mmol) and Et_3N (0.11 mL, 0.79 mmol) was added di-*t*-butyl dicarbonate (192 mg, 0.88 mmol) in 1,4-dioxane (0.9 mL), and the mixture was stirred for 1 h at 60 °C. After addition of aq 28% NH_3 (0.12 mL) followed by stirring for 30 min at 60 °C, the mixture was concentrated. The residue was purified by column chromatography (10:1 $CHCl_3$–AcOEt) to give **27** as crystals (124 mg, 86%), mp 94–95 °C; $[\alpha]_D^{24}$ +85° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ ~ 1.4 (m, 1 H, H-4*ax*), 1.48 and 1.49 (each s of 9 H, 2 Boc), 1.63 (m, 1 H, H-3*ax*), 1.7–1.85 (2 H, H-3*eq*, 4*eq*), 3.26 (s, 3 H, OMe), 3.2–3.4 (2 H, H-6a,b), 3.88 (m, 1 H, H-5), 3.93 (m, 1 H, H-2), 4.77 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1). Anal. Calcd for $C_{17}H_{32}N_2O_6$: C, 56.65; H, 8.95; N, 7.77. Found: C, 56.37; H, 8.98; N, 7.85.

*Methyl 3-*tert*-butoxycarbonylamino-3-deoxy-α-*D*-glucopyranoside* (**28**).—Compound **28** was prepared according to the literature [20], mp 172–173 °C (lit. [20], mp 170–171 °C); $[\alpha]_D^{23}$ +130° (*c* 1, CH_3OH) {lit. [20], $[\alpha]_D^{20}$ +136.5° (*c* 1, DMF)}; ^1H NMR (pyridine-d_5 at 80 °C): δ 1.43 (s, 9 H, Boc), 3.38 (s, 3 H, OMe), 3.93 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2), 3.99 (t, 1 H, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 4.05 (ddd, 1 H, $J_{4,5}$ 9, $J_{5,6a}$ 4.5, $J_{5,6b}$ 3 Hz, H-5), 4.16 (dd, 1 H, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 3, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.35 (ddd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9, $J_{3,NH}$ 8 Hz, H-3), 4.95 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1).

*Methyl 3-*tert*-butoxycarbonylamino-4,6-O-cyclohexylidene-3-deoxy-α-*D*-glucopyranoside* (**29**).—**28** (56 mg) was treated with 1,1-dimethoxycyclohexane as described for **3**, to give, after chromatography (30:1 $CHCl_3$–MeOH), **29** as an amorphous powder (65 mg, 89%), $[\alpha]_D^{24}$ +85° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.47 (s, 9 H, Boc), 1.2–2.2 (10 H, cyclohexylidene), 3.33 (s, 3 H, OMe), 4.09 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2), 4.93 (d 1 H, H-1). Anal. Calcd for $C_{18}H_{31}NO_7 \cdot 0.5H_2O$: C, 56.53; H, 8.43; N, 3.66. Found: C, 56.79; H, 8.17; N, 3.75.

*1,3-Bis(*N*-tert-butoxycarbonyl)-2-deoxystreptamine* (**30**).—To an aq solution (0.7 mL) of 2-deoxystreptamine (free base, 50 mg, 0.31 mmol) and Et_3N (0.17 mL, 1.2 mmol) was added di-*t*-butyl dicarbonate (168 mg, 0.77 mmol) in 1,4-dioxane (0.9 mL), and the mixture was stirred for 1 h at 60 °C. After addition of aq 28% NH_3 (0.07 mL) followed by stirring for 30 min at 60 °C, the mixture was concentrated. Evaporation of MeOH from the product (three times) gave **30** as colorless needles (101 mg, 88%). The analytical sample was prepared by recrystallization from MeOH, mp 212–213 °C (decomp); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.46 (s, 18 H, 2 Boc), 1.75 (q, 1 H, $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3}$ 12.5 Hz, H-2ax), 2.78 (dt, 1 H, $J_{1,2eq} = J_{2eq,3}$ 4.5, $J_{2ax,2eq}$ 12.5 Hz, H-2*eq*), 3.75–3.85 (m, 3 H, H-4, 5, 6), 3.85–4.05 (m, 2 H, H-1, 3). Anal. Calcd for $C_{16}H_{30}N_2O_7 \cdot 0.5H_2O$: C, 51.74; H, 8.41; N, 7.54. Found: C, 51.44; H, 8.64; N, 7.30.

*1,3-Bis(*N*-tert-butoxycarbonyl)-4,5(5,6)-O-cyclohexylidene-2-deoxystreptamine* (**31**).—To a solution of **30** (56 mg, 0.15 mmol) in dry DMF (1.2 mL)–CH_2Cl_2 (6 mL) were added 1,1-dimethoxycyclohexane (0.07 mL, 0.49 mmol) and anhyd pyridinium *p*-toluenesulfonate (8 mg) and the mixture was refluxed for 2 h in the presence of molecular sieves 5A. The mixture was poured into ice-cold aq $NaHCO_3$ (std, 10 mL) and extracted with $CHCl_3$ (2 mL × 5). The organic solution was washed with aq $NaHCO_3$ (std) and water, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography (30:1 $CHCl_3$–MeOH) to give **31** as a solid (43 mg, 64%); ^1H NMR (pyridine-d_5 at 80 °C): δ 1.2–1.4 (2 H) and 1.5–1.75 (8 H) (cyclohexylidene); 1.46 (s, 18 H, 2 Boc), 1.80 (q, 1 H, *J* 12 Hz × 3, H-2*ax*), 2.80 (dt, 1 H, *J* 4.5, 4.5, 12 Hz, H-2*eq*), 3.7–3.85 (2 H, H-4, 5), 3.96 (m, 1 H, H-1), 4.05–4.15 (2 H, H-3, 6). ^{13}C NMR (pyridine-d_5 at 80 °C): δ 24.00, 25.37, 36.80 and 37.05 (cyclohexylidene); 28.56 and 28.58 [2(CH_3)$_3$C(O)O], 37.54 (C-2), 50.23 (C-3), 54.99 (C-1), 73.86 (C-6), 79.56 (C-5), 82.34 (C-4). Assignments for ^1H and ^{13}C resonances were confirmed by ^1H–^1H COSY and ^1H–^{13}C HETCOR. Anal. Calcd for $C_{22}H_{38}N_2O_7$: C, 59.71; H, 8.66; N, 6.33. Found: C, 59.59; H, 8.75; N, 6.23.

Minimal inhibitory concentration (μg mL^{-1}) of **9**, **12**, *dibekacin,* **22**, *and arbekacin.*—Performed on Mueller–Hinton agar for 18 h at 37 °C. *Staphylococcus aureus* FDA 209*P*: 6.25, 25, < 0.2, 3.12, < 0.2, in the following order; *Bacillus subtilis* PCI 219: 1.56, 25, 0.39, 6.25, < 0.2; *Escherichia coli* K-12: 12.5, 50, 0.39, 12.5, 0.78; *Klebsiella pneumoniae* PCI 602: 25, > 100, 0.78, 25, 0.39; *Serratia* sp. 4 [AAD(2″)]: 25, 100, 50, 25, 1.56; *Pseudomonas aeruginosa* A3: 6.25, 25, < 0.2, 6.25, 0.39.

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