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CARBOHYDRATE RESEARCH

Synthesis of 1-*N*-[(2*S*,4*S*)- and (2*S*,4*R*)-5-amino-4-fluoro-2-hydroxypentanoyl]dibekacins (study on structure–toxicity relationships)

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Abstract

(2*S*,4*S*)- and (2*S*,4*R*)-5-azido-2-*O*-benzyl-4-fluoro-2-hydroxypentanoic acids (**15** and **19**) have been prepared from L-malic acid (**1**), and coupled to the H_2N-1 group of 3,2′,6′-tris(*N*-benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)dibekacin (**23**), to give, after reduction and deblocking, 1-*N*-[(2*S*,4*S*)- and (2*S*,4*R*)-5-amino-4-fluoro-2-hydroxypentanoyl]dibekacins (**26** and **27**). The fluorinated arbekacin analogs showed almost the same antibacterial activities as that of arbekacin, but lower toxicity. Comparision of the toxicity between **26** (and **27**) and the arbekacin analogs (**28**–**30**) with change of the 1*N*-side-chain indicates that the observed decrease in toxicity was a function of the chain length rather than the introduction of flourine.

Keywords: 5-Amino-4-fluoro-2-hydroxypentanoic acid; Arbekacin; Fluorination; Toxicity

1. Introduction

In a previous paper [1] we reported the synthesis of 1-*N*-[(2*R*,3*R*)- and (2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl] analogs of arbekacin {1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl](AHB)dibekacin} [2] in the hope of obtaining derivatives with decreased toxicity. This was based on the hypothesis that decreasing the basicity of the H_2N-4‴ group in arbekacin by introducing flourine, an electron-withdrawing atom, proximal to the amino group (in this case, at C-3‴) would decrease the toxicity of arbekacin, as observed in other fluorine-containing analogs of kanamycins [3–5]. However, contrary to our expectation, the results showed that no such decrease of toxicity occurred. This was explained [1] on the basis of a low decrease in basicity of the H_2N-4‴ group compared to that of arbekacin [p*K*a 10.2 (arbekacin)→8.7]; in other words, the Δp*K*a value (=1.5) did not reach 2.2 (p*K*a 8.0), the value expected to show a toxicity-decreasing effect under physiological conditions (pH∼7.0). The present study was designed to examine this concept [1], and describes the synthesis of dibekacin analogs containing 1-*N*-[(2*S*,4*S*)- and (2*S*,4*R*)-5-amino-4-fluoro-2-hydroxypentanoyl] residues which, compared to AHB, have a one-carbon extended chain.

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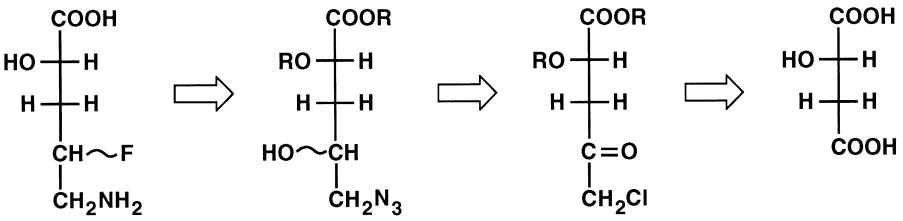
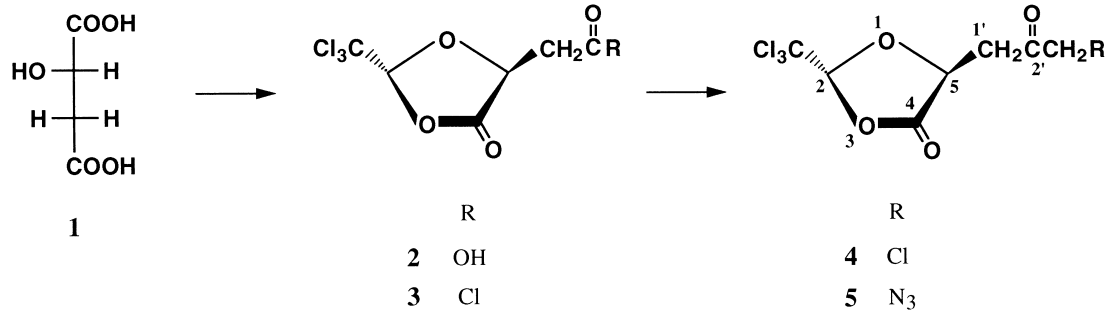


Scheme 1.

2. Results and discussion

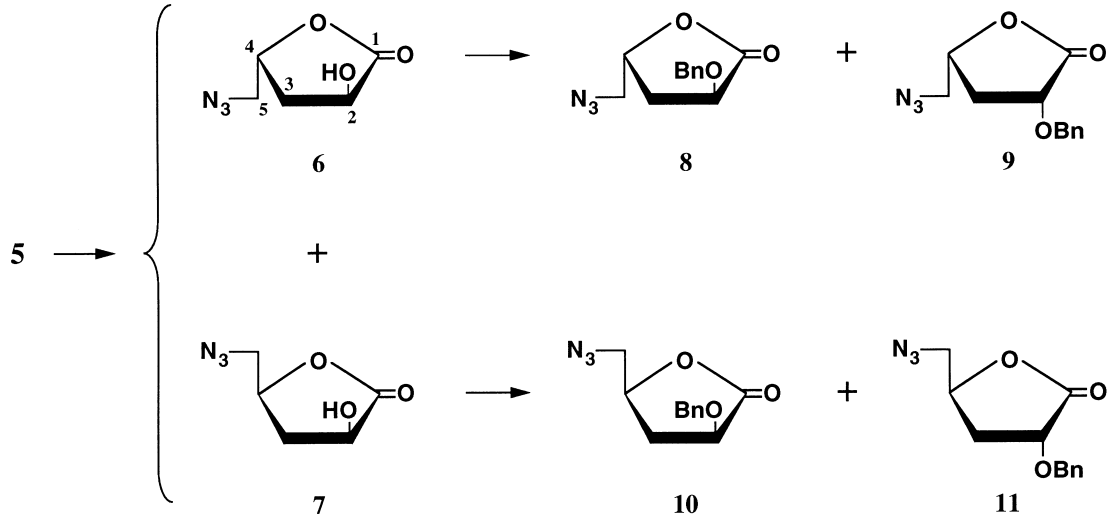
Synthesis.—First of all, preparation of (2*S*,4*R* and 2*S*,4*S*)-5-amino-4-fluoro-2-hydroxypentanoic acids was designed according to the retrosynthesis shown (Scheme 1). After the vicinal carboxyl and hydroxyl groups of L-malic acid (**1**) had been doubly protected with chloral [6,7], the monoacid **2** was refluxed with thionyl chloride, giving the corresponding acid chloride **3**. One-carbon elongation was performed by treatment of **3** with diazomethane to afford an intermediary diazoketone ($R\text{-}COC^{-}HN_2^{+}$), which was then treated with ethereal HCl to give the chloromethyl ketone **4**. Attempted displacement of the terminal chlorine in **4** by an azide group by NaN_3 in DMF (at room temperature) or in hot CH_3CN gave the corresponding azidoketone **5** in only a trace amount. However, treatment of **4** with NaN_3–Et_4NBr in CH_2Cl_2–CH_3CN, gave **5** in high yield. If, in this reaction, CH_2Cl_2 was omitted, **5** was produced in only a low yield; the reason is unknown. Next, conversion of **5** into the corresponding 2′-ol was attempted with the N_3 group remaining intact. As the azido group is known to be comparatively stable to $NaBH_4$ [8–10], compound **5** was initially



1

	R
2	OH
3	Cl

	R
4	Cl
5	N_3



6 **8** **9**

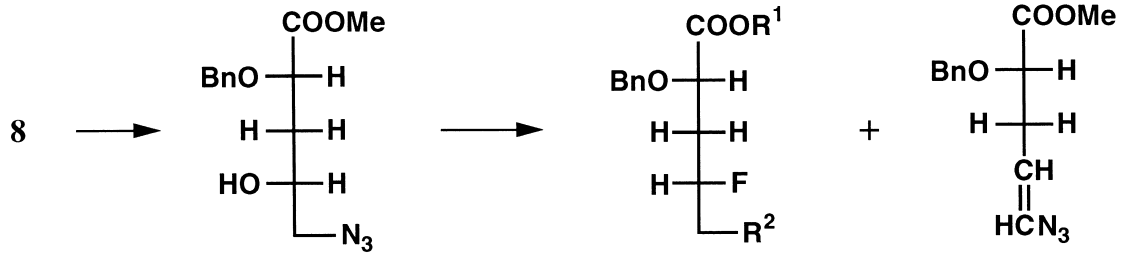
7 **10** **11**

treated with $NaBH_4$ ($NaBH_3CN$ or Me_4NBH_4) in MeOH (or THF), but a considerable amount of undesirable by-products (ninhydrin-negative) were produced. Next, $Zn(BH_4)_2$ in Et_2O was tried, whereupon the oxo group was selectively reduced with simultaneous cyclization, giving a mixture of γ-lactones (**6** (major) and **7**). The chirality of **6** and **7** at C-4 was determined by NOE experiments; in **6** irradiation of the higher-field half of the H-5 protons caused a signal increase of H-2 together with the H-3 (H-3a and 3b) protons, indicating the *R*-configuration at C-4. In **7**, the *S* configuration at C-4 was deduced from a ROESY experiment for **10**, because of the appearance, in **7**, of the H-2 and 4 signals at closely proximal positions (Table 1). Benzylation of the 2-hydroxyl group of **6** and **7** was performed by a conventional method ($PhCH_2Br$ in DMF in the presence of Ag_2O), whereupon, however, partial epimerization occurred at C-2, giving a mixture of 2-*O*-benzyl products (**8** and **9** from **6**; **10** and **11** from **7**) with the 2*R* isomers predominating. Each of the mixtures was separated

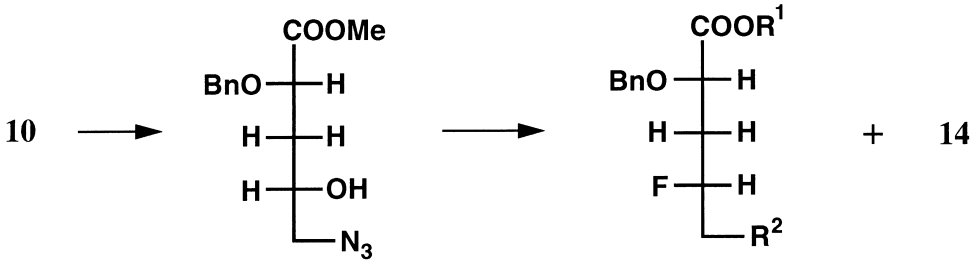
Table 1
^1H and ^{19}F NMR data for compounds **3**–**13**, **15**, and **17**–**19** in $CDCl_3$

Compound	Chemical shifts in ppm (*J* in Hz)								
	H-2	H-3a	H-3b	H-4	H-5a	H-5b	F-4	PhCH_2	Other signals
3	5.91 d $J_{2,5}$ 2	—	—	—	4.88 dt $J_{5,1'a}$ 4 $J_{5,1'b}$ 4	—	—	—	3.52 dd (H-1′a) 3.62 dd (H-1′b) $J_{1'a,1'b}$ 19
4	5.90 d $J_{2,5}$ 2	—	—	—	4.89 dt $J_{5,1'a}$ 3.5 $J_{5,1'b}$ 4	—	—	—	3.23 dd (H-1′a) 3.47 dd (H-1′b) $J_{1'a,1'b}$ 19 4.14 s (H-3′a,3′b)
5	5.91 d $J_{2,5}$ 2	—	—	—	4.88 dt $J_{5,1'a}$ 3.5 $J_{5,1'b}$ 4	—	—	—	3.10 dd (H-1′a) 3.29 dd (H-1′b) $J_{1'a,1'b}$ 19 4.05 s (H-3′a,3′b)
6	4.66 dd $J_{2,3a}$ 8 $J_{2,3b}$ 9	2.37 ddd $J_{3a,3b}$ 13.5 $J_{3a,4}$ 8.5	2.45 ddd $J_{3b,4}$ 3.5	4.77 dq $J_{4,5a}$ 4 $J_{4,5b}$ 3.5	3.49 dd $J_{5a,5b}$ 13	3.69 dd	—	—	2.68 br s (OH)
7	4.63 dd $J_{2,3a}$ 10.5 $J_{2,3b}$ 8.5	2.09 dt $J_{3a,3b}$ 13 $J_{3a,4}$ 10.5	2.68 ddd $J_{3b,4}$ 6	4.57 ddt $J_{4,5a}$ 6 $J_{4,5b}$ 3.5	3.51 dd $J_{5a,5b}$ 13.5	3.62 dd	—	—	3.87 br s (OH)
8 and **11**	4.27 dd $J_{2,3a}$ 7.5 $J_{2,3b}$ 5.5	2.28 ddd $J_{3a,3b}$ 13.5 $J_{3a,4}$ 6	2.33 ddd $J_{3b,4}$ 7	4.76 dddd $J_{4,5a}$ 4 $J_{4,5b}$ 3.5	3.42 dd $J_{5a,5b}$ 13.5	3.63 dd	—	4.69 d 4.93 d J_{gem} 11.5	
9 and **10**	4.26 dd $J_{2,3a}$ 9 $J_{2,3b}$ 8.5	2.10 dt $J_{3a,3b}$ 13 $J_{3a,4}$ 9	2.52 ddd $J_{3b,4}$ 6	4.47 ddt $J_{4,5a}$ 6 $J_{4,5b}$ 4.5	3.49 dd $J_{5a,5b}$ 13	3.54 dd	—	4.75 d 4.97 d J_{gem} 12	
12	4.17 dd $J_{2,3a}$ 5 $J_{2,3b}$ 7.5	1.96 ddd $J_{3a,3b}$ 14.5 $J_{3a,4}$ 4	1.99 dt $J_{3b,4}$ 7.5	4.01 m $J_{4,5a}$ 6 $J_{4,5b}$ 5	3.23 dd $J_{5a,5b}$ 12.5	3.26 dd	—	4.44 d 4.77 d J_{gem} 11	2.97 d (OH) $J_{4,OH}$ 2.5 3.77 s (CO_2Me)
13	4.19 dd $J_{2,3a}$ 11 $J_{2,3b}$ 3	1.90 dddd $J_{3a,3b}$ 15 $J_{3a,4}$ 2.5 $J_{3a,F}$ 35	2.20 dddd $J_{3b,4}$ 10 $J_{3b,F}$ 13	4.90 dddt $J_{4,5a}$ 6 $J_{4,5b}$ 3 $J_{4,F}$ 49	3.37 ddd $J_{5a,5b}$ 13.5 $J_{5a,F}$ 24	3.46 ddd $J_{5b,F}$ 24	−188.4 dddt	4.45 d 4.75 d J_{gem} 11	3.77 s (CO_2Me)
15	4.23 dd $J_{2,3a}$ 11 $J_{2,3b}$ 3	1.93 dddd $J_{3a,3b}$ 15 $J_{3a,4}$ 2.5 $J_{3a,F}$ 35	2.28 dddd $J_{3b,4}$ 10 $J_{3b,F}$ 12.5	4.89 dddt $J_{4,5a}$ 6 $J_{4,5b}$ 3 $J_{4,F}$ 49	3.39 ddd $J_{5a,5b}$ 13.5 $J_{5a,F}$ 23.5	3.46 ddd $J_{5b,F}$ 2	−188.6 dddt	4.51 d 4.79 d J_{gem} 11	
17	4.25 dd $J_{2,3a}$ 8.5 $J_{2,3b}$ 4	1.87 ddd $J_{3a,3b}$ 14.5 $J_{3a,4}$ 3	1.94 ddd $J_{3b,4}$ 9.5	3.98 m $J_{4,5a}$ 6.5 $J_{4,5b}$ 4	3.24 dd $J_{5a,5b}$ 12.5	3.32 dd	—	4.43 d 4.77 d J_{gem} 11	2.42 d (OH) $J_{4,OH}$ 4 3.77 s (CO_2Me)
18	4.10 t $J_{2,3a}$ 5.5 $J_{2,3b}$ 6	2.10 ddt $J_{3a,3b}$ 14.5 $J_{3a,4}$ 5.5 $J_{3a,F}$ 25	2.26 dddd $J_{3b,4}$ 7.5 $J_{3b,F}$ 17	4.87 dddt $J_{4,5a}$ 6 $J_{4,5b}$ 3 $J_{4,F}$ 48	3.35 ddd $J_{5a,5b}$ 13.5 $J_{5a,F}$ 23.5	3.38 ddd $J_{5b,F}$ 25	−185.8 ddq	4.43 d 4.76 d J_{gem} 11.5	3.78 s (CO_2Me)
19	4.15 t $J_{2,3a}$ 6 $J_{2,3b}$ 6	2.13 dddd $J_{3a,3b}$ 15 $J_{3a,4}$ 4.5 $J_{3a,F}$ 27	2.32 ddt $J_{3b,4}$ 8 $J_{3b,F}$ 15	4.93 dddd $J_{4,5a}$ 6 $J_{4,5b}$ 3.5 $J_{4,F}$ 48	3.36 ddd $J_{5a,5b}$ 13.5 $J_{5a,F}$ 23.5	3.39 ddd $J_{5b,F}$ 24.5	−186.0 dddt	4.51 d 4.79 d J_{gem} 11.5	

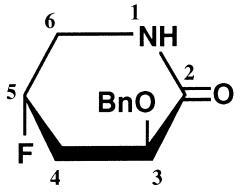
chromatographically. The structures were confirmed by their ^{1}H NMR spectra (Table 1) as well as by changes in the specific rotations: a change of **6**→**8** or **7**→**10** gave a levorotatory change, whereas a change of **6**→**9** or **7**→**11** gave a large dextrorotatory change. It is noteworthy that **8** and **11**, and **9** and **10**, showed identical ^{1}H NMR signals and had opposite rotations. Next, to free the HO-4 group, **8** and **10** were each treated with methanolic HCl, whereupon the desired open-chain esters **12** and **17** were obtained in high yields as syrups (however, long storage or further purification caused partial relactonization, giving the starting materials). Fluorination of **12** or **17** with diethylaminosulfur trifluoride (DAST) [11] gave the corresponding 4-fluoro derivatives (**13** and **18**) with



	R^1	R^2
13	Me	N_3
15	H	N_3
16	H	NH_2



	R^1	R^2
18	Me	N_3
19	H	N_3
20	H	NH_2



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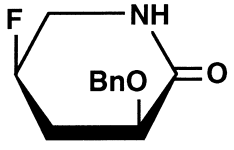

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Table 2
^{13}C NMR chemical shifts (δ, ppm) and coupling constants ($J_{C,F}$, Hz) for compounds **6**–**13**, **15**, **17**–**19**, **21**, and **22** in $CDCl_3$

Compound	C-1	C-2	C-3	C-4	C-5	C-6	Other signals
6	177.3	67.0	33.0	76.0	54.2		
7	176.4	68.1	33.5	75.1	53.6		
8 and **11**	173.9	72.9	32.5	76.2	54.0		72.3[a]
9 and **10**	173.8	72.9	32.3	75.0	53.9		72.4[a]
12	172.4	76.4	36.9	69.1	56.4		52.2[b]
							72.8[a]
13	172.5	74.0 d (3.7)	35.7 d (21.0)	89.0 d (174.2)	54.4 d (21.0)		52.2[b] 73.1[a]
15	176.9	73.7 d (3.7)	35.5 d (21.1)	88.8 d (174.5)	54.3 d (21.0)		73.4[a]
17	172.8	75.1	36.9	67.6	56.8		52.1[b]
							72.9[a]
18	172.1	73.7 d (5.6)	35.3 d (21.5)	89.3 d (173.2)	54.1 d (21.3)		52.2[b] 72.5[a]
19	176.1 d (3.0)	73.3 d (4.9)	35.0 d (21.2)	89.1 d (173.6)	54.0 d (21.5)		72.8[a]
21		171.3	71.1 d (5.6)	33.8 d (20.9)	85.1 d (173.2)	46.4 d (24.3)	73.5[a]
22		171.6	70.9 d (6.3)	34.5 d (22.4)	84.2 d (177.3)	45.5 d (26.2)	72.7[a]

[a,b] Signals for Ph*C*H_2 and COO*C*H_3, respectively.

inversion, accompanied by a small amount of 4-eno compound **14** (this was a mixture of *E* and *Z* isomers). Alkaline hydrolysis of **13** (or **18**) gave the respective free acids **15** (or **19**). To ascertain the structures of **15** and **19**, they were each reduced catalytically and the amino acids obtained (**16** and **20**) were cyclized according to the procedure of Pellegata and coworkers [12], with 1,1,1,3,3,3-hexamethyldisilazane to give the corresponding δ-lactams (**21** and **22**). The structures were confirmed by ^1H NMR spectra and NOE experiments: in **21**, large coupling constants $J_{3,4ax}$, $J_{4ax,F}$, and $J_{6ax,F}$ indicate that BnO-3 (equatorial) and F-5 (axial) are in opposite faces, and this was supported by the NOE experiments: irradiation of F-5 caused increases of the H-3*ax* and H-4*eq* signals. A $^4H_5$ (or $^4S_5$) structure was proposed as the most probable conformation for **21**. In **22**, a structure with the BnO-3 and F-5 in the same face was confirmed on account of the observation of an NOE between H-3 and H-5, although the correct conformation is not clear. These results indicate that **15** and **19** have the expected structures (Table 2).

Coupling of **15** (or **19**) with 3,2′,6′-tris[*N*-benzyloxycarbonyl(=Z)-3″-*N*-trifluoroacetyl]dibekacin **23** having the H_2N-1 group free was performed utilizing the active ester **24** (or **25**) of **15** (or **19**) according to conventional methodology [1,13]. The condensed 1-*N*-acyl derivatives were successively de(trifluoroacetyl)ated, reduced (N_3-5‴), and hydrogenolyzed (three Z groups) to give the final products (**26** and **27**).

Biological activity.—Antibacterial activities of **26** and **27** as compared with arbekacin (Table 4) indicate that both compounds had activities almost identical to that of arbekacin. This indicates that the activity is not influenced by the one-carbon elongation or the orientation of F-4‴. It is noteworthy that the more-active counterpart, 1-*N*-[(2*R*,3*R*)- [not

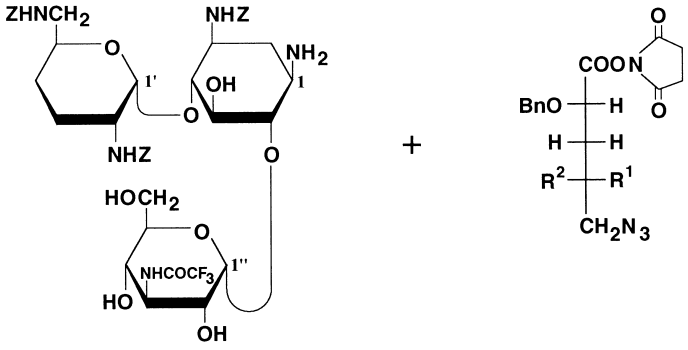
Table 3
^{13}C NMR chemical shifts (δ, ppm) and coupling constants ($J_{C,F}$, Hz) for compounds **26**–**30** and arbekacin (ABK) in DCl–D_2O (pD 3)

	26	**27**	**28**	**29**	**30**	ABK[a]
C-1	49.6	49.7	49.5	49.6	49.6	49.6
C-2	31.2	31.1	31.1	31.2	31.3	31.2
C-3	49.8	49.8	49.9	49.9	49.9	49.8
C-4	78.6	78.6	78.6	78.6	78.6	78.5
C-5	75.7	75.7	75.8	75.7	75.7	75.7
C-6	81.0	80.7	81.7	80.9	80.9	81.1
C-1′	96.0	96.0	96.0	96.0	95.9	95.9
C-2′	49.7	49.7	49.7	49.7	49.8	49.7
C-3′	21.4	21.4	21.5	21.5	21.5	21.5
C-4′	26.2	26.2	26.3	26.3	26.3	26.3
C-5′	66.9	66.8	66.9	66.9	66.9	66.9
C-6′	43.5	43.4	43.5	43.5	43.6	43.5
C-1″	98.9	98.7	99.2	98.8	98.7	98.9
C-2″	68.8	68.8	68.9	68.9	68.9	68.8
C-3″	56.0	56.0	56.1	56.1	56.1	56.1
C-4″	66.4	66.4	66.5	66.5	66.5	66.5
C-5″	73.0	73.0	73.1	73.1	73.1	73.0
C-6″	60.7	60.7	60.8	60.8	60.8	60.7
C-1‴	176.6	176.4	173.8	177.1	177.5	176.3
C-2‴	68.2d	68.9 d	68.7	71.8	72.1	70.5
C-3‴	37.0 d	36.8 d	42.9	31.3	33.9	31.7
C-4‴	88.4 d	89.4 d		24.1	22.8	37.9
C-5‴	43.9 d	43.7 d		40.1	27.3	
C-6‴					40.3	
$J_{C\text{-}2''',F}$	3.5	~4				
$J_{C\text{-}3''',F}$	19.5	19.4				
$J_{C\text{-}4''',F}$	170.4	169.6				
$J_{C\text{-}5''',F}$	20.3	19.6				

[a] Ref. [1].

(2*R*,3*S*)]-4-amino-3-fluoro-2-hydroxybutanoyl]dibekacin reported previously [1] has an F-3-D structure. In terms of acute toxicity, both **26** (LD_{50}~130 mg/kg, mice, intravenous injection) and **27** (LD_{50}~125) showed almost the same value, but this was considerably lower than that of arbekacin (LD_{50}~75). This indicates that antibacterial activity and toxicity do not necessarily change in parallel [4]. This lower toxicity of **26** and **27** as compared with arbekacin, however, is not to be ascribed to the decrease in basicity of H_2N-5‴ that results from the induction of the F-4‴ atom, as had been predicted in our previous work and was explained in detail in [1] (for example, arbekacin and an arbekacin analog having the (2*R*,3*R*)-4-amino-3-fluoro-2-hydroxybutanoyl residue have similar toxicities). This unexpected decrease in toxicity in the present derivatives **26** and **27** must, therefore, be ascribed to the difference in the chain-length or the position of the fluorine atom introduced.

To clarify the problem, three arbekacin analogs with varying lengths of the side chain, without fluorine, were conventionally prepared, namely,



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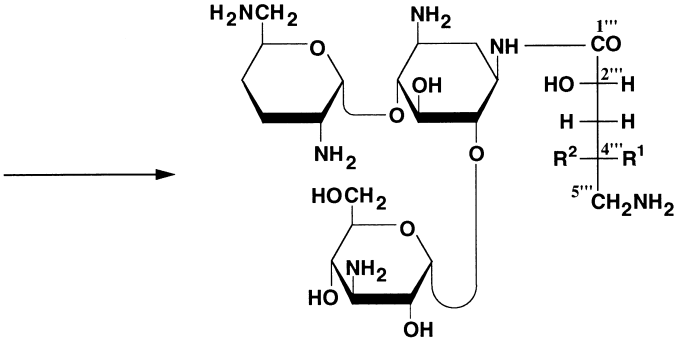
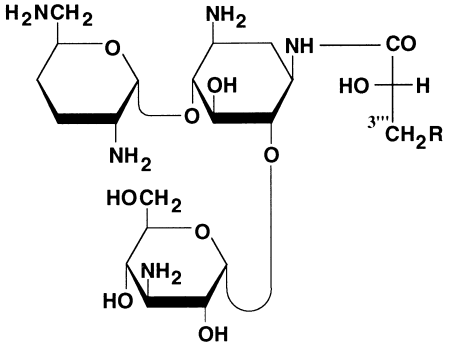


	R^1	R^2
24	F	H
25	H	F

	R^1	R^2
26	F	H
27	H	F



	R
28	NH_2
Arbekacin	CH_2NH_2
29	$CH_2CH_2NH_2$
30	$CH_2CH_2CH_2NH_2$

Table 4
Minimal inhibitory concentration[a] (μg/mL) of compounds **26**–**30** and arbekacin (ABK)

Test organism[b]	**26**	**27**	**28**	**29**	**30**	ABK
St. a. FDA 209 P	0.10	0.10	0.39	0.10	0.20	0.20
St. a. Smith	< 0.05	< 0.05	0.05	< 0.05	0.10	0.05
St. a. Ap01[c]	> 100	> 100	> 100	> 100	> 100	> 100
Micr. l. FDA16	3.13	1.56	3.13	6.25	> 100	3.13
Micr. l. PCI 1001	3.13	1.56	3.13	6.25	> 100	3.13
B. c. ATCC 10702	0.78	0.78	1.56	1.56	1.56	1.56
Coryn. b. 1810	1.56	0.39	0.78	3.13	50	0.39
E. c. NIHJ	0.39	0.39	0.39	0.39	1.56	0.39
E. c. K-12 R5[d]	> 100	> 100	> 100	> 100	> 100	> 100
E. c. K-12 ML1629[e]	0.78	1.56	1.56	1.56	6.25	1.56
E. c. K-12 ML1410 R81[e]	1.56	1.56	1.56	1.56	25	1.56
E. c. K-12 LA290 R55[f]	3.13	3.13	1.56	0.78	3.13	1.56
E. c. K-12 LA290 R64	0.39	0.39	0.39	0.39	1.56	0.39
E. c. W677	0.39	0.39	0.39	0.39	1.56	0.39
E. c. JR66/W677[f,g]	1.56	1.56	1.56	1.56	6.25	1.56
E. c. JR225[h]	0.78	1.56	0.78	0.78	3.13	0.78
Kl. p. PCI602	0.39	0.78	1.56	0.78	3.13	0.78
Kl. p. 22#3038[f,g]	0.78	0.78	0.78	0.78	3.13	0.78
Sh. s. JS11746	0.78	0.78	0.78	0.78	3.13	0.78
Sal. e. 1891	3.13	3.13	3.13	6.25	50	3.13
Serr. marc.	25	3.13	3.13	12.5	100	6.25
Prot. r. GN311	1.56	3.13	3.13	1.56	3.13	6.25
Prov. sp. Pv 16[i]	6.25	1.56	3.13	6.25	50	1.56
Prov. sp. 2991[i]	25	12.5	25	25	> 100	6.25
Ps. aerug. A3	0.39	0.39	0.78	0.39	0.78	0.39
Ps. aerug. H9[g]	1.56	1.56	1.56	1.56	6.25	1.56
Ps. aerug. GN315[d]	25	6.25	6.25	25	> 100	6.25

[a] Judged by the agar dilution streak method (Mueller–Hinton agar, 37 °C, 18 h).
[b] Abbreviations: *St. a., Staphylococcus aureus*; *Micr. l., Micrococcus luteus; B. c., Bacillus cereus*; *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*.
[c] Resistant strain producing AAD(4′).
[d] AAC(6′).
[e] APH(3′)-I.
[f] AAD(2″).
[g] APH(3′)-II.
[h] AAC(3).
[i] AAC(2′).

1-*N*-[(2*S*)-3-amino-2-hydroxypropanoyl]dibekacin (**28**) [14], 1-*N*-[(2*S*)-5-amino-2-hydroxypentanoyl]-dibekacin (**29**), and 1-*N*-[(2*S*)-6-amino-2-hydroxy-hexanoyl]dibekacin (**30**) (Table 3). The antibacterial activities (Table 4) of compounds **28**, **29**, and arbekacin were found to be almost the same, whereas **30** was less active. However, it was established that the acute toxicity of these compounds was significantly influenced by the chain-length, with decrease in toxicity with increase in length (**28**: ~65, **29**: ~120, **30**: ~125 mg/kg). A specific feature is that **26**, **27**, and **29** with the same chain-length, had analogous toxicities, indicating that the decrease in toxicity of **26** (and **27**) compared to arbekacin must be ascribed to the difference in chain length, and not to the introduction of fluorine at C-4‴.

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 measured with a Jasco A-202 grating spectrophotometer. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded at 250 (^{1}H, for **3**–**5** and **7**) and 235.3 MHz (^{19}F) with a Bruker WM 250 spectrometer, and at 500 (^{1}H), 125.8 (^{13}C), and 470.6 MHz (^{19}F, for **21**) with an AMX 500 spectrometer. Chemical shifts (δ) of ^{1}H, ^{13}C, and ^{19}F spectra were measured downfield from internal Me_4Si (for ^{1}H and ^{13}C) and internal Freon 11 (for ^{19}F), unless otherwise stated, and were confirmed, when necessary, by shift-correlated 2D spectra. Thin-layer chromatography (TLC) was

performed on Kieselgel 60 F_{254} (Merck), and column chromatography, on Wakogel C-200, unless stated otherwise. HPLC was performed on a C.I.G. pre-packed silica-gel column (Kusano Kagakukikai Co., Japan) using 1:3 hexane–EtOAc.

(2R,5S)-5-(2-Chloro-2-oxoethyl)-2-trichloromethyl-1,3-dioxolan-4-one (**3**).—A mixture of **2** [6,7] [11.9 g, 45 mmol; $[\alpha]_D^{23}$ +38.5° (*c* 5, EtOH), $[\alpha]_D^{23}$ +35° (*c* 1, $CHCl_3$); lit [6], $[\alpha]_D^{28}$ +39.1° (*c* 5.14, EtOH), lit [7], $[\alpha]_D^{20}$ + 33.03 (*c* 1.005, $CHCl_3$)] and $SOCl_2$ (20 mL, 270 mmol) was gently refluxed for 60 h. Evaporation of $SOCl_2$ gave a residue that crystallized from hexane to give **3** as needles (11.8 g, 93%); mp 74–75 °C (lit [6] 70–72 °C), $[\alpha]_D^{24}$ + 24° (*c* 1, $CHCl_3$); IR (KBr): 1780 (C=O), 1820 cm^{-1} [C=O (lactone)]. Anal. Calcd for $C_6H_4Cl_4O_4$: C, 25.56; H, 1.43; Cl, 50.30. Found: C, 25.79; H, 1.50; Cl, 50.19.

(2R,5S)-5-(3-Chloro-2-oxopropyl)-2-trichloromethyl-1,3-dioxolan-4-one (**4**).—To a solution of **3** (10.0 g, 35 mmol) in Et_2O (100 mL) was added 0.25 M ethereal CH_2N_2 (280 mL, 70 mmol), and the solution was kept for 30 min at room temperature. Ethereal 1.2 M HCl (70 mL) was added, and the mixture was kept for a further 30 min. TLC (6:1 toluene–EtOAc) of the solution showed a spot at R_f 0.55. After concentration, the residue was chromatographed (6:1 toluene–EtOAc) to give **4** as a crystalline solid (9.8 g, 93%); mp 75–76 °C (toluene–hexane), $[\alpha]_D^{23}$ + 33° (*c* 1, $CHCl_3$); IR (KBr): 1735 (C=O), 1815 cm^{-1} [C=O (lactone)]; ^{13}C NMR ($CDCl_3$; confirmed by the ^1H-^{13}C HMBC): δ 198.8 (C-2′), 170.3 (C-4), 105.2 (C-2), 97.7 (CCl_3), 70.5 (C-5), 47.5 (C-3′), 40.7 (C-1′). Anal. Calcd for $C_7H_6Cl_4O_4$: C, 28.41; H, 2.04; Cl, 47.92. Found: C, 28.67; H, 1.87; Cl, 47.98.

(2R,5S)-5-(3-Azido-2-oxopropyl)-2-trichloromethyl-1,3-dioxolan-4-one (**5**).—To a cold (0 °C) solution of **4** (6.0 g, 20 mmol) in 6:5 CH_2Cl_2–CH_3CN (110 mL) were added NaN_3 (1.7 g, 26 mmol) and Et_4NBr (4.5 g, 21 mmol), and the mixture was stirred for 3 h in the cold; TLC (6:1 toluene–EtOAc) of the organic layer showed a spot at R_f 0.45. After dilution with $CHCl_3$ (600 mL), the solution was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography (6:1 toluene–EtOAc) to give **5** as a crystalline solid (5.28 g, 86%), mp 105–106 °C (toluene–hexane), $[\alpha]_D^{23}$ + 38° (*c* 1, $CHCl_3$); IR (KBr): 1725, 1830, 2110 cm^{-1} (N_3). Anal. Calcd for $C_7H_6Cl_3N_3O_4$: C, 27.79; H, 2.00; Cl, 35.16; N, 13.89. Found: C, 28.09; H, 2.24; Cl, 35.37; N, 13.71.

5-Azido-3,5-dideoxy-L-erythro-*pentono-1,4-lactone* (**6**) *and 5-azido-3,5-dideoxy*-D-threo-*pentono-1,4-lactone* (**7**).—To a cold (−10 °C) solution of **5** (1.95 g, 6.45 mmol) in THF (60 mL) was added ~0.07 M ethereal $Zn(BH_4)_2$ (first 30 mL, and then another 30 mL after 1 h; ~4 mmol in total), and the mixture was kept for 2 h in the cold. TLC (1:1 toluene–EtOAc) of the organic layer showed spots at R_f 0.25 (**6**), 0.2 (**7**), and 0 (*cf.* **5**: R_f 0.7). After addition of AcOH (~0.8 mL), the mixture was extracted with EtOAc. The extracts were washed with aq Na_2SO_4 (satd), dried (Na_2SO_4), and concentrated to a syrup. HPLC of the syrup afforded **6** (565 mg, 56%) and **7** (357 mg, 35%) as syrups. Compound **6** had $[\alpha]_D^{22}$ −137° (*c* 1, $CHCl_3$); IR (neat): 1780 (C=O), 2110 cm^{-1} (N_3); NOE difference spectroscopy: irradiation of H-5a, H-2 (0.4%), H-3a (0.6%), H-3b (1.2%), and H-4 (1.8%) signals were increased. Anal. Calcd for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.74. Found: C, 38.32; H, 4.31; N, 26.41. Compound **7** had $[\alpha]_D^{22}$ + 59° (*c* 1, $CHCl_3$); IR (neat): 1780, 2110 cm^{-1}. Anal. Calcd for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.74. Found: C, 38.23; H, 4.75; N, 26.63.

5-Azido-2-O-*benzyl-3,5-dideoxy*-L-erythro-*pentono-1,4-lactone* (**8**) *and 5-azido-2*-O-*benzyl-3,5-dideoxy*-L-threo-*pentono-1,4-lactone* (**9**).—To a solution of **6** (395 mg) in DMF (8 mL) were added Ag_2O (1.16 g) and excess $PhCH_2Br$ (0.6 mL), and the mixture was stirred for 3 h at room temperature; TLC (4:1 toluene–EtOAc) of the organic layer showed two spots at R_f 0.55 (**8**, major) and 0.45 (**9**) (*cf.* **6**: R_f 0.05). Filtration followed by concentration in vacuo gave a syrup, which was extracted with $CHCl_3$. The extracts were washed with 0.05 M aq HCl and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (8:1 toluene–EtOAc) to give **8** (503 mg, 81%) and **9** (68 mg, 11%) as syrups. Compound **8** had $[\alpha]_D^{21}$ −160° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.17; H, 5.31; N, 16.75. Compound **9** had $[\alpha]_D^{20}$ −5° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.05; H, 5.26; N, 16.77.

5-Azido-2-O-*benzyl-3,5-dideoxy*-D-threo-*pentono-1,4-lactone* (**10**) *and 5-azido-2*-O-*benzyl-3,5-dideoxy*-D-erythro-*pentono-1,4-lactone* (**11**).—To a solution of **7** (1.08 g) in DMF (22 mL) were added Ag_2O (3.20 g) and excess $PhCH_2Br$ (1.64 mL), and the mixture was stirred for 3 h at room temperature. TLC (4:1 toluene–EtOAc) of the organic layer showed two spots at R_f 0.45 (**10**, major) and 0.55

(**11**). The mixture was treated as first described above to give **10** (1.36 g, 80%) and **11** (160 mg, 9%) as syrups. Compound **10** had $[\alpha]_D^{24}$ +4° (*c* 1, $CHCl_3$); the ^1H NMR spectrum was the same as that of **9**; in ROESY, a cross peak was observed between H-2 and H-4. Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.54; H, 5.37; N, 17.23. Compound **11** had $[\alpha]_D^{20}$ +161° (*c* 1, $CHCl_3$); the ^1H NMR spectrum was the same as that of **8**. Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.00; H, 5.52; N, 16.69.

*Methyl (2*S*,4*R*)-5-azido-2*-O-*benzyl-2,4-dihydroxypentanoate* (**12**).—A solution of **8** (4.58 g) in 0.1 M methanolic HCl (75 mL) was kept for 10 min at room temperature. TLC (4:1 toluene–EtOAc) of the solution showed two spots at R_f 0.35 (**12**) and 0.55 (**8**, minor). After addition of $CHCl_3$ (500 mL), the solution was washed with water, dried (Na_2SO_4), and concentrated. Chromatography (6:1 toluene–EtOAc) of the syrup gave **12** as a syrup (4.25 g, 82%) together with **8** recovered (640 mg). Compound **12** had IR (neat): 1740 (C=O), 2110 cm^{-1} (N_3).

*Methyl (2*S*,4*S*)-5-azido-2*-O-*benzyl-4-fluoro-2-hydroxypentanoate* (**13**) *and methyl (*S*)-5-azido-2*-O-*benzyl-2-hydroxy-4-pentenoate* (**14**).—To a solution of DAST (2.0 mL, 15.1 mmol) in 8:1 benzene–pyridine (45 mL) was added a solution of **12** (1.50 g, 5.37 mmol) in benzene (30 mL), and the mixture was kept for 1 h at room temperature and then for 2 h at 60 °C. TLC (10:2:1 cyclohexane–$CHCl_3$–acetone) of the solution showed spots at R_f 0.45 (**14**), 0.35 (**13**, major), 0.2 (trace), and 0.1. The mixture was poured into aq $NaHCO_3$ (satd, 300 mL) and toluene (300 mL), and after shaking for 10 min, the organic layer that separated was washed with water, dried (Na_2SO_4), and concentrated. Chromatography (10:2:1 cyclohexane–$CHCl_3$–acetone) of the residue gave **13** as a syrup (1.16 g, 77%), along with syrupy **14** (84 mg), ^1H NMR ($CDCl_3$): (the ratio of the *E* and *Z* isomers was ~1:2) δ 6.23 [dt, 1 H, H-5 (*Z*)], 5.94 [dt, 1 H, H-5 (*E*)], 5.34 [dt, 1 H, H-4 (*E*)], 4.94 [q, 1 H, H-4 (*Z*)], 4.44 and 4.71 [each d of 1 H, *J* 12 Hz, PhCH_2 (*Z*)], 4.43 and 4.72 [each d of 1 H, *J* 12 Hz, PhCH_2 (*E*)], 3.98 [dd, 1 H, H-2 (*Z*)], 3.95 [dd, 1 H, H-2 (*E*)], 3.75 (s, COOMe), 2.58 [dddd, 1 H, H-3b (*Z*)], 2.54 [ddt, 1 H, H-3a (*Z*)], 2.49 [m, 2 H, H-3a,3b (*E*)]; *J* (*E* isomer): $J_{2,3a}$ 7, $J_{2,3b}$ 5.5, $J_{3a,4} \approx J_{3b,4}$ 7.5, $J_{4,5}$ 13.5, $J_{3a,5} \approx J_{3b,5}$ ~1.5 Hz; (*Z* isomer): $J_{2,3a}$ 7, $J_{2,3b}$ 5.5, $J_{3a,3b}$ 15, $J_{3a,4} \approx J_{3b,4} \approx J_{4,5}$ 7.5, $J_{3a,5} \approx J_{3b,5}$ 1.5 Hz. Compound **13**, $[\alpha]_D^{24}$ −74° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{13}H_{16}FN_3O_3$: C, 55.51; H, 5.73; F, 6.75; N, 14.94. Found: C, 55.42; H, 5.81; F, 6.92; N, 14.93.

*(2*S*,4*S*)-5-Azido-2*-O-*benzyl-4-fluoro-2-hydroxypentanoic acid* (**15**).—To a solution of **13** (1.80 g) in MeOH (36 mL) was added M aq NaOH (9 mL), and the solution was kept for 1 h at room temperature. TLC (3:1 $CHCl_3$–MeOH) of the solution showed a single spot at R_f 0.5 (*cf.* **13**: R_f 0.9). The solution was concentrated to a low volume, diluted with water, and acidified with M aq HCl to pH ~1 under cooling. Extraction of the product with EtOAc followed by concentration gave **15** as a crystalline solid (1.70 g, 99%), mp 99–100 °C ($CHCl_3$–hexane), $[\alpha]_D^{24}$ −81° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{14}FN_3O_3$: C, 53.93; H, 5.28; F, 7.11; N, 15.72. Found: C, 53.74; H, 5.18; F, 6.86; N, 15.92.

*Methyl (2*S*,4*S*)-5-azido-2*-O-*benzyl-2,4-dihydroxypentanoate* (**17**).—A solution of **10** (1.56 g) in 0.1 M methanolic HCl (25.5 mL) was kept for 10 min at room temperature. TLC (4:1 toluene–EtOAc) of the solution showed two spots at R_f 0.4 (**17**) and 0.45 (**10**, minor). Processing as described for **12** gave a syrup, chromatography (6:1 toluene–EtOAc) of which gave **17** as a syrup (1.41 g, 80%) together with **10** recovered (230 mg). Compound **17** had IR (neat): 1740 (C=O), 2110 cm^{-1} (N_3).

*Methyl (2*S*,4*R*)-5-azido-2*-O-*benzyl-4-fluoro-2-hydroxypentanoate* (**18**).—To a solution of DAST (1.4 mL) in 8:1 benzene–pyridine (30 mL) was added a solution of **17** (1.00 g) in benzene (20 mL), and the mixture was treated as described for **13**. TLC (10:2:1 cyclohexane–$CHCl_3$–acetone) of the solution showed spots at R_f 0.45 (**14**), 0.35 (**18**, major), 0.2 (trace), and 0.1 (a mixture of **17** and several by-products). Similar purification as described for **13** gave **18** as a syrup (545 mg, 54%), together with **14** (125 mg). Compound **18** had $[\alpha]_D^{23}$ −60° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{13}H_{16}FN_3O_3$: C, 55.51; H, 5.73; F, 6.75; N, 14.94. Found: C, 55.63; H, 5.79; F, 6.98; N, 15.15.

*(2*S*,4*R*)-5-Azido-2*-O-*benzyl-4-fluoro-2-hydroxypentanoic acid* (**19**).—To a solution of **18** (350 mg) in MeOH (7 mL) was added M aq NaOH (1.8 mL), and the solution was kept for 1 h at room temperature. TLC (3:1 $CHCl_3$–MeOH) of the solution showed a single spot at R_f 0.5 (*cf.* **18**: R_f 0.9). Similar treatment as described for **15** gave **19** as a syrup (315 mg, 95%). An analytical sample (syrup) was prepared by column chromatography using

the lower phase of 20:1:1 $CHCl_3$–MeOH–20% aq AcOH, $[\alpha]_D^{22}$ −49° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{14}FN_3O_3$: C, 53.93; H, 5.28; F, 7.11; N, 15.72. Found: C, 54.15; H, 5.37; F, 7.12; N, 15.87.

(3S,5S)-3-Benzyloxy-5-fluoro-2-piperidinone (**21**).—A solution of **15** (200 mg) in 3:1 MeOH–H_2O (8 mL) was hydrogenated under H_2 in the presence of Pd-black for 40 min at room temperature. TLC (1:1 $CHCl_3$–MeOH) of the solution showed a single spot at R_f 0.2 (*cf.* **15**: R_f 0.65). Filtration followed by concentration gave **16** as a pale-yellow solid (168 mg, 93%). A mixture of the solid in CH_3CN (1.7 mL), 1,1,1,3,3,3-hexamethyldisilazane (3.1 mL), and 1.2 M ethereal HCl (0.6 mL; prepared by introducing HCl vapor into Et_2O) was refluxed overnight. TLC (1:3 toluene–EtOAc) of the solution showed a spot at R_f 0.25. Addition of MeOH (8 mL) followed by concentration gave a residue, which was purified by chromatography (1:3 toluene–EtOAc) to give **21** as a crystalline solid (90 mg, 58%), mp 119–120 °C (toluene–hexane), $[\alpha]_D^{22}$ −115° (*c* 1, $CHCl_3$); IR (KBr): 1630 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 5.95 (br s, 1 H, NH), 5.07 (dddq, 1 H, $J_{5,4ax}$ 3.5, $J_{5,4eq}$ 5, $J_{5,F}$ 48, $J_{5,6ax}$ 4, $J_{5,6eq}$ 3, $J_{5,NH}$ ~1 Hz, H-5), 4.72 and 5.02 (each d of 1 H, *J* 12 Hz, PhCH_2), 4.13 (dd, 1 H, $J_{3,4ax}$ 9, $J_{3,4eq}$ 5.5 Hz, H-3), 3.62 (dddd, 1 H, $J_{6ax,5}$ 4, $J_{6ax,F}$ 34, $J_{6ax,6eq}$ 13.5, $J_{6ax,NH}$ 2 Hz, H-6*ax*), 3.52 (dddt, 1 H, $J_{6eq,4eq}$ 2, $J_{6eq,5}$ 3, $J_{6eq,F}$ 18, $J_{6eq,6ax}$ 13.5, $J_{6eq,NH}$ 3 Hz, H-6*eq*), 2.47 (ddddd, 1 H, $J_{4eq,3}$ 5.5, $J_{4eq,4ax}$ 14.5, $J_{4eq,5}$ 5, $J_{4eq,6eq}$ 2, $J_{4eq,F}$ 12.5 Hz, H-4*eq*), 2.19 (dddd, 1 H, $J_{4ax,3}$ 9, $J_{4ax,4eq}$ 14.5, $J_{4ax,5}$ 3.5, $J_{4ax,F}$ 34 Hz, H-4*ax*); NOE difference spectroscopy: irradiation of F increased the signals of H-3 (27%), H-4*eq* (21%), H-5 (100%; taken as the reference for the increases), and H-6*eq* (23%). ^{19}F NMR ($CDCl_3$): δ −182.8 (dddt). Anal. Calcd for $C_{12}H_{14}FNO_2$: C, 64.56; H, 6.32; F, 8.51; N, 6.28. Found: C, 64.77; H, 6.24; F, 8.30; N, 6.43.

(3S,5R)-3-Benzyloxy-5-fluoro-2-piperidinone (**22**).—A solution of **19** (150 mg) in 3:1 MeOH–H_2O (6 mL) was hydrogenated in a similar manner as described for **21**. TLC (1:1 $CHCl_3$–MeOH) of the solution showed a single spot at R_f 0.2 (*cf.* **19**: R_f 0.65). Post-treatment as described for **16** gave **20** as a pale-yellow solid (128 mg, 95%). The solid was then treated as described for **21** to give **22** as a crystalline solid (73 mg, 62%); mp 85.5–86.5 °C (toluene–hexane), $[\alpha]_D^{23}$ −83° (*c* 1, $CHCl_3$); IR (KBr): 1645 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 6.09 (br s, 1 H, NH), 4.94 (m, 1 H, $J_{5,4a}$ 5.5, $J_{5,4b}$ 6, $J_{5,F}$ 49, $J_{5,6a}$ 4, $J_{5,6b}$ 5 Hz, $J_{5,NH} \leq 0.5$, H-5; irradiation of NH caused collapse to ddddd), 4.73 and 4.99 (each d of 1 H, *J* 12 Hz, PhCH_2), 3.88 (dd, 1 H, $J_{3,4a}$ 9, $J_{3,4b}$ 7 Hz, H-3), 3.54 (ddddd, 1 H, $J_{6b,4a}$ 1, $J_{6b,5}$ 5, $J_{6b,F}$ 14, $J_{6b,6a}$ 13.5, $J_{6b,NH}$ 4 Hz, H-6b), 3.43 (ddddd, 1 H, $J_{6a,4b} \leq 1$, $J_{6a,5}$ 4, $J_{6a,6b}$ 13.5, $J_{6a,F}$ 24, $J_{6a,NH}$ 3 Hz, H-6a), 2.52 (ddddd, 1 H, $J_{4b,3}$ 7, $J_{4b,4a}$ 14.5, $J_{4b,5}$ 6, $J_{4b,6a} \leq 1$, $J_{4b,F}$ 22 Hz, H-4b), 2.22 (ddddd, 1 H, $J_{4a,3}$ 9, $J_{4a,4b}$ 14.5, $J_{4a,5}$ 5.5, $J_{4a,6b}$ 1, $J_{4a,F}$ 21 Hz, H-4a); NOE difference spectroscopy: irradiation of H-3 increased the signals of H-4b (3.3%), H-5 (1.9%), and H-6a (2.2 %). ^{19}F NMR ($CDCl_3$): δ −178.3 (dddq; $J_{F,NH}$ ~1.5 Hz). Anal. Calcd for $C_{12}H_{14}FNO_2$: C, 64.56; H, 6.32; F, 8.51; N, 6.28. Found: C, 64.52; H, 6.29; F, 8.62; N, 6.35.

N-*Hydroxysuccinimide esters* (**24** *and* **25**) *of* **15** *and* **19**.—A mixture of **15** (or **19**) (1.0 mmol), *N*-hydroxysuccinimide (1.02 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (1.0 mmol) in dry EtOAc (5.5 mL) was stirred for 1 h at room temperature. The resultant precipitate was filtered off, washed with EtOAc, and the combined filtrate and washings were concentrated to give syrupy **24** (or **25**), which showed a single spot at R_f 0.45 in TLC (4:1 toluene–EtOAc), and was used without further purification.

1-N-[(2S,4S)-5-Amino-4-fluoro-2-hydroxypentanoyl]dibekacin (**26**).—To a solution of **23** [13] (716 mg, 0.75 mmol) in 2:1 THF–H_2O (40 mL) was added **24** (580 mg, ~1.6 mmol) in THF (10 mL) and, after the pH had been adjusted to ~8 by addition of aq $NaHCO_3$ (satd), the solution was kept for 1 h at room temperature. Concentration gave a residue, which was washed with water and EtOAc. The solid obtained (632 mg) was dissolved in M NH_3 in 2:1 THF–H_2O (36 mL) and the solution was kept for 40 h at room temperature [de(trifluoroacetyl)ation]. Concentration gave a residue, which was dissolved in 30:15: 1 1,4-dioxane–H_2O–AcOH (28 mL) and hydrogenated in the presence of Pd-black for 5 h. After filtration, the filtrate was concentrated, and the solid was chromatographed on a CM Sephadex C-25 column (aq 0→0.15 M NH_3) to give **26** as a solid, which was dried thoroughly in vacuo (0.2~1 mmHg) in a desiccator for 3 days in the presence of P_2O_5 (225 mg, 43%); $[\alpha]_D^{23}$ +71° (*c* 1, H_2O); ^1H NMR (DCl-D_2O, pD 3): δ (signals relating to the side chain are shown mainly) 5.83 (d, 1 H, H-1′), 5.22 (d, 1 H, H-1″), 5.11 (double multiplets, 1 H, H-4‴), 4.41 (dd, 1 H, H-2‴), 2.28 (dddd, 1 H, H-3‴b), 1.93 (dddd, 1 H, H-3‴a); $J_{1',2'} \approx J_{1'',2''}$ 3.8, $J_{2''',3'''a}$ 11, $J_{2''',3'''b}$ 3,

$J_{3'''a,3'''b}$ 15, $J_{3'''a,4'''}$ 2.5, $J_{3'''b,4'''}$ 10, $J_{3'''a,F}$ 37, $J_{3'''b,F}$ 13, $J_{4''',F}$ ~51 Hz. ^{19}F NMR (DCl-D_2O, Freon 11 as the external reference, pD 3): δ −192.5 (m). Anal. Calcd for $C_{23}H_{45}FN_6O_{10}\cdot 1.5\ H_2CO_3\cdot H_2O$: C, 42.30; H, 7.24; F, 2.73; N, 12.08. Found: C, 42.11; H, 7.30; F, 2.78; N, 11.98.

1-N-*[(2S,4R)-5-Amino-4-fluoro-2-hydroxypentanoyl]dibekacin* (**27**).—Compound **23** (530 mg, 0.56 mmol) was treated with **25** (410 mg, 1.1 mmol) in the manner described for **26** to give **27** as a solid (178 mg, 49%); $[\alpha]_D^{22}$ +81° (*c* 1, H_2O); ^{1}H NMR (DCl-D_2O, pD 3): δ 5.82 (d, 1 H, H-1′), 5.22 (d, 1 H, H-1″), 5.15 (double multiplets, 1 H, H-4‴), 4.42 (dd, 1 H, H-2‴), 2.25 (ddt, 1 H, H-3‴b), 2.18 (ddt, 1 H, H-3‴a); $J_{1',2'} \approx J_{1'',2''}$ 3.8, $J_{2''',3'''a}$ 7.5, $J_{2''',3'''b}$ 4.5, $J_{3'''a,3'''b}$ 15, $J_{3'''a,4'''}$ 7.5, $J_{3'''b,4'''}$ 4.5, $J_{3'''a,F}$ 18.5, $J_{3'''b,F}$ 30, $J_{4''',F}$ ~50 Hz. ^{19}F NMR (DCl–D_2O, Freon-11 as the external reference, pD 3): δ −190.3 (m). Anal. Calcd for $C_{23}H_{45}FN_6O_{10}\cdot H_2CO_3\cdot 0.5\ H_2O$: C, 43.96; H, 7.38; F, 2.90; N, 12.82. Found: C, 43.94; H, 7.51; F, 3.04; N, 13.07.

1-N-*[(S)-3-Amino-2-hydroxypropanoyl]dibekacin* (**28**).—A mixture of (*S*)-*N*-(benzyloxycarbonyl)-isoserine [15,16] (1.20 g, 5.02 mmol), *N*-hydroxysuccinimide (580 mg, 5.04 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (1.04 g, 5.04 mmol) in THF (25 mL) was stirred for 2 h at room temperature. The precipitate was filtered off and washed thoroughly with THF. The combined filtrate and washings were concentrated to give the active ester of the protected isoserine as a syrup. Subsequently the syrup was treated with **23** (2.74 g, 2.88 mmol) as described for **26** to give a solid, which was subjected to chromatography on a column of CM-Sephadex C-25 (aq 0→0.5 M NH_3) to give **28** as a solid (920 mg, 53%), $[\alpha]_D^{25}$ +89° (*c* 1, H_2O) (lit [14], no data reported); ^{1}H NMR (DCl–D_2O, pD 3): δ 5.84 (d, 1 H, H-1′), 5.21 (d, 1 H, H-1″), 4.52 (dd, 1 H, H-2‴), 3.44 (dd, 1 H, H-3‴b), 3.28 (dd, 1 H, H-3‴a), 2.32 (dt, 1 H, H-2*eq*), 1.94 (q, 1 H, H-2*ax*); $J_{1,2ax} \approx J_{2ax,2eq} \approx J_{2ax,3}$ 13, $J_{1,2eq} \approx J_{2eq,3}$ 4.5, $J_{1',2'} \approx J_{1'',2''}$ 3.8, $J_{2''',3'''a}$ 8.5, $J_{2''',3'''b}$ 4, $J_{3'''a,3'''b}$ 13 Hz. Anal. Calcd for $C_{21}H_{42}N_6O_{10}\cdot H_2CO_3$: C, 43.70; H, 8.00; N, 13.90. Found: C, 43.40; H, 7.68; N, 14.10.

1-N-*[(S)-5-Amino-2-hydroxypentanoyl]dibekacin* (**29**).—(*S*)-5-(Benzyloxycarbonyl)amino-2-hydroxypentanoic acid [15,17,18] (1.50 g, 5.61 mmol) was treated with *N*-hydroxysuccinimide (660 mg, 5.73 mmol) in the manner described for **24** to give the corresponding active ester as a syrup. Subsequently the syrup was treated with **23** (3.17 g, 3.34 mmol) as described for **28** to give **29** as a solid (1.45 g, 67%); $[\alpha]_D^{24}$ +82° (*c* 0.5, H_2O); ^{1}H NMR (DCl–D_2O, pD 3): δ 5.83 (d, 1 H, H-1′), 5.22 (d, 1 H, H-1″), 4.20 (dd, 1 H, H-2‴), 3.09 (slightly br t, 2 H, *J* 7.5 Hz, H-5‴a,5‴b), 2.29 (dt, 1 H, H-2*eq*), ~1.93 (H-3‴b), 1.91 (q, 1 H, H-2*ax*), 1.77–1.95 (H-4‴a,4‴b), 1.62–1.74 (m, 2 H, H-4′*ax*, 3‴a); $J_{1',2'} \approx J_{1'',2''}$ 3.8, $J_{2''',3'''a}$ 9, $J_{2''',3'''b}$ 3.5 Hz. Anal. Calcd for $C_{23}H_{46}N_6O_{10}\cdot H_2CO_3\cdot H_2O$: C, 44.57; H, 7.79; N, 13.00. Found: C, 44.47; H, 7.92; N, 12.55.

1-N-*[(S)-6-Amino-2-hydroxyhexanoyl]dibekacin* (**30**).—(*S*)-6-(Benzyloxycarbonyl)amino-2-hydroxyhexanoic acid [15,18] (1.50 g, 5.33 mmol) was treated with *N*-hydroxysuccinimide (625 mg, 5.43 mmol) in the manner described for **24** to give the active ester as a syrup. Subsequently the syrup was treated with **23** (3.20 g, 3.37 mmol) as described for **28** to give **30** as a solid (1.40 g, 63%), $[\alpha]_D^{25}$ +80° (*c* 1, H_2O); ^{1}H NMR (DCl–D_2O, pD 3): δ 5.85 (d, 1 H, H-1′), 5.24 (d, 1 H, H-1″), 4.20 (dd, 1 H, H-2‴), 3.07 (slightly br t, 2 H, *J* 7.5 Hz, H-6‴a,6‴b), 2.31 (dt, 1 H, H-2*eq*), 1.93 (q, 1 H, H-2*ax*), 1.88 (dddd, 1 H, H-3‴b), 1.76 (m, 2 H, H-5‴a,5‴b), ~1.68 (H-3‴a), 1.55 (m, 2 H, H-4‴a,4‴b); $J_{1',2'} \approx J_{1'',2''}$ 3.8, $J_{2''',3'''a}$ 9, $J_{2''',3'''b}$ 4, $J_{3'''a,3'''b}$ 14, $J_{3'''b,4'''a}$ 6.5 (or 10), $J_{3'''b,4'''b}$ 10 (or 6.5) Hz. Anal. Calcd for $C_{24}H_{48}N_6O_{10}\cdot H_2CO_3\cdot H_2O$: C, 45.45; H, 7.93; N, 12.72. Found: C, 45.71; H, 7.73; N, 12.52.

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