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Study on fluorination–toxicity relationships. Syntheses of 1-*N*-[(2*R*,3*R*)- and (2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl] derivatives of kanamycins

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ABSTRACT

(2*R*,3*R*)- And (2*R*,3*S*)-4-azido-3-fluoro-2-hydroxybutanoic acids (**11** and **22**) have been prepared from 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**) and 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**12**), respectively. They were then coupled to the H_2N-1 group of suitably protected kanamycin A or kanamycin B analogs to give, 1-*N*-[(2*R*,3*R*)- and (2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl]kanamycins (**32–35**). This group of compounds (**32–34**) exhibited similar antibacterial activity and toxicity level as those of the corresponding 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] (AHB) derivatives of kanamycins. The base strength of the H_2N-4‴ group of **32** and **34**, as determined by ^{13}C NMR spectroscopy (in D_2O) at varying pD values, was found to be lower when compared to the basicity for the corresponding AHB analogs. The relationship between observed toxicity and base strength of the H_2N-4‴ group is discussed.

INTRODUCTION

In pursuing the structure–toxicity relationships of aminoglycoside antibiotics, we have found that deoxyfluorination of certain hydroxyl groups vicinally situated to a specific amino group sometimes gave rise to compounds of decreased toxicity relative to the parent compounds. 3-Demethoxy-3-fluorosporaricin A (refs 1 and 2) and 3′-deoxy-3′-fluorokanamycin B (refs 3 and 4) lowered the toxicity of the parent compounds, respectively, with enhancement or, at worst, retention of antibacterial activity. Recently we have prepared 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives[5] of kanamycin B analogs, together with their 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivatives[6], and found that these compounds showed decreased toxicity. We considered that this reduction in toxicity may result from the decrease in basicity of a specific amino group, induced by the strongly electron-withdrawing fluorine atom. In this paper we describe the synthesis of 1-*N*-[(2*R*,3*R*)- and

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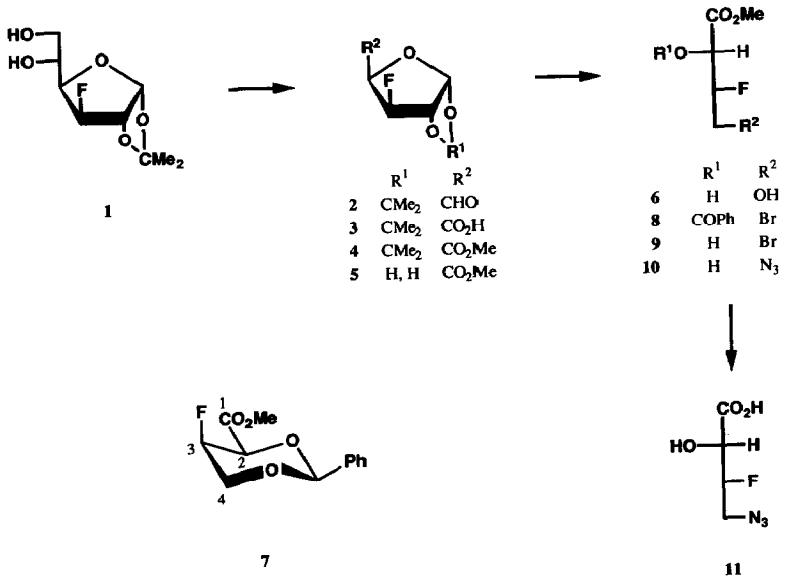
(2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl] derivatives of kanamycin A, tobramycin, and dibekacin[7,8]. This work was pursued with the hope of obtaining derivatives exhibiting decreased toxicity relative to compounds having the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] (AHB) residue, such as amikacin[9] or arbekacin[10], by decreasing the base strength of the strongly basic 4-amino group of the AHB residue.

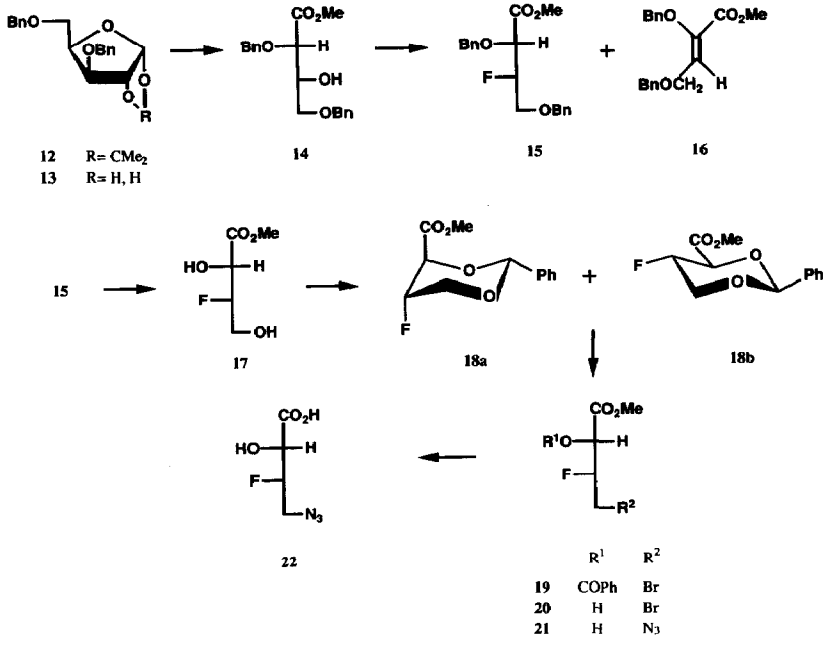
RESULTS AND DISCUSSION

Introduction of a fluorine atom at C-3 of AHB gives rise to chirality at this position; thus two 3-fluoro compounds of 3*R* and 3*S* configurations must be prepared. Synthesis of the former butanoic acid is described first. Periodate oxidation of 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose[11] (**1**) followed by oxidation of the resulting dialdose derivative **2** with $AgNO_3$–KOH gave 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranuronic acid (**3**). Esterification of **3** with CH_2N_2 (to give **4**), followed by removal of the isopropylidene group gave the free sugar **5**, which was successively treated with $NaIO_4$ and $NaBH_4$ to give the four-carbon ester **6**. Acetalation of **6** with α,α-dimethoxytoluene gave the cyclic acetal **7** having the six-membered ring. Its structure and conformation were deduced from the ^1H and ^{19}F NMR spectra, which showed a chair conformation with an axial fluorine (large $J_{2,F}$ and $J_{4ax,F}$ values) and an equatorial methoxycarbonyl group. The conformation of the ring was further substantiated by NOE experiments (see Experimental). NOEs were observed between any combination of the benzylidene methine, H-2, and one of the C-4 methylene protons. This indicates that the three hydrogens concerned are all axial, precluding skew conformations, and leads to the conclusion that **7** has 2*R*,3*R* structure. Hanessian reaction[12] of **7** with *N*-bromosuccinimide (NBS) gave methyl 2-benzoyloxy-4-bromo-3-fluorobutanoate **8** in high yield. After azide displacement of the bromine (NaN_3 in DMF, 44% yield), the corresponding 4-azido ester was subjected to base hydrolysis (0.5 M NaOH in 1:1 oxolane–water at room temperature); however it gave mainly unsaturated products lacking the fluorine, along with only ~7% (based on **8**) of the desired **11**. These unsaturated products were presumed to be 2,3- and 3,4-enoic acids, as judged from the ^1H NMR spectrum. This disappointing result may have been due to the presence of the weakly electron-withdrawing benzoyloxy group at C-2. Therefore, compound **8** was deprotected by heating with HBr in AcOH, and the resulting free acid was reesterified with CH_2N_2, to give the 4-bromo-2-hydroxy ester **9**. The latter was converted into the corresponding 4-azido ester **10**, alkaline hydrolysis of which furnished the free acid **11** (45% based on **8**), supporting the foregoing assumption.

Synthesis of the 3*S* compound is described next. 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose[13] (**12**) was deacetalated and the free sugar **13** was oxidized successively with $NaIO_4$ and $AgNO_3$–KOH to give the 2,4-di-*O*-benzyl-D-*threo*-trihydroxybutanoic acid, which was subsequently esterified with CH_2N_2.



Treatment of the resulting methyl ester **14** with diethylaminosulfur trifluoride[14] (DAST, Et_2NSF_3) in refluxing CH_2Cl_2 gave the 3-fluoro derivative **15** with inversion, together with an unsaturated product **16**. The *Z* configuration of **16** was deduced from the results of a NOESY experiment. NOE cross-peaks were observed between CO_2CH_3 and H-3, and between $PhCH_2O$-2 and CH_2-4. The use of cyclohexane, benzene–pyridine, CCl_4, diethyl ether, oxolane, or EtOAc (all at room temperature) in this reaction instead of boiling CH_2Cl_2 as the solvent gave a preponderance of **16** over **15** (only in benzene were equal amounts of **15** and **16** formed). As the separation of **15** from the mixture was difficult due to the similar mobilities of **15** and **16**, the mixture was treated with Br_2 in CCl_4 in order to convert **16** into the bromo derivatives of different mobilities. Compound **15** could then be isolated chromatographically in good yield. This was catalytically debenzylated to give the diol **17**, which was acetalated with α,α-dimethoxytoluene to give two cyclic compounds, **18a** (major) and **18b**. The structures were determined by their ^1H and ^{19}F NMR spectra, along with NOE experiments. In **18a**, small vicinal coupling-constants of $J_{2,3}$, $J_{3,4ax}$, and $J_{3,4eq}$ (~ 2 Hz each), and $J_{2,F}$ and $J_{4eq,F}$ (~ 15 Hz each), except for $J_{4ax,F}$ (40 Hz), indicate that **18a** adopts a chair conformation with equatorial phenyl and axial fluorine and methoxycarbonyl groups. An observed long-range coupling ($^4J_{2,4eq}$) also supports the conclusion. In **18b**, a chair conformation having three equatorial substituents was deduced from the ^1H and ^{19}F NMR spectra. The small coupling constants (0–6 Hz) observed between fluorine and each of the three vicinal protons supported[15–18] the antiperiplanar relationships between (equatorial) fluorine and each of the two ring-oxygen



atoms. It is noteworthy that the isomer **18a** having diaxial substituents was produced in excess over the triequatorial isomer **18b**; in both 0.5- and 2.5-h reaction times **18a** and **18b** were produced in the same ratio. This suggests that **18a** is the thermodynamically more-stable isomer *. Treatment of a mixture of **18a** and **18b** with NBS as described for **8** gave the methyl 2-benzoyloxy-4-bromo-3-fluorobutanoate **19**, which was transformed into the final product, (2*R*,3*S*)-4-azido-3-fluoro-2-hydroxybutanoic acid (**22**) through debenzoylation (to give **20**), displacement of the bromine by azide, and alkaline hydrolysis. The ^{1}H and ^{19}F NMR spectra of the compounds prepared are shown in Table I.

Coupling of the 3-fluoro acids (**11** and **22**) with kanamycins were performed by using the *N*-hydroxysuccinimide esters (**26** and **27**) of **11** and **22**. Thus 3″-*N*-(trifluoroacetyl)-3,6′-bis- (**23**) or -3,2′,6′-tris-*N*-(benzyloxycarbonyl) derivatives (**24** and **25**) of kanamycins, prepared according to the Zn-chelate–ethyl trifluoroacetate method[19], were condensed with the above active esters to give the 1-*N*-acyl derivatives (**28–31**). Deblocking followed by reduction ($N_3 \rightarrow NH_2$) gave the final products (**32–35**). The ^{13}C NMR data for **32–35** are shown in Table II together with those for amikacin (AMK) and arbekacin (ABK).

Antibacterial screening data for these compounds against standard as well as selected bacterial strains are shown in Table III. It may be noted that the

* On storage in a desiccator, **18b** gradually changed into a mixture of **18a**, **18b**, and **17**, whereas **18a** under the same conditions remained unchanged.

1-*N*-[(2*R*,3*R*)-4-amino-3-fluoro-2-hydroxybutanoyl] derivatives (**32–34**) show similar (slightly higher *) antibacterial activities, relative to the respective parent antibiotics (amikacin[9], 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3′-deoxykanamycin B, and arbekacin[10]). In contrast, the 1-*N*-[(2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl] derivative † (**35**) of 3′,4′-dideoxykanamycin B showed decreased activity, relative to arbekacin. This suggests that attachment of a fluorine with the *R* configuration at C-3 of the AHB residue enhances the activity relative to the parent compounds having the AHB residue.

The pK_a values of the H_3N^+-4‴ group of **32** and **34** were determined as a measure of influence of the F-3‴ atom upon the basicity of the amino group. This was performed by measuring the shift values of C-3‴ in the ^{13}C NMR spectra upon varying the pD values. As the C-3‴ signal is expected to incur upfield shift of 5–6 ppm[20] (β shift) upon protonation of H_2N-4‴, the pK_a values of protic salt H_3N^+-4‴ can be determined by measuring the midpoint (of the pD values in the D_2O solution) between the lines before and after protonation on the titration curve obtained for the C-3‴ shift – pD value (see Fig. 1). This result showed that the pK_a value (10.2) of H_3N^+-4‴ of amikacin or arbekacin was lowered to 8.7 in **32** and **34**, indicating that the attachment the F-3‴ atom of *R* configuration (and possibly F-3‴ of *S* configuration) clearly induced a decrease in the base strength of the H_2N-4‴ group. On the other hand the toxicities of **32** (LD_{50} 250) ** and **34** (LD_{50} 80) remained almost the same relative to the measured toxicity for the parent compounds, amikacin (LD_{50} 220) and arbekacin (LD_{50} 80), respectively. This result suggests that the toxicity of these compounds is not influenced by the base strength of the H_2N-4‴ group. This result was unexpected in view of toxicity–fluorination relationships of kanamycins obtained previously in our laboratory. In 3′-deoxy-3′-fluoro[3,4], and 5-deoxy-5-fluoro as well as 5-deoxy-5,5-difluoro analogs[5,6] of kanamycin, toxicities were lowered, relative to the respective parent compounds, possibly by the decrease in basicity[21] of the H_2N-2′ and -3 groups, respectively. How could the undiminished toxicities of **32** and **34** be reconciled with these observations? We consider that the toxicity of **32** and **34** (and related compounds) may be largely influenced by the proportions of ionic (H_3N^+) and nonionic (H_2N) forms in which the 4‴-amino group exists, and not simply by the

* A characteristic feature is that **32**, **33**, and **34**, and **35** showed slightly higher and weaker activities, respectively, against bacteria producing acetyltransferases [AAC(3), AAC(2′), and AAC(6′)] relative to the respective parent antibiotics (see Table III). This suggests that the F-3‴ influences the binding of the compounds to the acetyltransferases although the H_2N-3, -2′, and -6′ groups are located remote from F-3‴.

† Crude 1-*N*-[(2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl]-3′-deoxykanamycin B (details of preparation not published) also showed lower activity (as in **35**) than 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3′-deoxykanamycin B. This suggests that the attachment of (3‴*S*)-fluorine decreases the activity in general.

** Intravenous injection in mice, expressed as mg/kg.

TABLE I

^1H and ^{19}F NMR data for **3–11**, **14**, **15**, and **17–22** (δ in ppm J in Hz) in $CDCl_3$ or CD_3OD (for **6**, **11**, **17**, and **22**)

Compound	H-1	H-2	H-3	H-4a	H-4b	F-3	Other signals	$J_{2,3}$	$J_{3,4a}$	$J_{3,4b}$	$J_{2,F}$	$J_{3,F}$	$J_{4a,F}$	$J_{4b,F}$	Other couplings	
3	6.14 d	4.76 dd	5.26 dd	4.89 dd		−201.9 ddd	1.35, 1.51 (CMe_2) 9.32 (CO_2H)	0	2.7		9.5	49.5	31.5		$J_{1,2}$	3.5
4	6.13 d	4.73 dd	5.19 dd	4.84 dd		−201.7 ddd	1.34, 1.49 (CMe_2) 3.83 [a]	0	2.7		10	50	31.5		$J_{1,2}$	3.5
5 (α)	5.79 d	4.38 sl.br dd	5.20 ddd	4.96 dd		−197.1 ddd	3.82 [a]	1.5	3.5		13	50.5	31		$J_{1,2}$	3.5
5 (β)	5.43 s	4.45 sl.br dd	5.18 sl.br dd	4.98 dd		−193.1 ddd	3.84 [a]	<1	5		12	51.5	28		$J_{1,2}$	0
6		4.38 dd	4.80 dddd	3.78 ddd	3.82 ddd	−203.9 dddd	3.79 [a]	2.5	5	6.5	30	47.5	~23	~17		12 [b]
7		4.63 dd	4.86 dq	4.10 ddd	4.46 dt	−201.2 dddd	3.84 [a] 5.59 ($CHPh$)	1.5	1.5	1.5	32	46.5	39	13.5		13.5 [b]
8		5.74 dd	5.31 ddt	3.59 ddd	3.64 dt	−186.8 dddd	3.84 [a]	2.5	7	7	29.5	45.5	16	11		11 [b]
9		4.50 ddd	4.98 dddd	3.59 dt	3.66 ddd	−190.6 dddd	3.07 (d, OH) 3.88 [a]	1.5	6.5	7.5	31	46	10.5	16		10.5 [b] 7 [c]
10		4.27 ddd	4.90 dddd	3.51 ddd	3.77 dt	−201.5 dddd	3.08 (d, OH) 3.89 [a]	2	5	7.5	31	47	25	13		13 [b] 6.5 [c]
11		4.27 dd	4.94 dddd	3.50 ddd	3.73 dt	−200.3 dddd		2	4	8	31	47.5	28.5	13.5		13.5 [b]
14		4.14 d	4.12 ddd	3.54 dd	3.58 dd		2.56 (d, OH) 3.72 [a]	3.5	6	5					$J_{3,OH}$	9.5 [b] 7.5

TABLE I (continued)

Compound	H-1	H-2	H-3	H-4a	H-4b	F-3	Other signals	$J_{2,3}$	$J_{3,4a}$	$J_{3,4b}$	$J_{2,F}$	$J_{3,F}$	$J_{4a,F}$	$J_{4b,F}$	Other couplings	
15		4.31 dd	4.92 dq	3.78 dd		−195.4 ddt	3.71 [a]	5	5	5	11.5	46.5	23	23		
17		4.41 dd	4.66 dq	3.66–3.90		−197.0 ddt	3.76 [a]	5	5	5	13.5	47	~23	~23		
18a		4.90 dt	4.89 dq	4.10 ddd	4.38 ddt	−190.5 ddt	3.87 [a] 5.88 (CHPh)	2	1.5	2	16	46	40	14	$J_{2,4b}$	13.5 [b] 2
18b		4.39 dd	4.89 ddt	3.84 ddd	4.50 dd	−200.5 dt	3.84 [a] 5.56 (CHPh)	9.5	9.5	5.5	5.5	48.5	6	~0		11 [b]
19		5.66 dd	5.18 ddt	3.62–3.85		−185.2 ddt	3.82 [a]	3.5	~6	~6	17.5	46	20	17.5		
20		4.51 ddd	4.88 ddt	3.52–3.72		−184.9 dq	3.18 (d, OH) 3.87 [a]	4	6	6	18	46.5	18	18		6.0 [c]
21		4.45 dt	4.81 ddt	3.49 ddd	3.70 ddd	−193.9 ddt	3.21 (d, OH) 3.87 [a]	4	4.5	7	16	47	24	17.5		13.5 [b] 4.5 [c]
22		4.39 dd	4.82 dddd	3.46 ddd	3.70 ddd	−193.2 dddd		4.5	3.5	7.5	15	47.5	28	18.5		14 [b]

[a] Signals for CO_2Me (s, 3 H). [b] $J_{4a,4b}$. [c] $J_{2,OH}$.

TABLE II

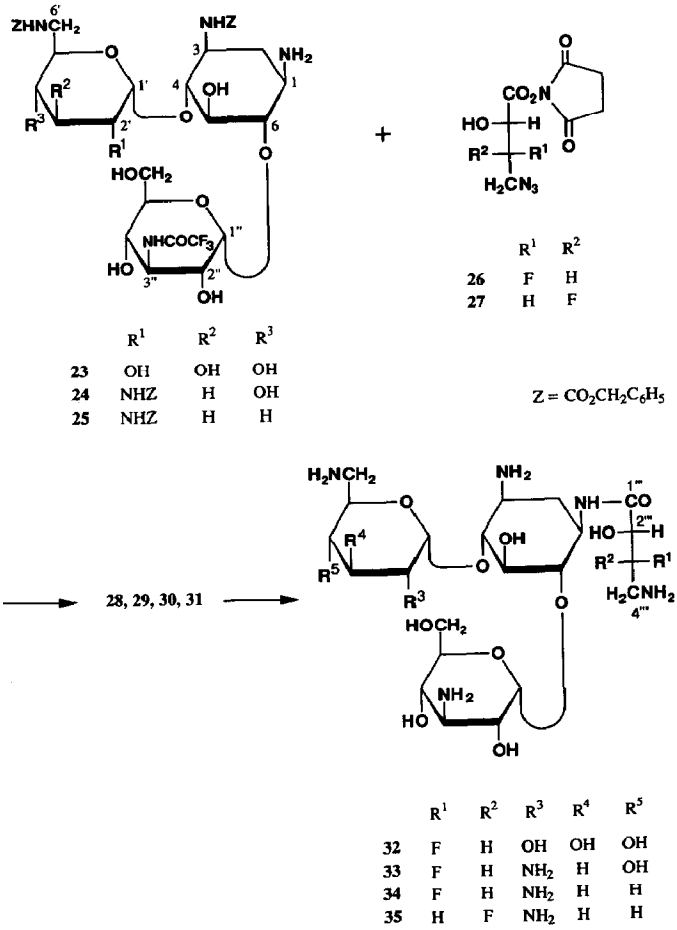
^{13}C NMR chemical shifts [a] (δ [b], ppm) and coupling constants ($J_{C,F}$ Hz) for **32–35**, amikacin (AMK), and arbekacin (ABK) in DCl–D_2O (pD 3)

	Compound					
	32	**33**	**34**	**35**	AMK	ABK
C-1	49.9	49.8	49.8	49.7	49.7	49.6
C-2	30.9	31.1	31.1	31.1	31.0	31.2
C-3	48.7	49.7	49.8	49.7	48.7	49.8
C-4	79.8 [c]	78.8	78.4 [c]	78.5	80.0 [c]	78.5
C-5	73.5	75.7	75.8	75.8	73.3	75.7
C-6	80.2 [c]	79.7	79.8 [c]	80.5	81.1 [c]	81.1
C-1′	96.4	95.0	95.9	96.0	96.3	95.9
C-2′	71.7	48.8	49.7	49.7	71.7	49.7
C-3′	73.2	30.2	21.4	21.4	73.2	21.5
C-4′	71.7	65.4	26.3	26.2	71.7	26.3
C-5′	69.6	71.2	66.8	66.8	69.6	66.9
C-6′	41.2	40.8	43.5	43.4	41.2	43.5
C-1″	98.2	98.1	98.1	98.6	98.9	98.9
C-2″	68.9	68.8	68.9	68.8	68.9	68.8
C-3″	56.0	55.9	55.9	55.9	56.2	56.1
C-4″	66.4	66.4	66.4	66.4	66.4	66.5
C-5″	73.0	73.1	73.1	73.1	72.9	73.0
C-6″	60.7	60.7	60.8	60.7	60.7	60.7
C-1‴	172.9 d	173.0	173.0	172.4 d	176.3	176.3
C-2‴	72.0 d	72.0 d	72.0 d	72.1 d	70.5	70.5
C-3‴	90.8 d	90.8 d	90.9 d	91.5 d	31.7	31.7
C-4‴	41.4 d	41.4 d	41.4 d	40.5 d	37.9	37.9
$J_{C\text{-}1''',F}$	3.1	~ 0	~ 0	7.2		
$J_{C\text{-}2''',F}$	19.1	18.9	19.2	22.3		
$J_{C\text{-}3''',F}$	176.9	176.7	176.7	176.7		
$J_{C\text{-}4''',F}$	20.6	20.7	20.7	20.5		

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

base strength as such. In blood or tissue fluid, the H_2N-4‴ group will largely be protonated, independent of the presence or absence of F-3‴, because the pH value is normally 6–8; when the H_3N^+-4‴ group has pK_a 8.7 as in 3-fluoro-AHB (meaning that equimolar proportions of H_3N^+ and H_2N forms exist at pH 8.7), only one-sixth or less of the amino groups are in the H_2N form at values of pH 8 or below. Therefore, in order to diminish in practical terms the toxicity of aminoglycoside antibiotics by decreasing the basicity of a certain amino group, such a decrease should at least reach a pK_a value of ~ 8, as was just the case for the 3′-deoxy-3′-fluoro and 5-deoxy-5-fluoro (and 5-deoxy-5,5-difluoro) analogs of kanamycin.

Another problem which should be considered in terms of fluorination–biological activity (antibacterial and toxicity) is the spatial arrangement of the fluorinated AHB residues (3*R* and 3*S*) compared to that of AHB residue in amikacin and arbekacin. In the NMR spectra of the fluorinated residue in **32–34**, the coupling



constants (Table IV) for the corresponding protons in the three compounds are almost identical, and the magnitudes indicate that bond relationships between C-2‴–H-2‴ and C-3‴–F, between C-3‴–F and C-4‴–H-4‴a, and between C-3‴–H-3‴ and C-4‴–H-4‴b (see Fig. 2) are antiperiplanar. This indicates a zigzag conformation for the chain of C-1‴–C-2‴–C-3‴–C-4‴–N (five atoms are on the same plane), as shown in Fig. 2A. In contrast, the conformation of the corresponding residue of **35** was estimated as shown in Fig. 2B. The reliability of these assigned conformations (A and B) were examined by MM3(89) calculations using structurally related models, namely 1-*N*-[(2*R*,3*R*)- and (2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl]-2-deoxy-6-*O*-methylstreptamines. The results showed that the projection angles relating to H-2‴–C-2‴–C-3‴–H-3‴ and H-3‴–C-3‴–C-4‴–H-4‴a for the A portion of the former model are −62 and +63°, respectively, and the angles relating to H-2‴–C-2‴–C-3‴–H-3‴ and H-3‴–C-3‴–C-4‴–H-4‴b for

TABLE III

Minimal inhibitory concentration [a] (μg mL^{-1}) of **32–35**, amikacin (AMK), and 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivatives (TBAH and ABK) of 3′-deoxykanamycin B (tobramycin) and 3′,4′-dideoxykanamycin B

Test organism [b]	AMK	**32**	TBAH	**33**	ABK	**34**	**35**
St.a. FDA 209 P	0.8	1.6	< 0.2	0.4	0.4	0.4	0.4
St.a. Smith	0.4	0.8	< 0.2	< 0.2	0.4	< 0.2	0.4
St.a. Ap01 [c]	3.1	6.2	1.6	0.8	1.6	1.6	1.6
Micr. l. FDA16	6.2	3.1	0.8	0.8	0.8	0.4	12.5
Micr. l. PCI 1001	3.1	3.1	0.8	1.6	0.8	0.4	6.2
Coryn. b. 1810	0.8	0.8	< 0.2	0.4	0.4	< 0.2	6.2
E.c. NIHJ	0.8	0.4	0.4	0.4	0.4	< 0.2	0.4
E.c. K-12 R5 [d]	25	12.5	12.5	3.1	12.5	3.1	50
E.c. K-12 ML1629 [e]	1.6	1.6	0.8	0.8	0.8	0.4	1.6
E.c. K-12 ML1410 R81 [e]	3.1	1.6	0.8	0.8	0.8	0.8	1.6
E.c. K-12 LA290 R55 [f]	1.6	1.6	0.8	0.4	0.4	0.4	1.6
E.c. K-12 LA290 R64	0.8	0.4	0.4	0.4	0.4	< 0.2	0.4
E.c. W677	0.4	0.4	< 0.2	< 0.2	0.4	< 0.2	0.8
E.c. JR66/W677 [f,g]	3.1	1.6	0.8	0.4	1.6	0.8	1.6
E.c. JR225 [h]	1.6	0.8	0.4	< 0.2	0.4	< 0.2	0.8
Kl.p. PCI602	1.6	0.8	0.8	0.4	0.8	0.4	0.8
Kl.p. 22#3038 [f,g]	3.1	3.1	1.6	1.6	1.6	1.6	1.6
Sh.s. JS11746	3.1	1.6	0.8	0.8	1.6	0.8	1.6
Sal.e. 1891	1.6	3.1	1.6	1.6	0.8	0.8	3.1
Serr. marc.	3.1	3.1	6.2	3.1	6.2	3.1	12.5
Prot.r. GN311	1.6	0.4	0.8	0.4	3.1	0.8	1.6
Prov. sp Pv 16 [i]	1.6	1.6	0.8	0.8	1.6	0.8	6.2
Prov. sp. 2991 [i]	1.6	0.8	3.1	1.6	6.2	1.6	25
Ps. aerug. A3	0.8	0.8	< 0.2	< 0.2	< 0.2	< 0.2	0.8
Ps. aerug. H9 [g]	3.1	6.2	0.8	1.6	1.6	1.6	1.6
Ps. aerug. GN315 [d]	25	25	3.1	3.1	3.1	3.1	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*; *Coryn. b.*, *Corynebacterium bovis*; *E.c.*, *Escherichia coli*; *Kl. p.*, *Klebsiella pneumoniae*; *Sh. s.*, *Shigella sonnei*; *Sal. e.*, *Salmonella enteritidis*; *Serr. marc.*, *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), and [i] AAC(2′).

the B portion of the latter model are −68 and +179°, respectively, supporting the structures A and B, although several other energy-minimum conformations are found to exist by the MM3 calculations. The conformation of the AHB residue in amikacin and arbekacin was, however, not determined based on the NMR data because of inability to discriminate between the two H-3‴ hydrogens (Table IV). Thus a similar model compound, 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-6-*O*-methylstreptamine was selected, and the energy-minimum conformations were searched by using MM3(92). The result showed that the conformations as depicted in both A and B (see Fig. 2, in which each of the F atoms in A and B is substituted by H) exist as two of the nine energy-minimum conformations, with no eclipse of any of the substituents vicinally situated. The foregoing conformational study gives

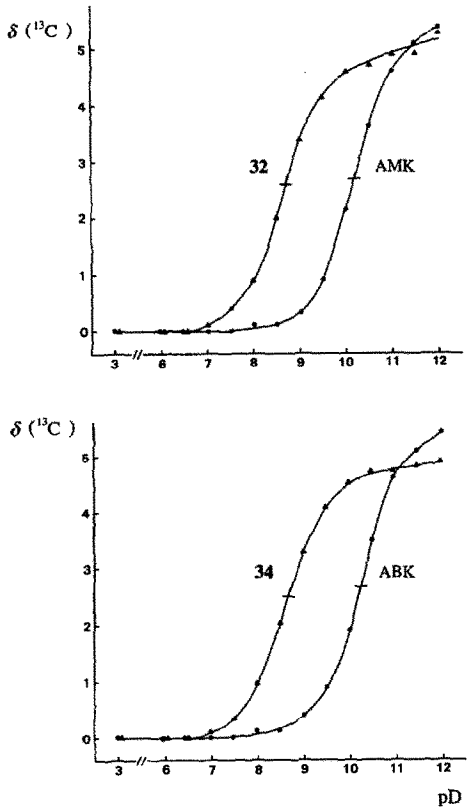


Fig. 1. Titration curves for pD–^{13}C-3‴ shift values (the values in strongly acidic region are taken as zero) of **32** and amikacin (AMK) (upper), and **34** and arbekacin (ABK) (down). The midpoints between the levels of strongly acidic and strongly basic regions are indicated by a short line on the curves.

little information on chemical modification–biological activity relationships; however, it may be stated that compounds having a zigzag conformation as shown in A should give slightly better antibacterial activity (Table III) than the compounds having a conformation of type B.

TABLE IV

Coupling constants (J in Hz) for the fluorinated AHB residues [a] of **32–34** and **35**, and the AHB residue of amikacin (AMK) and arbekacin (ABK) measured in DCl–D_2O (pD 3)

	$J_{2''',3'''}$	$J_{2''',F}$	$J_{3''',4'''a}$	$J_{3''',4'''b}$	$J_{4'''a,F}$	$J_{4'''b,F}$
32, **33**, **34**	2	31 ~ 31.5	2.5 ~ 3	8.5 ~ 9	31 ~ 32	~ 18
35	4	18	2.5	9	31	18
AMK, ABK	4, 9		7, 7			

[a] $J_{3''',F}$ 47 ~ 48 Hz for **32–35**.

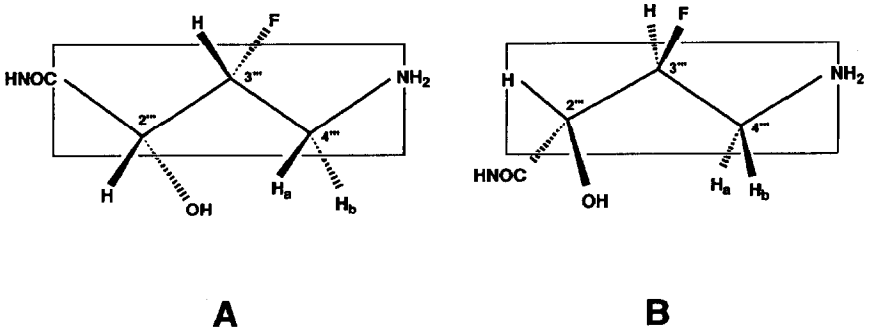


Fig. 2. The assigned conformations of the residues of (A) compounds **32–34** and (B) of compound **35**.

EXPERIMENTAL

General.—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. NMR spectra (^1H at 250, ^{13}C at 125.8, and ^{19}F at 235.3 MHz) were recorded with Bruker WM 250 and AMX 500 spectrometers unless stated otherwise. Chemical shift (δ) of ^1H, ^{13}C, and ^{19}F spectra were measured downfield from internal Me_4Si (for ^1H and ^{13}C) or internal Freon 11 (for ^{19}F), unless stated otherwise, 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 stated otherwise.

General procedure to determine the pK_a values of H_3N^+-4''' by ^{13}C NMR spectroscopy.—Solutions of arbekacin or amikacin, **32** or **34** (each as base, 0.075 mmol) in D_2O (5 mL) were acidified to pD ~ 1 with DCl in D_2O and the solution was freeze-dried. The solid obtained was dissolved in D_2O (0.5 mL) and the solution was neutralized stepwise with 0.5 M NaOD in D_2O. The pD values were measured by using a TOA ion meter IM-40S (see Fig. 1).

3-Deoxy-3-fluoro-1,2-O-isopropylidene-α-D-xylofuranuronic acid (**3**).—An aqueous solution (120 mL) of **1** (8.30 g, 37.4 mmol) and $NaIO_4$ (8.0 g, 37.4 mmol) was kept for 30 min at room temperature. TLC (10:1 $CHCl_3$–MeOH) then showed a main spot at R_f 0.55. Extraction of the mixture with EtOAc followed by concentration of the solution gave **2** as a syrup (6.53 g). To a stirred mixture of the syrup and $AgNO_3$ (12.3 g, 72.4 mmol) in water (65 mL) was added (within 15 min) 2.2 M aq KOH (66 mL), and stirring was continued for a further 30 min. The precipitate was filtered, washed with 1 M aq KOH, and the ice-cold filtrate was acidified to pH 1 with 6 M aq HCl. TLC (10:1 $CHCl_3$–MeOH) showed a spot at R_f 0.05. The aqueous solution was thoroughly extracted with $CHCl_3$ and the extract dried (Na_2SO_4) and concentrated to give **3** (6.59 g, 86%) as a crystalline solid; mp 131–132°C ($CHCl_3$–hexane); $[\alpha]_D^{22}$ – 52° (*c* 1, $CHCl_3$). Anal. Calcd for $C_8H_{11}FO_5$: C, 46.60; H, 5.38; F, 9.22. Found: C, 46.82; H, 5.44; F, 8.98.

Methyl 3-deoxy-3-fluoro-1,2-O-isopropylidene-α-D-xylofuranuronate (**4**).—To a solution of **3** (6.00 g) in diethyl ether (120 mL) was added CH_2N_2 in diethyl ether until the solution showed a single spot (R_f 0.55; **3**: R_f 0.05) in TLC (3:1 toluene–EtOAc). Concentration gave **4** (6.31 g, 98%) as a crystalline solid; mp 71–72°C (benzene–hexane); $[\alpha]_D^{22}$ − 45° (*c* 1, $CHCl_3$). Anal. Calcd for $C_9H_{13}FO_5$: C, 49.09; H, 5.95; F, 8.63. Found: C, 49.31; H, 5.96; F, 8.53.

Methyl 3-deoxy-3-fluoro-D-xylofuranuronate (**5**).—A solution of **4** (290 mg) in 90% aq CF_3CO_2H (3 mL) was kept for 4 h at room temperature. Evaporation followed by coevaporation of added water gave a syrup, which was purified by column chromatography (1:2 toluene–EtOAc) to give **5** (213 mg, 90%) as a crystalline solid; mp 80–81°C (EtOAc–hexane); $[\alpha]_D^{20}$ − 15° (5 min) → +2° (final) (*c* 1, MeOH). Anal. Calcd for $C_6H_9FO_5$: C, 40.00; H, 5.04; F, 10.55. Found: C, 40.30; H, 4.79; F, 10.67.

Methyl (2R,3R)-3-fluoro-2,4-dihydroxybutanoate (**6**).—An aqueous solution (15 mL) of **5** (1.00 g, 5.55 mmol) and $NaIO_4$ (1.31 g, 6.12 mmol) was kept for 19 h at room temperature. In TLC (8:1 $CHCl_3$–MeOH), a spot (R_f 0.55, minor, *O*-formyl intermediate?) appeared at an early stage and finally disappeared to give a single spot (R_f 0.4; **5**: R_f 0.35). Thorough extraction of the mixture with EtOAc followed by concentration of the solution gave a syrup (800 mg). To a solution of the syrup in MeOH (16 mL) was added $NaBH_4$ at 30 min intervals (60 mg × 5) at room temperature. After 2.5 h the solution was neutralized with 1 M aq HCl and concentrated. The residue was extracted with oxolane and to the solution was added CH_2N_2 in diethyl ether to give a methylated product, which was purified by column chromatography with EtOAc, affording **6** (571 mg, 68%) as a crystalline solid; mp 89–90°C (needles from EtOAc–hexane); $[\alpha]_D^{22}$ − 2° (*c* 1, MeOH); ^{13}C NMR (at 62.9 MHz in MeOH-d_4): δ 52.9 (CO_2CH_3), 61.7 (d, C-4), 71.4 (d, C-2), 95.0 (d, C-3), and 173.5 (d, C-1); $J_{C\text{-}1,F}$ 3.7, $J_{C\text{-}2,F}$ 20.1, $J_{C\text{-}3,F}$ 177.3, and $J_{C\text{-}4,F}$ 24.5 Hz. Anal. Calcd for $C_5H_9FO_4$: C, 39.48; H, 5.96; F, 12.49. Found: C, 39.64; H, 6.00; F, 12.53.

Methyl (2R,3R)-2,4-O-benzylidene-3-fluoro-2,4-dihydroxybutanoate (**7**).—To a solution of **6** (1.00 g, 6.57 mmol) in dry DMF (20 mL) were added α,α-dimethoxytoluene (3.0 g, 19.7 mmol) and anhyd *p*-toluenesulfonic acid (230 mg), and the mixture was heated at 50°C for 1.5 h under slightly decreased pressure (20–30 hPa; to partially remove liberated MeOH). After addition of 5% aqueous $NaHCO_3$ (5 mL), the mixture was concentrated. The residue was extracted with $CHCl_3$ and the solution washed with water, dried (Na_2SO_4), and concentrated to give **7** (1.48 g, 94%) as a crystalline solid; mp 107.5–108.5°C (benzene–hexane); $[\alpha]_D^{22}$ − 45° (*c* 1, $CHCl_3$); phase-sensitive NOESY: cross peaks for H-2 and C*H*Ph, and H-4*ax* and C*H*Ph were observed; NOE difference spectroscopy: upon irradiation of H-4*ax*, signal increases of H-2 (2.3%), H-3 (5.6%), H-4*eq* (21.8%), and C*H*Ph (6.2%) were observed. Anal. Calcd for $C_{12}H_{13}FO_4$: C, 60.00; H, 5.45; F, 7.91. Found: C, 60.05; H, 5.54; F, 7.61.

Methyl (2R,3S)-2-benzoyloxy-4-bromo-3-fluorobutanoate (**8**).—A mixture of **7**

(1.00 g, 4.16 mmol), NBS (815 mg, 4.58 mmol), and $BaCO_3$ (1.36 g) in dry CCl_4 (20 mL) was refluxed for 1 h. TLC (10:1 toluene–EtOAc) showed a main spot at R_f 0.6 with several minor spots. The mixture was concentrated and the residue was extracted with toluene. The products were chromatographed with 12:1 toluene–EtOAc to give **8** (1.14 g, 86%) as a syrup, which crystallized on storage in a refrigerator; mp 67–68°C; $[\alpha]_D^{22}$ – 16° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{12}BrFO_4$: C, 45.16; H, 3.79; Br, 25.04; F, 5.95. Found: C, 45.30; H, 3.63; Br, 25.07; F, 5.69.

Methyl (2R,3S)-4-bromo-3-fluoro-2-hydroxybutanoate (**9**).—To a suspension of **8** (1.00 g) in water (5 mL), was added 30% HBr in AcOH (10 mL), and the mixture was heated for 10 h at 90°C. TLC (EtOAc) of the solution showed a main spot at R_f 0.15 along with a slight spot (R_f 0.3, the corresponding 2-benzoyloxy acid). Concentration gave a residue, that was dissolved in oxolane (100 mL) and the solution was treated with CH_2N_2 in diethyl ether to give a main product having R_f 0.25 in TLC (12:1 toluene– EtOAc). It was purified by column chromatography with 12:1 toluene–EtOAc to give **9** (440 mg, 65%) as a syrup, which crystallized on storage in a refrigerator; mp 31–32°C; $[\alpha]_D^{22}$ + 19° (*c* 1, $CHCl_3$). Anal. Calcd for $C_5H_8BrFO_3$: C, 27.93; H, 3.75; Br, 37.16; F, 8.84. Found: C, 28.33; H, 3.73; Br, 37.08; F, 8.90.

Methyl (2R,3R)-4-azido-3-fluoro-2-hydroxybutanoate (**10**).—A mixture of **9** (54 mg, 0.25 mmol) and NaN_3 (20 mg, 0.31 mmol) in dry DMF (1 mL) was heated for 50 min at 80°C. Concentration gave a residue, that was extracted with EtOAc. The solution was washed with water, dried (Na_2SO_4), and concentrated to give **10** (36.0 mg, 81%) as a syrup. An analytical sample was obtained by column chromatography (12:1 toluene–EtOAc); $[\alpha]_D^{23}$ – 18° (*c* 1, $CHCl_3$); IR (neat): 1750 (CO_2Me) and 2130 cm^{-1} (N_3). Anal. Calcd for $C_5H_8FN_3O_3$: C, 33.90; H, 4.55; F, 10.73; N, 23.72. Found: C, 34.10; H, 4.73; F, 10.81; N, 23.45.

(2R,3R)-4-Azido-3-fluoro-2-hydroxybutanoic acid (**11**).—To a solution of **10** (723 mg) in MeOH (15 mL) was added 0.6 M aq NaOH (15 mL), and after 10 min, the solution was concentrated to half its volume, neutralized with 1 M aq HCl under cooling until acidic, and the mixture was extracted with EtOAc. The solution was dried (Na_2SO_4), and concentrated to give **11** (575 mg, 86%) as a syrup. An analytical sample was obtained by column chromatography (lower layer of 2:1:1 $CHCl_3$–MeOH–20% aq AcOH) to give a syrup, which crystallized on storage in a refrigerator; mp 73.5–74.5°C; $[\alpha]_D^{23}$ – 18° (*c* 0.5, MeOH); ^{13}C NMR (at 62.9 MHz in MeOH-d_4): δ 52.2 (d, C-4), 71.5 (d, C-2), 93.7 (d, C-3), and 174.0 (d, C-1); $J_{C\text{-}1,F}$ 4.5, $J_{C\text{-}2,F}$ 20.1, $J_{C\text{-}3,F}$ 179.2, and $J_{C\text{-}4,F}$ 23.9 Hz. Anal. Calcd for $C_4H_8FN_3O_3$: C, 29.45; H, 3.71; F, 11.65; N, 25.76. Found: C, 29.39; H, 3.72; F, 11.95; N, 25.43.

3,5-Di-O-benzyl-D-xylofuranose (**13**).—A solution of **12** (4.57 g) in 0.25 M HCl in 3:1 1,4-dioxane–water (92 mL) was heated for 1 h at 90°C. The whole mixture was extracted with $CHCl_3$ (400 mL), and the organic solution was washed with aq $NaHCO_3$ and the crude product obtained was chromatographed (2:1 toluene–EtOAc) to give **13** (3.20 g, 79%) as a syrup; mp 71–73°C (precipitated from

benzene–hexane); $[\alpha]_D^{21}$ + 3° (3 min) → +8° (final) (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 20 h after preparation): δ 4.18 [ddd, ~0.7 H, H-2 (α anomer)], 4.23 [slightly br t, ~0.3 H, H-2 (β anomer)], 5.10 [slightly br d, ~0.3 H, H-1 (β)], and 5.48 [t, ~0.7 H, H-1 (α)]; *J* values: α anomer, $J_{1,2}$ 4.5, $J_{1,HO-1}$ 5, $J_{2,3}$ 3, and $J_{2,HO-2}$ 6 Hz; β anomer, $J_{1,2}$ ~0, $J_{1,HO-1}$ 11.5, $J_{2,3}$ 3, and $J_{2,HO-2}$ 4.5 Hz. Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 69.31; H, 6.71.

Methyl (2S,3R)-2,4-bis(benzyloxy)-3-hydroxybutanoate (**14**).—A mixture of **13** (10.0 g, 30.3 mmol) and $NaIO_4$ (7.12 g, 33.3 mmol) in 2:1 1,4-dioxane–water (300 mL) was stirred for 2 h at room temperature. TLC (2:1 toluene–EtOAc) of the solution showed a single spot at R_f 0.55. Concentration followed by extraction with $CHCl_3$ gave a pale-yellow syrup. To a stirred mixture of the syrup and $AgNO_3$ (11.6 g, 63.8 mmol) in 4:1 1,4-dioxane–water (400 mL) was added 2 M aq KOH (90 mL) and stirring was continued for 30 min. After filtration, the solution was neutralized with 6 M aq HCl (20 mL) and the whole mixture was extracted with $CHCl_3$. TLC (2:1 toluene–EtOAc) of the solution showed a spot at R_f 0.05. The syrupy product obtained after evaporation was dissolved in diethyl ether and treated with CH_2N_2 in diethyl ether to give the methyl ester (R_f 0.35 in TLC with 2:1 toluene–EtOAc). Purification by column chromatography (2:1 toluene–EtOAc) gave **14** (9.00 g, 90%) as a syrup; $[\alpha]_D^{23}$ −65° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 69.29; H, 6.78.

Methyl (2R,3S)-2,4-bis(benzyloxy)-3-fluorobutanoate (**15**) *and (Z)-methyl 2,4-bis(benzyloxy)but-2-enoate* (**16**).—To a refluxing solution of DAST (2.79 mL, 21.1 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise a solution of **14** (5.03 g, 15.2 mmol) in dry CH_2Cl_2 (25 mL), and refluxing was continued for a further 15 min. TLC (5:1 hexane–EtOAc) of the solution showed two spots at R_f 0.25 (**15**, major) and 0.3 (**16**) (cf. **14**: R_f 0.1). The solution was poured into a mixture of aq $NaHCO_3$ (satd, 300 mL) and $CHCl_3$ (500 mL) and, after shaking for 10 min, the organic solution separated was washed with water, dried (Na_2SO_4), and concentrated to give a pale-brown syrup (5.00 g). To a solution of the syrup in CCl_4 (100 mL) was added 10% Br_2 in CCl_4 (2.5 mL) and the mixture was kept for 30 min at room temperature. TLC (5:1 hexane–EtOAc) showed spots at R_f 0.45, 0.25 (**15**), and 0.1. Concentration gave a residue, that was chromatographed ($CHCl_3$) to give **15** (3.04 g, 60%) as a syrup; $[\alpha]_D^{24}$ −42° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{19}H_{21}FO_4$: C, 68.66; H, 6.37; F, 5.72. Found: C, 68.64; H, 6.37; F, 5.88.

In another experiment, the pale-brown syrup (1.50 g) obtained after reaction was subjected to HPLC (SSC-Silica of Senshu Scientific Co. Ltd., 5:1 hexane–EtOAc) to give a syrup of **15** (557 mg, 38%) and a syrup of **16** (195 mg, 14%). Compound **16**, IR (neat): 1655 (C=C) and 1730 cm^{-1} (C=O); ^1H NMR (at 500 MHz in $CDCl_3$): δ 3.80 (s, 3 H, CO_2Me), 4.10 (d, 2 H, H-4a,4b), 4.39 (s, 2 H, PhCH_2O-4), 4.87 (s, 2 H, PhCH_2O-2), 6.38 (t, 1 H, $J_{3,4a} = J_{3,4b}$ = 6 Hz, H-3), and 7.20–7.40 (10 H, Ph × 2); ^{13}C NMR ($CDCl_3$): δ 52.1 (CO_2CH_3), 64.4 (C-4), 72.6 (PhCH_2O-4), 74.3 (PhCH_2O-2), 125.4 (C-3), 145.3 (C-2), and 163.8 (CO_2Me). Anal. Calcd for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45. Found: C, 73.15; H, 6.52.

Methyl (2R,3S)-3-fluoro-2,4-dihydroxybutanoate (**17**).—To a solution of **15** (1.90 g) in 3:1 1,4-dioxane–water (80 mL) was added Raney Ni (0.1 mL), and after shaking for a while (to remove inactivating impurities for Pd), the mixture was filtered. To the filtrate, AcOH (0.05 mL) was added, and the solution was hydrogenated under H_2 in the presence of Pd-black for 6 h at room temperature. TLC (8:1 $CHCl_3$–MeOH) of the solution showed a major spot at R_f 0.25 with disappearance of spots at R_f 0.9 (**15**), 0.7 and 0.6 (both monobenzyl derivatives); a spot at R_f 0.2 (the 1,4-lactone of **17**, which sometimes appeared under slightly more acidic conditions) was also not observed. After filtration, the solution was neutralized ($NaHCO_3$), concentrated, and the residue was chromatographed (8:1 $CHCl_3$–MeOH) to give **17** (744 mg, 86%) as a syrup; $[\alpha]_D^{21}$ + 8° (*c* 1, MeOH); ^{13}C NMR (at 62.9 MHz in MeOH-d_4): δ 52.7 (CO_2CH_3), 61.3 (d, C-4), 71.2 (d, C-2), 95.3 (d, C-3), and 173.2 (d, C-1); $J_{C\text{-}1,F}$ 6.9, $J_{C\text{-}2,F}$ 24.5, $J_{C\text{-}3,F}$ 177.3, and $J_{C\text{-}4,F}$ 22.6 Hz. Anal. Calcd for $C_5H_9FO_4$: C, 39.48; H, 5.96; F, 12.49. Found: C, 39.09; H, 5.88; F, 12.54.

Methyl (2R,3S)-2,4-O-benzylidene-3-fluoro-2,4-dihydroxybutanoate (**18a** *and* **18b**).—Compound **17** (1.01 g, 6.64 mmol) was treated with α,α-dimethoxytoluene (3.0 g, 19.7 mmol) in the presence of anhyd *p*-toluenesulfonic acid (115 mg) in dry DMF (20 mL) as described for **7**, to give a mixture of products. TLC (8:1 toluene–EtOAc) of the mixture showed two spots at R_f 0.58 (**18b**) and 0.45 (**18a**). The products were separated by chromatography (12:1 toluene–EtOAc) to give crystalline solids of **18a** (909 mg, 57%) and **18b** (340 mg, 21%). Compound **18a**; mp 70–71°C (needles from toluene–hexane); $[\alpha]_D^{20}$ + 2° (*c* 1, $CHCl_3$); phase-sensitive NOESY: a cross peak for H-4*ax* and C*H*Ph was observed; NOE difference spectroscopy: upon irradiation of H-4*ax*, signal increases of H-3 (3.5%), H-4*eq* (19.0%), and C*H*Ph (6.9%) were observed. Anal. Calcd for $C_{12}H_{13}FO_4$:C, 60.00; H, 5.45; F, 7.91. Found: C, 60.02; H, 5.37; F, 7.84.

Compound **18b**; mp 63–64°C (needles from hexane); $[\alpha]_D^{22}$ + 3° (*c* 1, $CHCl_3$); phase-sensitive NOESY: cross peaks for H-2 and H-4*ax*, H-2 and C*H*Ph, and H-4*ax* and C*H*Ph were observed; NOE difference spectroscopy: upon irradiation of H-4*ax*, signal increases of H-2 (4.4%), H-4*eq* (20.9%), and C*H*Ph (5.1%) were observed. Anal. Calcd for $C_{12}H_{13}FO_4$: C, 60.00; H, 5.45; F, 7.91. Found: C, 60.30; H, 5.42; F, 7.76.

Methyl (2R,3R)-2-benzoyloxy-4-bromo-3-fluorobutanoate (**19**).—A mixture of **18** (1.25 g, 5.20 mmol, a mixture of **18a** and **18b**), NBS (1.02 g, 5.73 mmol), and $BaCO_3$ (1.70 g) in dry CCl_4 (25 mL) was refluxed for 2 h. TLC (10:1 toluene–EtOAc) of the solution showed two spots at R_f 0.6 (**19**, major) and 0.55 [4-benzoyloxy-2-bromo derivative; ^1H NMR ($CDCl_3$): δ 3.82 (CO_2Me), 4.60 (dd, 1 H, H-2), 4.66 (dd, 2 H, H-4s), and 5.18 (ddt, 1 H, H-3); $J_{2,3}$ 6, $J_{3,4s}$ 4.5, $J_{2,F}$ 16, $J_{3,F}$ 47, and $J_{4s,F}$ 21.5 Hz]. The mixture was concentrated and the residue was extracted with toluene. The products were separated by chromatography (toluene) to give **19** (1.13 g, 68%) as a syrup, which crystallized on storage in a refrigerator, but gave a syrup again at room temperature; $[\alpha]_D^{23}$ + 20° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{12}H_{12}BrFO_4$: C, 45.16; H, 3.79; Br, 25.04; F, 5.95. Found: C, 45.34; H, 3.68; Br, 24.88; F, 6.04.

Methyl (2R,3R)-4-bromo-3-fluoro-2-hydroxybutanoate (**20**).—To a suspension of **19** (1.34 g) in water (6.8 mL) was added 30% HBr in AcOH (13.5 mL) and the mixture was heated for 5 h at 90°C. The product obtained was then treated with CH_2N_2 as described for **9** to give, after chromatography (12:1 toluene–EtOAc), a syrup of **20** (554 mg, 61%); $[\alpha]_D^{21}$ + 34° (*c* 1,$CHCl_3$). Anal. Calcd for $C_5H_8BrFO_3$: C, 27.93; H, 3.75; Br, 37.16; F, 8.84. Found: C, 28.13; H, 3.70; Br, 37.37; F, 9.00.

Methyl (2R,3S)-4-azido-3-fluoro-2-hydroxybutanoate (**21**).—A mixture of **20** (198 mg, 0.921 mmol), NaN_3 (90 mg, 1.38 mmol), and Bu_4NBr (200 mg) in dry CH_3CN (4 mL) was gently refluxed for 18 h. Concentration, followed by work-up as described for **10** gave a product, that was purified by chromatography (12:1 toluene–EtOAc) to give **21** (123 mg, 75%) as a syrup, which was unstable, and gradually decomposed on storage; IR (KBr): 1740 (CO_2Me) and 2110 cm^{-1} (N_3).

(2R,3S)-4-Azido-3-fluoro-2-hydroxybutanoic acid (**22**).—To a solution of **21** (243 mg) in MeOH (4.8 mL) was added 0.6 M aq NaOH (4.8 mL) and the solution was treated as described for **11** to give **22** (207 mg, 93%) as a slightly unstable syrup. An analytical sample was prepared as described for **11**; $[\alpha]_D^{22}$ + 13° (*c* 1, MeOH); ^{13}C NMR (at 62.9 MHz in MeOH-d_4): δ 51.7 (d, C-4), 71.7 (d, C-2), 94.1 (d, C-3), and 173.7 (d, C-1); $J_{C\text{-}1,F}$ 8.4, $J_{C\text{-}2,F}$ 23.3, $J_{C\text{-}3,F}$ 178.7, and $J_{C\text{-}4,F}$ 22.6 Hz. Anal. Calcd for $C_4H_8FN_3O_3$: C, 29.45; H, 3.71; F, 11.65; N, 25.76. Found: C, 29.34; H, 4.09; F, 11.63; N, 25.49.

N-*Hydroxysuccinimide esters* (**26** *and* **27**) *of* **11** *and* **22**.—A mixture of **11** or **22** (1 mmol), *N*-hydroxysuccinimide (1.05 mmol), and dicyclohexylcarbodiimide (1 mmol) in dry EtOAc (6.5 mL) was stirred for 1 h at room temperature. The precipitate was filtered off, washed with EtOAc, and the filtrate was concentrated to give **26** or **27** as a syrup, which showed, in TLC (lower layer of 2:1:1 $CHCl_3$–MeOH–20% aq AcOH), a main spot at R_f 0.6 (**26** and **27**); the esters were sensitive to moisture, and were used without purification.

*3,2',6'-Tris(*N*-benzyloxycarbonyl)-3'-deoxy-3"-*N*-(trifluoroacetyl)kanamycin B* (**24**).—Prepared * according to the literature[19]; $[\alpha]_D^{21}$ + 15° (*c* 1, pyridine); ^1H NMR (pyridine-d_5): δ 7.1–7.6 (m, 15 H, CO_2CH_2Ph); ^{19}F NMR (pyridine-d_5): δ −74.2. Anal. Calcd for $C_{44}H_{54}F_3N_5O_{16}$: C, 54.71; H, 5.63; F, 5.90; N, 7.25. Found: C, 54.55; H, 5.79; F, 5.55; N, 7.25.

General procedure for coupling of N*-hydroxysuccinimide esters* (**26** *or* **27**) *with 3,6'-bis(*N*-benzyloxycarbonyl)-3"-*N*-(trifluoroacetyl)kanamycin A* (**23**), *3,2',6'-tris(*N*-benzyloxycarbonyl)-3"-*N*-(trifluoroacetyl) derivatives* (**24** *and* **25**) *of 3'-deoxykanamycin B or 3',4'-dideoxykanamycin B.*—To a solution of **23** (**24** or **25**) (1 mmol) in 2:1 oxolane–water (30 mL) was added **26** (or **27**) (1.8 mmol) in oxolane (10 mL) and the solution, after adjustment to pH ~ 8 by addition of aq $NaHCO_3$ (satd), was kept for 1 h at room temperature. Concentration gave crude 1-*N*-acyl derivatives (**28**–**31**). For the analytical sample, part of the product was purified by

* Prepared by Dr. Y. Takagi and Miss H. Sohtome of our laboratory.

chromatography (lower layer of 4:3:2 $CHCl_3$–MeOH– water) unless otherwise stated.

1-N-*[(2R,3R)-4-Azido-3-fluoro-2-hydroxybutanoyl]-3,6'-bis(*N-*benzyloxycarbonyl)-3"*-N-*(trifluoroacetyl)kanamycin A* (**28**).—The crude product obtained as just described was dissolved in water-saturated 1-butanol and the solution was washed with 1-butanol-saturated water. Concentration gave **28** (965 mg, 95%) as a solid. For an analytical sample, the solid (100 mg) was purified by column chromatography (lower layer of 1:1:1 $CHCl_3$–MeOH–20% aq AcOH) to give a solid (65.8 mg, 63%); $[\alpha]_D^{24}$ + 72° (*c* 1, pyridine); IR (KBr): 1520 (amide II), 1680 (amide I), and 2100 cm^{-1} (N_3); ^1H NMR (pyridine-d_5): δ 3.88 (ddd, 1 H, H-4‴a), 4.08 (ddd, 1 H, H-4‴b), 4.78 (dd, 1 H, H-2‴), 5.52 (apparently double quintets, 1 H, H-3‴), 5.57 (slightly br d, 1 H, *J* ~3 Hz, H-1' or 1") and 5.81 (d, *J* 3.5 Hz, 1 H, H-1" or 1'); $J_{2''',3'''}$ 4, $J_{3''',4'''a}$ 3.5, $J_{3''',4'''b}$ 7.5, $J_{4'''a,4'''b}$ 13.5, $J_{2''',F}$ 24.5, $J_{3''',F}$ ~48, $J_{4'''a,F}$ 29, and $J_{4'''b,F}$ 19 Hz; ^{19}F NMR (pyridine-d_5): δ −74.1 (s, 3 F, CF_3CO), and −196.2 (br sextet, 1 F, F-3‴). Anal. Calcd for $C_{40}H_{51}F_4N_7O_{18} \cdot H_2O$: C, 47.48; H, 5.28; F, 7.51; N, 9.69. Found: C, 47.48; H, 5.45; F, 7.45; N, 9,89.

1-N-*[(2R, 3R)-4-Azido-3-fluoro-2-hydroxybutanoyl]-3,2',6'-tris(*N-*benzyloxycarbonyl)-3'-deoxy-3"*-N-*(trifluoroacetyl)kanamycin B* (**29**).—The crude product obtained was washed thoroughly with water and dried to give **29** (1.11 g, 97%) as a solid. An analytical sample (73% after purification) had; $[\alpha]_D^{21}$ + 42° (*c* 1, pyridine); IR (KBr): 1525, 1700, and 2100 cm^{-1}; ^1H NMR (pyridine-d_5): δ 3.86 (ddd, 1 H, H-4‴a), 4.04 (ddd, 1 H, H-4‴b), 4.75 (dd, 1 H, H-2‴), and 5.47 (d quintets, H-3‴); $J_{2''',3'''}$ 4, $J_{3''',4'''a}$ 3.5, $J_{3''',4'''b}$ 7, $J_{4'''a,4'''b}$ 13.5, $J_{2''',F}$ 24, $J_{3''',F}$ 48, $J_{4'''a,F}$ ~28, and $J_{4'''b,F}$ 19 Hz. Anal. Calcd for $C_{48}H_{58}F_4N_8O_{18} \cdot 2H_2O$: C, 50.26; H, 5.45; F, 6.63; N, 9.77. Found: C, 50.44; H, 5.58; F, 6.91; N, 9.41.

1-N-*[(2R,3R)-4-Azido-3-fluoro-2-hydroxybutanoyl]-3,2',6'-tris(*N-*benzyloxycarbonyl)-3',4'-dideoxy-3"*-N-*(trifluoroacetyl)kanamycin B* (**30**).—The crude product obtained was treated as described for **29**; yield, 96%. An analytical sample (73%) had; $[\alpha]_D^{24}$ + 47° (*c* 1, pyridine); IR (KBr): 1520, 1670, and 2100 cm^{-1}, ^1H NMR (pyridine-d_5): δ 3.87 (ddd, 1 H, H-4‴a), 4.07 (ddd, 1 H, H-4‴b), 4.78 (dd, 1 H, H-2‴), and 5.50 (d quintets, 1 H, H-3‴); $J_{2''',3'''}$ 4, $J_{3''',4'''a}$ 3.5, $J_{3''',4'''b}$ 7, $J_{4'''a,4'''b}$ 13.5, $J_{2''',F}$ 24, $J_{3''',F}$ ~49, $J_{4'''a,F}$ ~28, and $J_{4'''b,F}$ 19 Hz. Anal. Calcd for $C_{48}H_{58}F_4N_8O_{17} \cdot H_2O$: C, 51.80; H, 5.43; F, 6.83; N, 10.07. Found: C, 51.58; H, 5.34; F, 6.92; N, 9.75.

1-N-*[(2R,3S)-4-Azido-3-fluoro-2-hydroxybutanoyl]-3,2',6'-tris(*N-*benzyloxycarbonyl)-3',4'-dideoxy-3"*-N-*(trifluoroacetyl)kanamycin B* (**31**).—The crude product was treated as described for **29**; yield, 97%. An analytical sample (57%) had; $[\alpha]_D^{21}$ +55° (*c* 0.5, pyridine); IR (KBr): 1530, 1700, and 2110 cm^{-1}; ^1H NMR (pyridine-d_5): δ 3.93 (slightly br dd, 1 H, H-4‴a), 4.13 (ddd, 1 H, H-4‴b), 4.93 (dd, 1 H, H-2‴), and 5.50 (d of br dt, 1 H, H-3‴); $J_{2''',3'''}$ 3, $J_{3''',4'''a}$ ~2, $J_{3''',4'''b}$ 8, $J_{4'''a,4'''b}$ 14, $J_{2''',F}$ 15, $J_{3''',F}$ ~49, $J_{4'''a,F}$ ~34, and $J_{4'''b,F}$ ~19 Hz. Anal. Calcd for $C_{48}H_{58}F_4N_8O_{17}$: C, 52.65; H, 5.34; F, 6.94; N, 10.23. Found: C, 52.44; H, 5.63; F, 6.73; N, 10.05.

*General procedure for preparation of 1-*N*-[(2*R*,3*R*) and (2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl]kanamycins* (**32–35**) *from the 1-*N*-acyl derivatives.*—A solution of **28** (**29**, **30**, or **31**) (1 mmol) in 1 M NH_3 in 3:1 oxolane–water (50 mL) was kept for 40 h at room temperature [de(trifluoroacetyl)ation]. Concentration gave a residue, which was dissolved in 20:20:1 1,4-dioxane–water–AcOH (40 mL) and hydrogenated in the presence of Pd-black for 3 h at room temperature. Filtration, followed by concentration of the solution gave a solid that was subjected to chromatography on CM Sephadex C-25 ($0 \rightarrow 0.15$ M aq NH_3) to give the final product.

*1-*N*-[(2*R*,3*R*)-4-Amino-3-fluoro-2-hydroxybutanoyl]kanamycin A* (**32**).—Yield 72%; $[\alpha]_D^{22}$ + 93°(*c* 1, water); ^{1}H NMR (DCl–D_2O, pD 3, at 500 MHz): δ 1.88 (q, 1 H, H-2*ax*), 2.29 (dt, 1 H, H-2*eq*), 4.46 (dd, 1 H, H-2‴), 5.25 (d, 1 H, H-1″), 5.31 (dddd, 1 H, H-3‴), and 5.57 (d, 1 H, H-1′); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3} = 13$, $J_{1,2eq} = J_{2eq,3} = J_{1',2'} = 4$, and $J_{1'',2''}$ 3.5 Hz. ^{19}F NMR (DCl–D_2O, Freon 11 as external reference, pD 3): δ −205.0 (ddt). Anal. Calcd for $C_{22}H_{42}FN_5O_{13} \cdot H_2CO_3 \cdot H_2O$: C, 40.41; H, 6.78; F, 2.78; N, 10.24. Found: C, 40.82; H, 6.99; F, 2.89; N, 10.03.

*1-*N*-[(2*R*,3*R*)-4-Amino-3-fluoro-2-hydroxybutanoyl]-3′-deoxykanamycin B* (**33**).—Yield 59%; $[\alpha]_D^{22}$ + 81° (*c* 0.5, water); ^{1}H NMR (DCl–D_2O, pD 3, at 500 MHz): δ 1.92 (q, 1 H, H-2*ax*), 2.07 (q, 1 H, H-3′*ax*), 4.46 (dd, 1 H, H-2‴), 5.25 (d, 1 H, H-1″), 5.31 (dddd, 1 H, H-3‴), and 5.79 (d, 1 H, H-1′); $J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3} = 13$, $J_{1',2'}$ 3.8, $J_{2',3'ax} = J_{3'ax,3'eq} = J_{3'ax,4'} = 12$, and $J_{1'',2''}$ 3.8 Hz. ^{19}F NMR (DCl–D_2O, Freon 11 as external reference, pD 3): δ −205.1 (ddt). Anal. Calcd for $C_{22}H_{43}FN_6O_{11} \cdot H_2CO_3 \cdot 1.5H_2O$: C, 40.89; H, 7.16; F, 2.81; N, 12.44. Found: C, 40.77; H, 7.09; F, 2.60; N, 12.07.

*1-*N*-[(2*R*,3*R*)-4-Amino-3-fluoro-2-hydroxybutanoyl]-3′,4′-dideoxykanamycin B* (**34**).—Yield 65%; $[\alpha]_D^{23}$ + 79° (*c* 0.5, water); ^{1}H NMR (DCl–D_2O, pD 3, at 500 MHz): δ 1.64 (ddt, 1 H, H-4′*ax*), 1.92 (q, 1 H, H-2*ax*), 2.30 (dt, 1 H, H-2*eq*), 4.46 (dd, 1 H, H-2‴), 5.25 (d, 1 H, H-1″), 5.31 (dddd, 1 H, H-3‴), and 5.81 (d, 1 H, H-1′); $J_{1',2'}$ 3.8, $J_{4'ax,4'eq}$ 14, and $J_{1'',2''}$ 3.8 Hz. ^{19}F NMR (DCl–D_2O, Freon 11 as external reference, pD 3): δ −205.1 (ddt). Anal. Calcd for $C_{22}H_{43}FN_6O_{10} \cdot 2H_2CO_3 \cdot H_2O$: C, 40.45; H, 6.93; F, 2.67; N, 11.79. Found: C, 40.57; H, 6.78; F, 2.51; N, 11.54.

*1-*N*-[(2*R*,3*S*)-4-Amino-3-fluoro-2-hydroxybutanoyl]-3′,4-dideoxykanamycin B* (**35**).—Yield 51%; $[\alpha]_D^{22}$ + 79° (*c* 1, water); ^{1}H NMR (DCl–D_2O, pD 3, at 500 MHz): δ 1.64 (ddt, 1 H, H-4′*ax*), 1.88 (q, 1 H, H-2*ax*), 1.96 (dq, 1 H, H-4′*eq*), 2.30 (dt, 1 H, H-2*eq*), 4.56 (dd, 1 H, H-2‴), 5.11 (dddd, 1 H, H-3‴), 5.22 (d, 1 H, H-1″), and 5.80 (d, 1 H, H-1′). ^{19}F NMR (DCl–D_2O, Freon 11 as external reference, pD 3): δ −199.2 (ddt). Anal. Calcd for $C_{22}H_{43}FN_6O_{10} \cdot 2H_2CO_3 \cdot H_2O$: C, 40.45; H, 6.93; F, 2.67; N, 11.79. Found: C, 40.53; H, 6.97; F, 2.70; N, 11.89.

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