AMIKACIN ANALOGS WITH A FLUORINATED AMINO ACID SIDE CHAIN†

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The synthesis and biological activity of kanamycin A derivatives with an ω-amino-α-fluoroalkanoyl side chain on the 1-amino group are described. The fluorinated amino acids (**4**) for the side chain were prepared by fluorination of the α-hydroxy esters (**2**) with diethylaminosulfur trifluoride (DAST) with accompanying the Walden inversion. The reaction products varied with the amino protective groups employed, chain length of the alkanoic acids and the presence or absence of base. The fluorinated side chain was introduced to 1-free-NH_2 kanamycin A (**12**) by the conventional active ester method and subsequent deblocking reactions afforded the desired final products (**13**~**17**). Of the derivatives prepared, 1-*N*-[(*S*)-4-amino-2-fluorobutyryl]kanamycin A (2‴-deoxy-2‴-fluoroamikacin, **14**) showed the best overall activity profile, nearly the same as that of amikacin. Preparation and antibacterial activity of several aminoglycoside antibiotics with the 1-*N*-(*S*)-4-amino-2-fluorobutyryl side chain are also discussed.

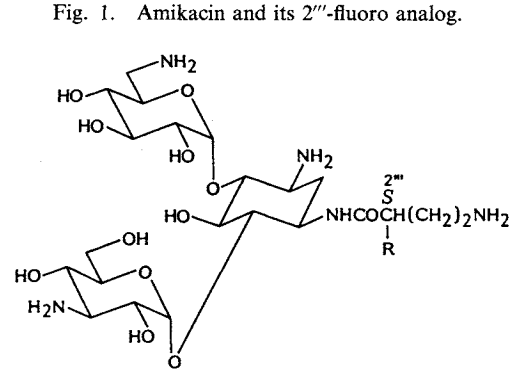
Aminoglycoside antibiotics have been clinically used for the treatment of a wide range of Gram-negative infections. Usually their potential nephrotoxicity limits the doses that may be used. Consequently, aminoglycosides exhibiting lowered nephrotoxicity and at least the same level of antibacterial activity as currently used antibiotics such as amikacin[1], would be very desirable.

Introduction of a fluorine atom is one promising method for modification of amikacin, as some fluorinated drugs exhibit improved biological activity or toxicity[2]. For several years, there have been described in the literature various fluorinated aminoglycosides showing a similar level of antibacterial activity[3~5] or reduced toxicity[6] as compared to the parent compounds. However most were obtained by fluorination of the sugar moiety; only one paper has reported on the introduction of a fluorine atom in the amino acid side chain moiety, giving 1-*N*-[(*R*)- and (*RS*)-3-amino-2-fluoropropanoyl]kanamycin A, which were much less active than amikacin[7].

Based upon these data, we were interested in preparing amikacin analogs with an ω-amino-α-fluoroalkanoyl side chain. Of the derivatives prepared, 1-*N*-[(*S*)-4-amino-2-fluorobutyryl]kanamycin A (the 2‴-fluoro analog of amikacin, **14**) was found to show almost the same biological activity as that of amikacin.

This paper describes the preparation and antibacterial activity of **14** and its related derivatives.

Fig. 1. Amikacin and its 2‴-fluoro analog.



Amikacin R=OH
14 R=F

† A part of this work was presented at 58th Annual Meeting of the Chemical Society of Japan, Kyoto, Apr. 4, 1989.

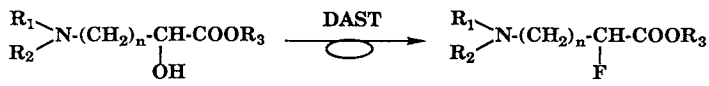
Chemistry

Fluorinated amino acid derivatives in the present study were prepared by treatment of the appropriate *N*-protected ω-amino-α-hydroxyalkanoic esters with diethylaminosulfur trifluoride (DAST) (Scheme 1).

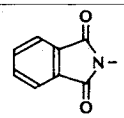
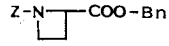
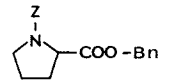
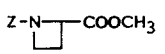
Two types of *N*-protective groups were examined on the reaction of the hydroxy esters **2** with DAST with the results summarized in Table 1. When a phthaloyl group (series **a**) was used as *N*-protective group, the reaction proceeded rather well in the butyric **2a** (n=2) and valeric **2a** (n=3) acid derivatives to give the desired fluoro esters **3a** (n=2) and **3a** (n=3), respectively. But the propionic acid derivative **2a** (n=1) afforded a mixture of products as shown by TLC. In the latter case, increased acidity of β-methylene protons adjacent to the *N*-phthalimide group may accelerate the elimination of the β-proton, which accompanies the leaving of the α-substituent, or other side reactions. In the case of the phthaloyl protective group, there seems to be no distinct effects with pyridine as shown in Table 1.

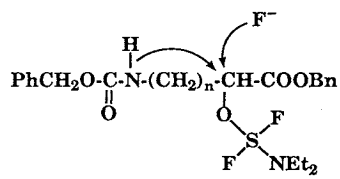
In contrast to the above results, when an *N*-benzyloxycarbonyl (Z) protective group (series **b** and **c**) was used, the products greatly varied in the presence or absence of pyridine. The propionyl derivative (*RS*)-**2b** (n=1) afforded the desired product (*RS*)-**3b** (n=1) only in the presence of pyridine, as was already demonstrated by TAKAGI *et al.*[7]. In the cases of **2b** (n=2) and **2b** (n=3), the reaction without pyridine

Table 1. Reaction of *N*-protected α-hydroxy esters (**2**) with DAST.



2 **3**

	R_1, R_2N	R_3	Reactant	Product	
				Without pyridine	With pyridine (10 equiv)
a)	 (N-Pht)	–Me	**2a** (n=1)	Multi products	Multi products
			2a (n=2)	**3a** (n=2) (41%)	**3a** (n=2) (38%)
			2a (n=3)	**3a** (n=3) (23%)	**3a** (n=3) (34%)
b)	$PhCH_2OCO-NH-$ (N-Z)	$-CH_2Ph$ (Bn)	**2b** (n=1)	Multi products	**3b** (n=1) (50%)[7]
			2b (n=2)	 (**6**, 77%)	Multi products
			2b (n=3)	 (**7**, quant)	**3b** (n=3) (14%) + **7** (3%)
c)	N-Z	–Me	**2c** (n=2)	 (**6'**, 87%)	Multi products including **6'**



A

Table 2. Specific rotations and CD spectra of ω-amino-α-fluoro acids and the corresponding α-hydroxy acids.

$$NH_2-(CH_2)_n-\overset{*}{C}H(R)-COOH$$

n	*	$[\alpha]_D^{H_2O}$		$[\theta]_{max}^{H_2O}$ (nm)	
		R=F (4)	R=OH	R=F (4)	R=OH
1	*S*	−32°[†]	−35°[14)]	+920 (204)	+380 (206)
2	*S*	−26°	−30°[13)]	+800 (213)	+300 (218)
2	*R*	+22°	+28°[13)]	−800 (213)	−300 (218)
3	*S*	−16°	−16°[14)]	ND	ND

† +29° for the corresponding (*R*)-isomer[8)].
ND: Not determined.

afforded azetidine (**6**) and pyrrolidine (**7**) derivatives as a major product, respectively, which might be formed *via* an intermediate (**A**) by intramolecular nucleophilic attack of the amide nitrogen prior to intermolecular attack of the fluoride ion. In the presence of pyridine, **2b** (n=2) gave a reaction mixture showing many spots on TLC and **2b** (n=3) afforded the fluoro compound **3b** (n=3) together with a small amount of **7**. No clear explanation can be given for the effect of pyridine in these reactions. The exchange of the ester protective group from benzyl to methyl (**2c** (n=2)) gave an azetidine derivative (**6′**) similar to **2b** (n=2).

The *N*-phthaloyl fluoro esters thus obtained, **3a** (n=2) and **3a** (n=3), were converted to the corresponding ω-amino-α-fluoro acids, **4** (n=2) and **4** (n=3), respectively. Optically active (*S*)-**4** (n=1)[a] was prepared from D-serine by Route B which was an application of the reported procedure with the (*R*)-isomer from L-serine[8)]. The α,α-difluoro derivative (**10**) was prepared by Route C shown in Scheme 1.

Stereochemistry of the optically active 2-fluoro derivatives was confirmed by comparing the specific rotations and CD curves of the 2-fluoro acids (**4**) with those of the corresponding 2-hydroxy derivatives and also with $[\alpha]_D$ of authentic (*R*)-3-amino-2-fluoropropanoic acid ((*R*)-**4** (n=1))[8)], with the results summarized in Table 2. These results indicate that the reaction of the hydroxy esters **2** with the DAST reagent proceeds by SN2 displacement mechanism accompanied by the Walden inversion[9)], affording (*S*)- and (*R*)-2-fluoro derivatives (**3**) from (*R*)- and (*S*)-2-hydroxy esters (**2**), respectively as shown in Scheme 1.

All the *N*-protected amino fluoro acids (**5** and **11**) were combined with the partially *N*-protected kanamycin A (**12**)[10)] by the conventional active ester method and then deblocked to afford the fluoro analogs of amikacin as shown in Scheme 2.

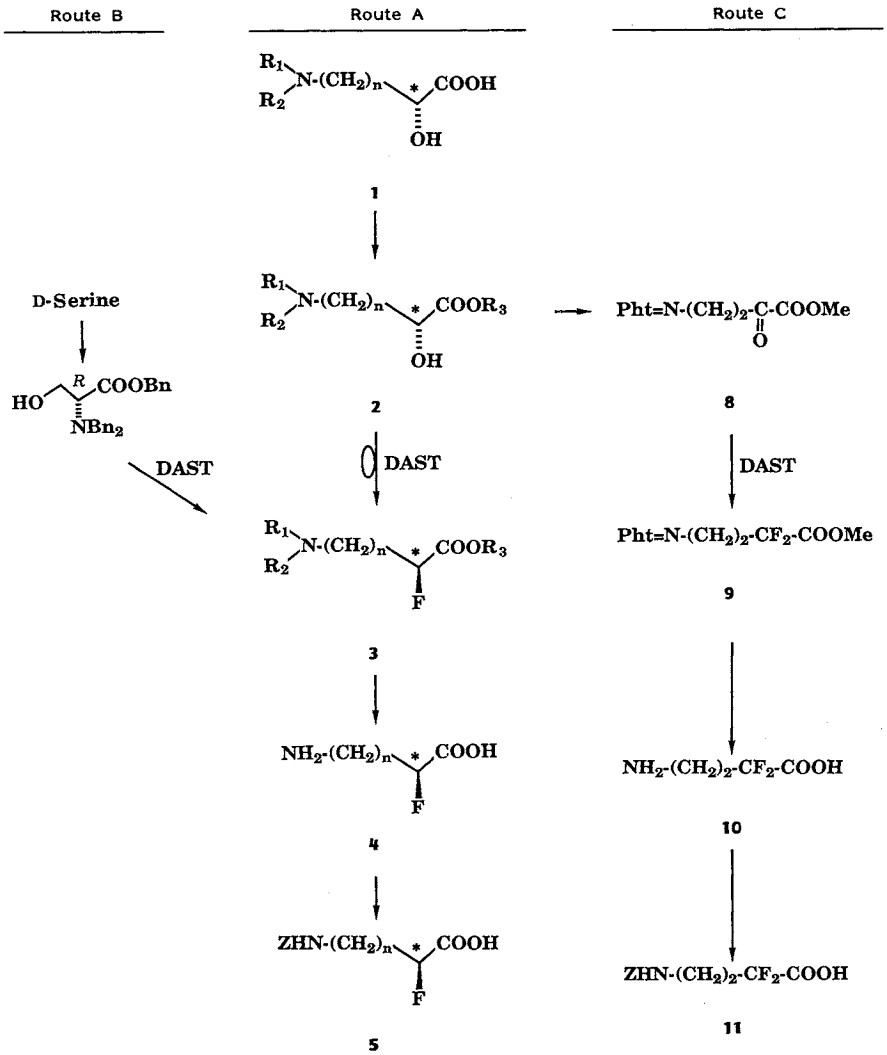
(*S*)-4-Amino-2-fluorobutyric acid moiety (*S*)-**4** (n=2), the most promising side chain in terms of biological activity was also introduced into other aminoglycoside antibiotics, such as kanamycin B, tobramycin, dibekacin, and gentamicin B, by conventional methods to give **18**, **19**, **20**, and **21**, respectively (Fig. 2).

Biological Activity

The MICs of nine new derivatives together with reference compounds were determined by the 2-fold serial agar dilution method in Mueller-Hinton agar against 32 strains of test organisms. As shown in the footnote of Table 3, the test organisms are classified in five groups. The geometric mean of MICs on each group is calculated and the results shown in Tables 3 and 4.

[a] The corresponding (*R*)- and (*RS*)-isomers have been prepared [7)] by Route A of Scheme 1.

Scheme 1. Preparation of fluorinated amino acids.

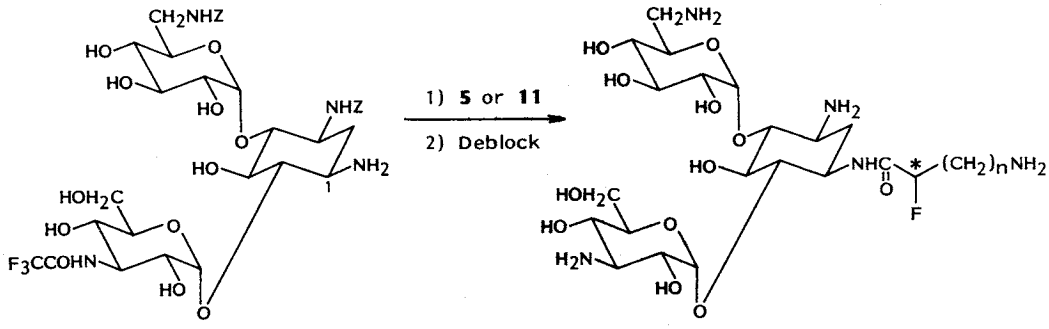


* *S* or *R*; n=1, 2, 3.

a R_1, R_2=phthaloyl R_3=Me
b R_1, R_2=Z, H R_3=Benzyl (Bn)
c R_1, R_2=Z, H R_3=Me
d R_1, R_2, R_3=Bn

As shown in Table 3, the (*S*)-4-amino-2-fluorobutyryl derivative of kanamycin A (**14**) was the best of the fluoro derivatives prepared so far, and was nearly as active as amikacin against all of the organism groups including *Pseudomonas aeruginosa*. However, the corresponding (*R*)-isomer (**15**), lower and higher homologs (**13** and **16**), and the difluoro derivative (**17**) showed markedly decreased activity as compared to amikacin. There was a great discrepancy in the antibacterial activity of the fluorobutyryl (**14**) and fluoropropanoyl derivative (**13**), although there is only a slight difference between the corresponding 2-hydroxy derivatives[11]. As was already speculated in the literature[7], one of the reasons might be the high electronegativity of the fluorine atom substituted on the β-carbon to the ω-amino group in **13**, which decreased the basicity of the amino group resulting in a decrease of its biological activity. This is supported

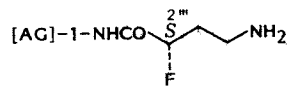
Scheme 2. Preparation of amikacin derivatives with a fluorinated amino acid side chain.



12

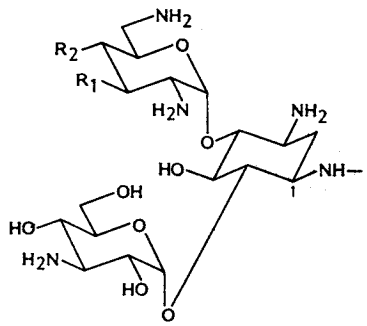
Compound	n	*
13	1	*S*
14	2	*S*
15	2	*R*
16	3	*S*
17	2	F F

Fig. 2. Aminoglycosides with an (*S*)-4-amino-2-fluorobutyric acid side chain.

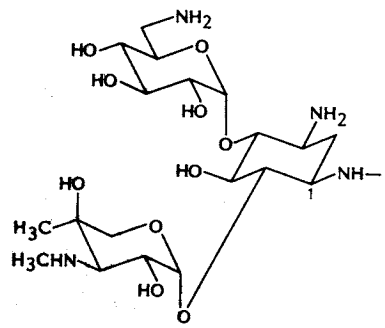


Compound	[AG]
18	Kanamycin B
19	Tobramycin
20	Dibekacin
21	Gentamicin B

[AG]–=

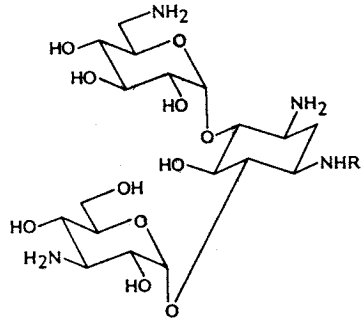


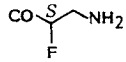
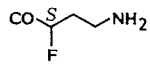
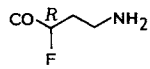
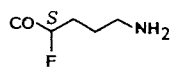
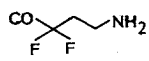
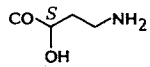
Kanamycin B $R_1=R_2=OH$
Tobramycin $R_1=H$ $R_2=OH$
Dibekacin $R_1=R_2=H$



Gentamicin B

Table 3. *In vitro* activity of amikacin derivatives.



Compound	R	Geometric mean of MIC (μg/ml)				
		Gp-S (5 strains)	Gp-R (4)	Gn-S (8)	Gn-R (8)	Pa (7)
13		4.1	>50	9.6	21	21
14		0.60	42	1.6	4.4	7.6
15		3.6	>50	15	27	25
16		1.0	>50	4.4	6.8	19
17		2.4	>50	8.1	30	28
Amikacin		0.65	30	1.4	3.7	5.2

Gp-S: Kanamycin A-sensitive *Staphylococcus aureus* (3 strains) and *S. epidermidis* (2). Gp-R: Kanamycin A-resistant *S. aureus* (1), *S. epidermidis* (1), *Enterococcus faecalis* (1) and *E. faecium* (1). Gn-S: Kanamycin A-sensitive *Escherichia coli* (2), *Proteus mirabilis* (1), *P. vulgaris* (1), *Morganella morganii* (1), *Klebsiella pneumoniae* (1), *Citrobacter freundii* (1), and *Serratia marcescens* (1). Gn-R: Kanamycin A-resistant *E. coli* (5), *K. pneumoniae* (1), *S. marcescens* (1) and *Enterobacter cloacae* (1). Pa: *Pseudomonas aeruginosa* (7).

Table 4. *In vitro* activity of fluoro derivatives of aminoglycosides.

Compound	[AG]	R	Geometric means of MIC (μg/ml)				
			Gp-S (5 strains)	Gp-R (4)	Gn-S (8)	Gn-R (8)	Pa (7)
14	Kanamycin A	F	0.60	42	1.6	4.4	7.6
Amikacin		OH	0.65	30	1.4	3.7	5.2
18	Kanamycin B	F	0.26	35	1.9	8.1	6.9
22[17,18]		OH	0.26	30	1.1	4.8	5.7
19	Tobramycin	F	0.20	21	1.3	2.2	2.8
23[19]		OH	0.20	13	0.93	1.7	1.6
20	Dibekacin	F	0.22	3.7	1.7	3.4	5.7
24[18]		OH	0.11	2.2	0.85	1.7	2.1
21	Gentamicin B	F	0.90	30	2.0	5.3	21
25[20]		OH	1.1	18	1.1	2.0	8.4

Abbreviations: See Table 3.

by the p*K*a values of the fluoro- and hydroxy-amino acids involved (Table 5).

Table 4 shows the antibacterial activity of aminoglycosides with the (*S*)-4-amino-2-fluorobutyryl side chain (**14** and **18**~**21**) and those with the corresponding 2-hydroxybutyryl side chain (amikacin and **22**~**25**). All the 2-fluoro derivatives showed almost the same activity as that of the corresponding 2-hydroxy derivatives. In terms of antibacterial activity, the 2-fluoro group may play the same role as the 2-hydroxy group in the 4-aminobutyric acid side chain.

Table 5. p*K*a Values of ω-amino-α-fluoro and α-hydroxyalkanoic acids.

$NH_2(CH_2)_n$-CH(R)-COOH

n	R	p*K*a	
1	F	1.9	8.8
	OH	2.5	9.4
2	F	2.0	10.0
	OH	3.0	10.3
		(2.9)	(9.9)[a]
3	F	2.3	10.4
	OH	3.2	10.7
2	$-CF_2-$	ND	9.7

[a] Reported values[21].
ND: Not determined.

The acute toxicity of **14** (LD_{50} 280 mg/kg, iv, mice) was found to be the same as that of amikacin (LD_{50} 280 mg/kg).

Experimental

MP's were determined with a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were recorded on an Analect FX-6160 FT-IR spectrometer and UV spectra on a Shimadzu UV-260 spectrophotometer. Optical rotations were measured by a Jasco DIP-140 polarimeter and circular dichroism spectra on a Jasco J600 spectropolarimeter. NMR spectra were recorded on a Jeol CL-60HL, Varian FT-80A or Joel GX-400 spectrometer. Mass spectra were measured on a JMS-AX505H mass spectrometer. p*K*a Values were determined by a Kyoto Electronics potentiometric automatic titrator AT-118. HPLC analyses were performed on a Shimadzu LC-6A under the following conditions. Column: SSC-ODS-262 (6 × 100 mm); mobile phase: 20~25% MeOH/phosphate buffer (pH 2); detection: OPA/fluorescence.

MICs were determined on solid medium by the standard 2-fold agar dilution method in Mueller-Hinton Agar (Difco) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml. LD_{50} values (mice, iv) were determined according to the reported procedure[12] using 5 mice for each dosing level.

Methyl (*R*)-2-Hydroxy-4-phthalimidobutyrate ((*R*)-**2a** (n=2))

A solution of (*R*)-2-hydroxy-4-phthalimidobutyric acid[13] ((*R*)-**1a** (n=2), 53.5 g, 0.21 mol) in 1.3 N HCl-methanol (1.5 liters) was kept at room temperature for 16 hours. The solvent was evaporated and the residue was dissolved in EtOAc (1.5 liters), washed with aqueous sodium bicarbonate solution (1 liter) and aqueous sodium chloride solution (1 liter), successively, dried ($MgSO_4$) and concentrated to afford a colorless solid, which was triturated with *n*-hexane to yield 51.1 g (92%) of the title compound. MP 125~127°C; $[\alpha]_D^{23}$ −9.3° (*c* 5.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1740, 1700; UV λ_{max}^{MeOH} nm (ε) 241 (9,800), 293 (1,800); ^1H NMR (80 MHz, $CDCl_3$) δ 1.9~2.4 (2H, m, 3-CH_2), 3.20 (1H, d, *J*=6 Hz, OH), 3.73 (3H, s, CH_3), 3.90 (2H, t, *J*=7 Hz, 4-CH_2), 4.27 (1H, m, 2-CH), 7.72 (4H, m, aromatic-H).

Anal Calcd for $C_{13}H_{13}NO_5$: C 59.31, H 4.98, N 5.32.
Found: C 59.33, H 4.95, N 5.19.

Methyl (*S*)-2-Fluoro-4-phthalimidobutyrate ((*S*)-**3a** (n=2))

To a solution of the hydroxy ester (*R*)-**2a** (n=2) (50 g, 0.19 mol) in dichloromethane (500 ml) was added DAST (75 ml, 0.57 mol) at 0°C. The solution was stirred at room temperature for 24 hours and poured into a cold aqueous sodium bicarbonate solution. The organic layer was separated, washed with water, dil HCl, water and an aqueous sodium chloride solution, successively, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (500 g), eluted with toluene and toluene-EtOAc (10:1), successively. The desired fractions were combined and concentrated *in vacuo*; the residue was triturated with ether and *n*-hexane to yield 20.8 g (41%) of the title compound as colorless

needles. MP 92~94°C; $[\alpha]_D^{25}$ +2.5° (*c* 5.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1740, 1710; UV λ_{max}^{MeOH} nm (ε) 240 (9,900), 293 (1,800); ^1H NMR (60 MHz, $CDCl_3$) δ 1.8~2.7 (2H, m, 3-CH_2), 3.75 (3H, s, CH_3), 3.86 (2H, t, *J*=7 Hz, 4-CH_2), 4.98 (1H, dt, *J*=6 and 48 Hz, 2-CH), *ca.* 7.7 (4H, m, Ph).

Anal Calcd for $C_{13}H_{12}NO_4F$: C 58.87, H 4.56, N 5.28.
Found: C 58.70, H 4.37, N 5.27.

When the same reaction was carried out in the presence of pyridine (10 equiv), the same product (*S*)-**3a** (n=2) (100 mg, yield 38%) was obtained from 263 mg of (*R*)-**2a** (n=2).

(*S*)-4-Amino-2-fluorobutyric Acid ((*S*)-**4** (n=2))

A suspension of the ester (*S*)-**3a** (n=2) (20 g, 0.75 mol) in 6 N HCl was heated under reflux overnight. After cooling, the precipitate was removed by filtration. The filtrate was concentrated *in vacuo*. The residue was subjected to a column of Amberlite IRA 120 (H^+ form, 800 ml). The column was washed with water and eluted with 2 N NH_4OH. The combined desired fractions were concentrated to a small volume and added to ethanol to crystallize 8.6 g (94%) of (*S*)-**4** (n=2) as colorless prisms. MP 240~242°C; $[\alpha]_D^{25}$ −26° (*c* 2.5, H_2O); IR ν_{max} (KBr) cm^{-1} 1590, 1570, 1400; ^1H NMR (60 MHz, D_2O) δ 1.9~2.7 (2H, m, 3-CH_2), 3.23 (2H, t, *J*=7 Hz, 4-CH_2), 4.99 (1H, dt, *J*=6 and 49 Hz, 2-CH).

Anal Calcd for $C_4H_8NO_2F$: C 39.67, H 6.66, N 11.57.
Found: C 39.83, H 6.69, N 11.49.

(*S*)-4-Benzyloxycarbonylamino-2-fluorobutyric Acid ((*S*)-**5** (n=2))

To a stirred solution of 1.4 g (11.6 mmol) of (*S*)-**4** (n=2) and 1 g (25 mmol) of sodium hydroxide in 50 ml of water was added dropwise a solution of 4 g (23 mmol) of carbobenzoxy chloride in 5 ml of ether. and the mixture was stirred at room temperature for 1 hour. The mixture was washed with 20 ml of ether and the aqueous layer was acidified with dil HCl and extracted twice with 50 ml of EtOAc. The combined extracts were washed with water and brine and dried over $MgSO_4$. After evaporating to dryness, the oily residue was triturated with *n*-haxane to give 2.67 g (91.5%) of the title compound. MP 82~84°C; $[\alpha]_D^{23}$ −2.7° (*c* 5.0, MeOH); IR ν_{max} (KBr) cm^{-1} 3300, 1690, 1550; UV λ_{max}^{MeOH} nm (ε) 252 (150), 258 (178); ^1H NMR (80 MHz, $CDCl_3$) δ 1.7~2.5 (2H, m, 3-CH_2), 3.34 (2H, t, *J*=7 Hz, 4-CH_2), 4.90 (1H, m, 2-CH), 5.10 (2H, s, CH_2Ph), 7.3 (5H, s, phenyl-H).

Anal Calcd for $C_{12}H_{14}NO_4F$: C 56.48, H 5.53, N 5.49.
Found: C 56.62, H 5.57, N 5.66.

(*R*)-4-Benzyloxycarbonylamino-2-fluorobutyric Acid ((*R*)-**5** (n=2))

By the procedures similar to those described above, (*S*)-2-hydroxy-4-phthalimidobutyric acid ((*S*)-**1a** (n=2))[13] was converted to the title compound (*R*)-**5** (n=2). See Table 6.

Table 6. Yield and physico-chemical properties of (*R*)-4-benzyloxycarbonylamino-2-fluorobutyric acid ((*R*)-**5** (n=2)) and its intermediate compounds, (*S*)-**2a** (n=2), (*R*)-**3a** (n=2) and (*R*)-**4** (n=2).

Compound	Yield (%)	MP (°C)	$[\alpha]_D$ (solvent)	Analysis					
				Found			Calcd (molecular formula)		
				C	H	N	C	H	N
(*S*)-**2a** (n=2)	87	123~124	+9.8° ($CHCl_3$)	59.54	4.93	5.22	59.31	4.98 ($C_{13}H_{13}NO_5$)	5.32
(*R*)-**3a** (n=2)	49	90~94	−2.1° ($CHCl_3$)	58.92	4.50	5.17	58.87	4.56 ($C_{13}H_{12}NO_4F$)	5.28
(*R*)-**4** (n=2)	87	240~243	+22° (H_2O)	39.82	6.59	11.52	39.67	6.66 ($C_4H_8NO_2F$)	11.57
(*R*)-**5** (n=2)	93	82~84	+2.9° (MeOH)	56.39	5.40	5.43	56.48	5.53 ($C_{12}H_{14}NO_4F$)	5.49

Reaction of Benzyl (*R*)-4-Benzyloxycarbonylamino-2-hydroxybutyrate ((*R*)-**2b** (n=2)) with DAST: Formation of Benzyl *N*-Benzyloxycarbonylazetidine-2-carboxylate (**6**)

(a) The starting material of (*R*)-**2b** (n=2) was prepared by esterification of the corresponding carboxylic acid ((*R*)-**1b** (n=2))[13] with benzyl alcohol according to the reported procedure[7] in 83% yield as an oil. $[\alpha]_D^{25}$ −7.5° (*c* 1.4, $CHCl_3$); IR ν_{max} (film) cm^{-1} 3370, 1720, 1690, 1520, 1445, 1260; ^1H NMR (80 MHz, $CDCl_3$) δ *ca.* 2.0 (2H, m, 3-CH_2), 3.34 (2H, q, *J*=7 Hz, 4-CH_2), 4.25 (1H, dd, *J*=4 and 8 Hz, 2-CH), 5.06, 5.16 (4H, each s, 2×CH_2Ph), 7.30 (10H, s, 2×Ph); FAB(+)-MS *m/z* 344 (M+H).

(b) To a cooled solution of 1.2 g (3.5 mmol) of (*R*)-**2b** (n=2) in 12 ml of dry dichloromethane was added 2 ml (15.1 mmol) of DAST and the mixture was stirred at room temperature for 4 hours. The same work-up procedures and purification by silica gel column chromatography afforded 880 mg (77%) of the title compound **6** as an oil. $[\alpha]_D^{25}$ +21° (*c* 1.5, $CHCl_3$); IR ν_{max} (film) cm^{-1} 1740, 1690, 1200; ^1H NMR (400 MHz, $CDCl_3$) δ *ca.* 1.98~2.08 (1H, m, 3-CH), 2.10~2.18 (1H, m, 3-CH), 3.29~3.36 (1H, m, 4-CH), 3.38~3.47 (1H, m, 4-CH), 4.88 (1H, m, 2-CH), 5.10 (2H, s)*, and 5.22, 5.26 (2H, ABq, *J*=12.4 Hz)*, (*: N−OCOCH_2Ph or COOCH_2Ph), 7.2~7.5 (10H, m, phenyl-H); EI-MS *m/z* 325 $(M)^+$.

The same reaction in the presence of pyridine (10 equiv) afforded a mixture of products on TLC.

Reaction of Methyl (*R*)-4-Benzyloxycarbonylamino-2-hydroxybutyrate ((*R*)-**2c** (n=2)) with DAST

A sample of (*R*)-**2c** (n=2) (150 mg, 0.56 mmol), prepared from (*R*)-**1c** (n=2)[13] with HCl-MeOH by the conventional method, was treated with DAST (0.22 ml, 1.69 mmol) by the same procedure as described above to afford the corresponding methyl ester of azetidine derivative **6'** (130 mg, yield 87%) as an oil, which was rather unstable to chromatographic purification on silica gel column. IR ν_{max} (film) cm^{-1} 1735, 1690, 1530; ^1H NMR (80 MHz, $CDCl_3$) δ *ca.* 2.0 (2H, m, 3-CH_2), *ca.* 3.4 (2H, m, 4-CH_2), 3.75 (3H, s, COOMe), *ca.* 4.55 (1H, m, 2-C*H*), 5.10 (2H, s, CH_2Ph), 7.33 (5H, s, Ph); EI-MS *m/z* 249 $(M)^+$.

The same reaction in the presence of pyridine (10 equiv) also afforded multiple products on TLC.

Benzyl (*S*)-3-Dibenzylamino-2-fluoropropanoate ((*S*)-**3d** (n=1))

Title compound (*S*)-**3d** (n=1) was prepared from D-serine according to the reported procedures of preparing the corresponding (*R*)-isomer[8]. 2.71 g (yield 46% from D-serine) as a syrup. $[\alpha]_D^{25}$ −0.9° (*c* 3.9, $CHCl_3$); IR ν_{max} (film) cm^{-1} 1755, 1655, 1620; ^1H NMR (80 MHz, $CDCl_3$) δ 3.02 (2H, dd, *J*=5 and 25 Hz, 3-H), 3.52, 3.82 (4H, ABq, *J*=14 Hz, NCH_2Ph), 5.02, 5.23 (2H, ABq, *J*=13 Hz, COOCH_2Ph), 5.06 (1H, dt, *J*=5 and 50 Hz, 2-H), *ca.* 7.3 (15H, m, Ph).

Anal Calcd for $C_{24}H_{24}NO_2F \cdot \frac{1}{4}H_2O$: C 75.47, H 6.47, N 3.67.
Found: C 75.54, H 6.38, N 3.63.

(*S*)-3-Amino-2-fluoropropanoic Acid ((*S*)-**4** (n=1))

A solution of (*S*)-**3d** (n=1) (130 mg) in MeOH (3 ml)-H_2O (0.3 ml) was subjected to hydrogenolysis on 10% Pd-C (70 mg) under H_2 atmosphere at room temperature for 8 hours. The catalyst was removed by filtration and washed with water. The combined filtrate and washings were evaporated to a small volume and then lyophilized to afford 37 mg of the title compound (yield quantitative). MP 205~210°C; $[\alpha]_D^{24}$ −32° (*c* 1.0, H_2O) (literature[8] for (*R*)-isomer $[\alpha]_D$ +29.1° (*c* 1.05, H_2O)); ^1H NMR (80 MHz, D_2O) δ *ca.* 3.0~4.0 (2H, m, 3-H), 5.17 (1H, ddd, *J*=4, 7 and 50 Hz, 2-H).

Anal Calcd for $C_3H_6NO_2F$: C 33.64, H 5.66, N 13.08.
Found: C 33.33, H 5.65, N 12.84.

(*S*)-3-Benzyloxycarbonylamino-2-fluoropropanoic Acid ((*S*)-**5** (n=1))

By the procedure used to prepare (*S*)-**5** (n=2) from (*S*)-**4** (n=2), (*S*)-**4** (n=1) (480 mg) was converted to the title compound (*S*)-**5** (n=1) (682 mg, yield 63%). MP 102~104°C; $[\alpha]_D^{25}$ −9° (*c* 1.0, EtOAc) (literature[7] for the corresponding (*R*)-isomer: MP 93~96°C, $[\alpha]_D^{25}$ +6° (*c* 1.7, EtOAc)); IR ν_{max} (KBr) cm^{-1} 3350, 1740, 1685, 1650, 1540; ^1H NMR (80 MHz, $CDCl_3$) δ *ca.* 3.6~3.9 (2H, m, 3-CH_2), 4.98 (1H, dt, *J*=4.6 and 48.4 Hz, 2-H), 5.13 (2H, s, CH_2Ph), 5.85 (2H, s, NH and COOH), 7.32 (5H, s, Ph).

Anal Calcd for $C_{11}H_{12}NO_4F$: C 54.77, H 5.01, N 5.81.
Found: C 54.57, H 5.02, N 5.76.

(*S*)-5-Benzyloxycarbonylamino-2-fluorovaleric Acid ((*S*)-**5** (n=3))

With the procedure employed in the transformation of (*R*)-**1a** (n=2) to (*S*)-**5** (n=2), (*R*)-**1a** (n=3) was converted to (*S*)-**5** (n=3).

(a) Methyl (*R*)-2-Hydroxy-5-phthalimidovalerate ((*R*)-**2a** (n=3)): Yield 920 mg (35%); mp 108~109°C; $[\alpha]_D^{28}$ −2.0° (*c* 5.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3480, 2950, 2920, 1770, 1740, 1720; ^1H NMR (80 MHz, $CDCl_3$) δ 1.5~2.0 (4H, m, 3, 4-CH_2), 3.5~4.4 (6H, m, COOMe, 5-CH_2, 2-CH), 7.5~7.9 (4H, m, Ph).

Anal Calcd for $C_{14}H_{15}NO_5$: C 60.64, H 5.45, N 5.05.
Found: C 60.53, H 5.47, N 4.94.

(b) Methyl (*S*)-2-Fluoro-5-phthalimidovalerate ((*S*)-**3a** (n=3)): (*R*)-**2a** (n=3) (552 mg, 2.0 mmol) was treated with DAST (0.85 ml, 6.5 mmol) at room temperature for 2 hours to afford 130 mg of the title compound (yield 23%) as prisms. MP 74~76°C; $[\alpha]_D^{28}$ −7.9° (*c* 5.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1770, 1740, 1690; ^1H NMR (80 MHz, $CHCl_3$) δ 1.6~2.2 (4H, m, 3, 4-CH_2), 3.6~3.9 (5H, m, COOMe, 5-CH_2), 4.92 (1H, dt, *J*=48 and 6 Hz, 2-CH), 7.6~7.9 (4H, m, Ph).

Anal Calcd for $C_{14}H_{14}NO_4F$: C 60.21, H 5.05, N 5.02.
Found: C 60.07, H 5.03, N 4.81.

The same reaction in the presence of pyridine (10 equiv) also gave the same product (96 mg, yield 34%) starting from 277 mg (1.0 mmol) of (*R*)-**2a** (n=3).

(c) (*S*)-5-Amino-2-fluorovaleric Acid ((*S*)-**4** (n=3)): Yield 259 mg (quantitative); mp 135~137°C; $[\alpha]_D^{25}$ −16° (*c* 0.25, H_2O); IR ν_{max} (KBr) cm^{-1} 1730, 1590; ^1H NMR (400 MHz, D_2O) δ *ca.* 1.8~2.0 (4H, m, 3, 4-CH_2), 3.09 (2H, t, *J*=7.5 Hz, 5-CH_2), 5.12 (1H, ddd, *J*=4.4, 6.6 and 49.1 Hz, 2-CH).

Anal Calcd for $C_5H_{10}NO_2F \cdot 3H_2O$: C 31.74, H 8.52, N 7.40.
Found: C 32.06, H 8.53, N 7.74.

(d) (*S*)-**5** (n=3): Yield 303 mg (87%); mp 58~61°C; $[\alpha]_D^{24}$ −2.1° (*c* 2.5, EtOAc); IR ν_{max} (KBr) cm^{-1} 1720, 1680; ^1H NMR (60 MHz, $CDCl_3$) δ 1.5~2.5 (4H, m, 3, 4-CH_2), 3.25 (2H, m, 5-CH_2), 4.90 (1H, dt, *J*=6 and 49 Hz, 2-CH), 5.10 (2H, s, CH_2Ph), 7.30 (5H, s, Ph).

Anal Calcd for $C_{13}H_{16}NO_4F$: C 57.99, H 5.99, N 5.20.
Found: C 57.72, H 6.07, N 5.26.

Reaction of Benzyl (*R*)-5-Benzyloxycarbonylamino-2-hydroxyvalerate ((*R*)-**2b** (n=3)) with DAST: Formation of Benzyl *N*-Benzyloxycarbonylpyrrolidine-2-carboxylate (**7**)

(a) The starting material of (*R*)-**2b** (n=3) was prepared by α-hydroxylation of D-ornithine[14], followed by conventional esterification.

i) (*R*)-5-Benzyloxycarbonylamino-2-hydroxyvaleric Acid ((*R*)-**1b** (n=3)): Yield 3.98 g (59%); mp 108~110°C (literature[14] for (*S*)-isomer. MP 107~108.5°C); $[\alpha]_D^{28}$ −2.2° (*c* 2.5, MeOH), IR ν_{max} (KBr) cm^{-1} 1720, 1680; ^1H NMR (60 MHz, DMSO-d_6) δ 1.55 (4H, m, 3, 4-CH_2), 3.00 (2H, m, 5-CH_2), 3.92 (1H, m, 2-CH), 5.00 (2H, s, CH_2Ph), 7.32 (5H, s, Ph).

Anal Calcd for $C_{13}H_{17}NO_5$: C 58.42, H 6.41, N 5.24.
Found: C 58.46, H 6.30, N 5.23.

ii) (*R*)-**2b** (n=3): Yield 1.48 g (quantitative); $[\alpha]_D^{24}$ +9.7° (*c* 2.5, $CHCl_3$); IR ν_{max} (film) cm^{-1} 1720, 1700; ^1H NMR (60 MHz, CCl_4) δ 1.60 (4H, m, 3, 4-CH_2), 3.06 (2H, m, 5-CH_2), 4.10 (1H, m, 2-CH), 4.95, 5.05 (2H each, s, 2×CH_2Ph), 7.18, 7.22 (5H each, s, 2×Ph).

Anal Calcd for $C_{20}H_{23}NO_5 \cdot \frac{1}{4}H_2O$: C 66.38, H 6.54, N 3.87.
Found: C 66.20, H 6.51, N 3.79.

(b) To a solution of (*R*)-**2b** (n=3) (357 mg, 1.0 mmol) in CH_2Cl_2 (7 ml) was added DAST (0.66 ml, 5 mmol) and the mixture was stirred at room temperature for 1.5 hours. The usual work-up and silica gel chromatography afforded 342 mg (quantitative) of the title compound **7** as a yellow oil. IR ν_{max} (film) cm^{-1} 1740, 1700; ^1H NMR (60 MHz, CCl_4) δ 1.95 (4H, m, 3, 4-CH_2), 3.50 (2H, m, 5-CH_2), 4.30 (1H, m, 2-CH), 4.95, 5.08 (2H each, s, 2×CH_2Ph), 7.25 (10H, s, 2×Ph).

(c) The same reaction of (*R*)-**2b** (n=3) (3.25 g, 9.1 mmol) with DAST (6.0 ml, 45.5 mmol) in CH_2Cl_2 (65 ml) in the presence of pyridine (7.3 ml, 90 mmol) at room temperature for 2 days afforded, after silica gel column chromatography, 466 mg (yield 14%) of (*S*)-**3b** (n=3) and 84 mg (yield 3%) of **7**. (*S*)-**3b** (n=3); IR ν_{max} (film) cm^{-1} 1760, 1740; ^1H NMR (60 MHz, CCl_4) δ 1.4~2.3 (4H, m, 3, 4-CH_2), 3.08 (2H, t, *J*=6 Hz, 5-CH_2), 4.80 (1H, dt, *J*=49 and 5 Hz, 2-CH), 4.97, 5.10 (2H each, s, 2×CH_2Ph), 7.22

(10H, s, 2×Ph).

Methyl 2-Oxo-4-phthalimidobutyrate (**8**)[15]

To a solution of (*RS*)-**2a** (n=2)[13] (2.63 g, 10 mmol) in a mixture of DMSO (13 ml) and toluene (13 ml) containing pyridine (0.81 ml, 10 mmol) and TFA (0.39 ml, 5 mmol) was added dicyclohexylcarbodiimide (DCC, 3.10 g, 15 mmol) and the whole mixture was stirred at room temperature for 1 hour and then diluted with EtOAc (100 ml). The insolubles formed were removed by filtration and washed with EtOAc. The combined filtrate and washings were washed with H_2O (3×50 ml) and saturated aqueous NaCl, dried and evaporated. The residual syrup was crystallized from ether (3 ml) and *n*-hexane (1 ml) to afford the title compound (2.57 g, yield 98%). MP 127°C; IR ν_{max} (KBr) cm^{-1} 1770, 1710; ^1H NMR (60 MHz, $CDCl_3$) δ 3.27 (2H, t, J=7 Hz, 3-CH_2), 3.88 (3H, s, COOMe), 4.07 (2H, t, 4-CH_2), *ca.* 7.8 (4H, m, Ph).

Anal Calcd for $C_{13}H_{11}NO_5$: C 59.77, H 4.24, N 5.36.
Found: C 59.70, H 4.21, N 5.30.

Methyl 2,2-Difluoro-4-phthalimidobutyrate (**9**)

To a solution of **8** (2.10 g, 8.05 mmol) in CH_2Cl_2 (40 ml) was slowly added DAST (7.44 ml, 56.3 mmol); the mixture was stirred at room temperature under argon atmosphere overnight. Usual work-up procedures, followed by purification on silica gel afforded 1.12 g (yield 50%) of the title compound **9**. MP 67°C; IR ν_{max} (KBr) cm^{-1} 1760, 1710; ^1H NMR (60 MHz, $CDCl_3$) δ *ca.* 2.0~3.2 (2H, m, 3-CH_2), 3.80 (3H, s, $COOCH_3$), *ca.* 3.5~4.2 (2H, m, 4-CH_2), *ca.* 7.7 (4H, m, Ph).

Anal Calcd for $C_{13}H_{11}NO_4F_2$: C 55.13, H 3.91, N 4.95.
Found: C 55.42, H 3.92, N 5.16.

4-Benzyloxycarbonylamino-2,2-difluorobutyric Acid (**11**)

A sample **9** (1.00 g) was converted to the title compound *via* 4-amino-2,2-difluorobutyric acid (**10**) by the same procedures employed for (*S*)-**3a** (n=2) to (*S*)-**5** (n=2) to afford 880 mg (yield 91%) of **11** as crystals.

10: MP 245~247°C; IR ν_{max} (KBr) cm^{-1} 1650, 1610, 1560; ^1H NMR (80 MHz, D_2O) δ 2.55 (2H, m, J=7 and 16.5 Hz, 3-CH_2), 3.37 (2H, t, J=7 Hz, 4-CH_2). FAB(+)-MS *m/z* 140 (M+H).

11: MP 82°C; IR ν_{max} (KBr) cm^{-1} 3390, 1760, 1660, 1630; ^1H NMR (60 MHz, $CDCl_3$) δ *ca.* 2.0~2.7 (2H, m, 3-CH_2), *ca.* 3.4 (2H, m, 4-CH_2), 5.13 (2H, s, CH_2Ph), 7.35 (5H, s, Ph).

Anal Calcd for $C_{12}H_{13}NO_4F_2$: C 52.75, H 4.80, N 5.13
Found: C 52.65, H 4.75, N 5.34.

Acylation of 1-Free-NH_2 Kanamycin A Derivative (**12**) with Fluoroamino Acids

Coupling reactions of *N*-protected kanamycin A derivative (**12**)[10] with fluoroamino acids (*S*)-**5** (n=1), (*S*)-**5** (n=2), (*R*)-**5** (n=2), (*S*)-**5** (n=3) and **11** by conventional active ester method, followed by deprotection afforded the final products **13**, **14**, **15**, **16** and **17**, respectively. The representative procedure for **14**, along with the physical data of these compounds are given below.

(*S*)-2‴-Deoxy-2‴-fluoroamikacin (**14**)

(a) A mixture of 3.06 g (12 mmol) of (*S*)-**5** (n=2), 1.66 g (14.4 mmol) of *N*-hydroxysuccinimide and 3 g (14.6 mmol) of DCC in 100 ml of dry THF was stirred at room temperature for 4 hours. The resulting urea was removed by filtration and the filtrate was concentrated to *ca.* 40 ml, which was added to a mixture of 6.78 g (8 mmol) of 3,6′-di-*N*-benzyloxycarbonyl-3″-*N*-trifluoroacetylkanamycin A (**12**) and 2 ml (14.2 mmol) of triethylamine in THF (100 ml) and H_2O (100 ml); the mixture was stirred at room temperature for 20 hours. After concentrating to 40 ml, the concentrate was diluted with 500 ml of water and the resulting precipitate was collected by filtration and air-dried to give 8.3 g (>100%) of the acylated product, which was dissolved in 400 ml of THF and 200 ml of 14% ammonia and stirred at room temperature for 2 days. The mixture was evaporated to dryness and the residue was hydrogenated over 1 g of 10% palladium on charcoal in a mixture of MeOH (200 ml), H_2O (100 ml), THF (100 ml) and AcOH (10 ml) at room temperature overnight under hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in 50 ml of water and the mixture was charged on

a 200-ml of column of Amberlite CG-50 (H^+ form) ion-exchange resin. The column was washed with water (1 liter) and then eluted with 0.15, 0.3 and 0.5 N ammonia, successively. The desired fractions eluting with 0.3 and 0.5 N ammonia, according to TLC monitoring, were collected and concentrated to 20 ml and lyophilized to give 3.07 g (59% from **12**) of the title compound (**14**) as the carbonate. Estimated purity 90% (by HPLC); mp 200°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1650, 1560; ^1H NMR (80 MHz, D_2O+DCl) δ 5.17 (1H, d, $J=4$ Hz, 1″-H), 5.58 (1H, d, $J=4$ Hz, 1′-H), 5.2~5.5 (1H, m, 2‴-CH).

Anal Calcd for $C_{22}H_{42}N_5O_{12}F \cdot H_2CO_3$: C 42.52, H 6.83, N 10.78.
Found: C 42.51, H 6.89, N 10.67.

TLC: Rf 0.38 (CH_2Cl_2 - MeOH - conc NH_4OH - H_2O, 1 : 4 : 2 : 1).

(b) The carbonate obtained above (550 mg, 0.79 mmol) was dissolved in 3 ml of *N*-sulfuric acid and the solution was added to cooled ethanol (50 ml).The resulting precipitate was collected by filtration, washed with ethanol to give 672 mg of the product, which was dissolved in 10 ml of water; the mixture was adjusted to pH 6 by the addition of Dowex 1-X2 resin, and then was filtered and concentrated to 5 ml. The concentrate was chromatographed on a Sephadex G-10 column (140 ml) by eluting with water. The desired fractions were collected, concentrated to 5 ml, and lyophilized to give 612 mg (95%) of the title compound (**14**) as the sulfate. Esimated purity >90% (by HPLC); mp >230°C (gradually dec); $[\alpha]_D^{23}$ +79.5° (*c* 2.5, H_2O); IR ν_{max} (KBr) cm^{-1} 1650, 1540, 1060; ^1H NMR (400 MHz, D_2O) δ 1.86 (1H, q, $J=12.6$ Hz, 2-H_{ax}), 5.18 (1H, d, $J=3.7$ Hz, 1″-H), 5.19 (1H, ddd, $J=48.4$, 9.0 and 3.4 Hz, 2‴-CH), 5.61 (1H, d, $J=4.0$ Hz, 1′-H); ^{13}C NMR see Table 7.

Anal Calcd for $C_{22}H_{42}N_5O_{12}F \cdot 2H_2SO_4 \cdot 2H_2O$: C 32.23, H 6.15, N 8.54, S 7.82.
Found: C 32.26, H 6.18, N 8.61, S 7.85.

Karl Fischer Calcd 4.4%. Found 4.0%.

1-*N*-[(*S*)-3-Amino-2-fluoropropanoyl]kanamycin A (**13**)

Yield 322 mg (42%) as the sulfate; Estimated purity 85% by HPLC; mp 195°C; $[\alpha]_D^{24}$ +81° (*c* 2.2, H_2O); IR ν_{max} (KBr) cm^{-1} 3747, 3336, 1674, 1538; ^1H NMR (400 MHz, D_2O) δ *ca.* 1.8~2.3 (2H, m, 2-H), 5.14 (1H, d, $J=3.7$ Hz, 1″-H), 5.38 (1H, ddd, $J=3.3$, 8.2 and 48.2 Hz, 2‴-H), 5.60 (1H, d, $J=4.0$ Hz, 1′-H).

Anal Calcd for $C_{21}H_{40}N_5O_{12}F \cdot 2H_2SO_4 \cdot 3H_2O$: C 30.62, H 6.12, N 8.50, S 7.78.
Found: C 30.27, H 6.18, N 8.76, S 7.60.

(*R*)-2‴-Deoxy-2‴-fluoroamikacin (**15**)

Yield 167 mg (15%) as the carbonate; Estimated purity 95% (by HPLC); mp 200°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1650, 1560; ^1H NMR (80 MHz, D_2O+DCl) δ 4.9~5.4 (1H, m, 2‴-H), 5.12 (1H, d, $J=3$ Hz, 1″-H), 5.55 (1H, d, $J=3$ Hz, 1′-H).

Anal Calcd for $C_{22}H_{42}N_5O_{12}F \cdot 1\frac{1}{2}H_2CO_3$: C 41.47, H 6.66, N 10.29.
Found: C 41.70, H 6.78, N 10.31.

TLC: Rf value 0.41 (CH_2Cl_2 - MeOH - conc NH_4OH - H_2O, 1 : 4 : 2 : 1).

1-*N*-[(*S*)-5-Amino-2-fluorovaleryl]kanamycin A (**16**)

Yield 62 mg (15%) as the carbonate; Estimated purity 80% by HPLC; mp 171~174°C; $[\alpha]_D^{25}$ +90° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1650; ^1H NMR (80 MHz, D_2O) δ 5.0~5.2 (1H, m, 2‴-CH), 5.12 (1H, d, $J=4.0$ Hz, 1″-H), 5.33 (1H, d, $J=3.5$ Hz, 1′-H).

Anal Calcd for $C_{23}H_{44}N_5O_{12}F \cdot 1\frac{1}{2}H_2CO_3 \cdot H_2O$: C 41.29, H 6.93, N 9.83.
Found: C 41.36, H 6.82, N 9.78.

2‴,2‴-Difluoroamikacin (**17**)

Yield 121 mg (20%) as the carbonate; mp 165°C (dec); Estimated purity 80% (by TLC; Rf 0.43 (CH_2Cl_2 - MeOH - conc NH_4OH, 1 : 3 : 2)); IR ν_{max} (KBr) cm^{-1} 3340, 1680, 1570, 1480; ^1H NMR (80 MHz, D_2O) δ *ca.* 1.5~2.5 (4H, m, 3‴-H, 2-H), *ca.* 5.1 (1H, m, 1″-H), 5.30 and 5.50 (1H, m, 1′-H).

Anal Calcd for $C_{22}H_{41}N_5O_{12}F_2 \cdot H_2CO_3 \cdot H_2O$: C 40.29, H 6.62, N 10.21.
Found: C 40.39, H 6.49, N 10.39.

Aminoglycosides having (*S*)-4-Amino-2-fluorobutyric Acid Side Chain (**18~21**)

By a procedure similar to that described in the preparation of **14**, 3,2′,6′-tri-*N*-benzyloxycarbonyl-3″-

Table 7. ^{13}C NMR (100 MHz, D_2O, pD≦2).

	δ (ppm)						Coupling with F (**14** and **18**~**21**)
	Amikacin[22]	**14**	**18**	**19**	**20**	**21**	
C-1	49.5	49.5	49.6	49.7	49.7	49.4	
C-2	30.9	30.8	31.1	30.1	31.0	31.2	
C-3	48.7	48.9	49.4	48.7	49.5	49.0	
C-4	79.8	79.5	78.2	78.3	77.8	79.7	
C-5	73.2	73.8	75.6	75.5	75.7	74.3	
C-6	81.2	81.4	81.0	81.1	81.1	81.0	
C-1′	96.2	96.7	96.2	94.4	95.4	97.3	
C-2′	71.7	71.9	54.4	49.4	49.4	71.7	
C-3′	73.1	73.0	70.0	30.9	21.4	72.9	
C-4′	71.7	71.7	71.9	65.7	26.3	71.9	
C-5′	69.5	69.5	69.2	71.1	68.8	69.5	
C-6′	41.2	41.3	41.2	40.9	43.5	41.3	
C-1″	98.7	99.1	99.0	99.0	99.0	99.3	
C-2″	68.8	68.8	68.7	68.7	66.8	66.8	
C-3″	56.2	56.2	56.0	56.0	56.0	65.2	
C-4″	66.4	66.4	66.5	66.5	66.4	70.8	
C-5″	72.7	72.8	72.9	72.9	72.9	67.9	
C-6″	60.7	60.6	60.6	60.6	60.6	—	
C-1‴	176.1	171.8	171.8	171.7	171.8	171.8	d, J=19~21 Hz
C-2‴	70.5	90.9	90.0	90.0	90.0	90.0	d, J=182~183 Hz
C-3‴	31.6	30.3	30.3	30.3	30.3	30.4	d, J=21~22 Hz
C-4‴	37.9	36.8	36.8	36.8	36.8	36.8	d, J=3~4 Hz
3″-N-Me						35.9	
4″-C-Me						21.8	

N-trifluoroacetylkanamycin B[16] (862 mg), 3,2′,6′-tri-*N*-benzyloxycarbonyl-3″-*N*-trifluoroacetyltobramycin[16] (966 mg), 3,2′,6′-tri-*N*-benzyloxycarbonyl-3″-*N*-trifluoroacetyldibekacin[10] (950 mg) and 3,6′-di-*N*-benzyloxycarbonylgentamicin B (113 mg) (prepared according to a conventional procedure[10]), were acylated with (*S*)-**5** (n=2) to afford the corresponding 1-*N*-[(*S*)-4-amino-2-fluorobutyryl] derivatives, **18**, **19**, **20** and **21**, respectively.

1-*N*-[(*S*)-4-Amino-2-fluorobutyryl]kanamycin B (**18**)

Yield 334 mg (46%); Estimated purity >90% (by HPLC); mp >210°C (gradually dec); $[\alpha]_D^{24}$ +76.4° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1640, 1540; ^1H NMR (400 MHz, D_2O) δ 1.92 (1H, q, J=12.8 Hz, 2-H_{ax}), 2.2~2.4 (3H, m, 2-H_{eq}, 3‴-H), 5.18 (1H, ddd, J=3.7, 8.8 and 48.4 Hz, 2‴-H), 5.18 (1H, d, J=3.7 Hz, 1″-H), 6.03 (1H, d, J=4.0 Hz, 1′-H); ^{13}C NMR see Table 7.

Anal Calcd for $C_{22}H_{43}N_6O_{11}F \cdot 2\frac{1}{2}H_2SO_4 \cdot 6H_2O$: C 28.11, H 6.43, N 8.94, S 8.53.
Found: C 28.28, H 6.59, N 9.11, S 8.56.
Karl Fischer: Calcd 11.50, Found 11.84.

1-*N*-[(*S*)-4-Amino-2-fluorobutyryl]tobramycin (**19**)

Yield 256 mg (36%); Estimated purity 90% (by HPLC); mp >190°C (gradually dec); $[\alpha]_D^{24}$ +75.3° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1650 (sh), 1610; ^1H NMR (400 MHz, D_2O) δ 1.94 (1H, q, J=12.8 Hz, 2-H_{ax}), 2.08 (1H, q, J=11.6 Hz, 3′-H_{ax}), 2.2~2.5 (4H, m, 2-H_{eq}, 3′-H_{eq} and 3‴-H), 5.18 (1H, ddd, J=3.7, 8.8 and 48.4 Hz, 2‴-H), 5.18 (1H, d, J=3.7 Hz, 1″-H), 5.85 (1H, d, J=3.7 Hz, 1′-H); ^{13}C NMR see Table 7.

Anal Calcd for $C_{22}H_{43}N_6O_{10}F \cdot 2\frac{1}{2}H_2SO_4 \cdot 6H_2O$: C 28.60, H 6.55, N 9.10, S 8.68.
Found: C 28.80, H 6.73, N 9.35, S 8.65.

1-*N*-[(*S*)-4-Amino-2-fluorobutyryl]dibekacin (**20**)

Yield 286 mg (38%); Estimated purity 90% (by HPLC); mp >200°C (gradually dec); $[\alpha]_D^{24}$ +73.2° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1650 (sh), 1610; ^1H NMR (400 MHz, D_2O) δ 5.17 (1H, ddd, J=3.7,

8.8 and 48.4 Hz, 2‴-H), 5.17 (1H, d, J=3.7 Hz, 1″-H), 5.85 (1H, d, J=3.3 Hz, 1′-H); ^{13}C NMR see Table 7.

Anal Calcd for $C_{22}H_{43}N_6O_9F \cdot 2\frac{1}{2}H_2SO_4 \cdot 5H_2O$: C 29.69, H 6.57, N 9.44, S 9.01
Found: C 29.74, H 6.56, N 9.62, S 8.99.

1-*N*-[(*S*)-4-Amino-2-fluorobutyryl]gentamicin B (**21**)

Yield 65 mg (56%); Estimated purity 85% (by HPLC); mp >190°C (gradually dec); $[\alpha]_D^{25}$ +100° (*c* 0.5, H_2O); IR ν_{max} (KBr) cm^{-1} 1650 (sh), 1610; ^{1}H NMR (400 MHz, D_2O) δ 1.35 (3H, s, 4″-CH_3), 1.87 (1H, q, J=12.5 Hz, 2-H_{ax}), 2.93 (3H, s, 3″-NCH_3), 5.17 (1H, d, J=4.0 Hz, 1″-H), 5.20 (1H, ddd, J=3.7, 8.8 and 48.4 Hz, 2‴-H), 5.65 (1H, d, J=4.0 Hz, 1′-H); ^{13}C NMR see Table 7.

Anal Calcd for $C_{23}H_{44}N_5O_{11}F \cdot 2H_2SO_4 \cdot 4\frac{1}{2}H_2O$: C 31.98, H 6.77, N 8.11, S 7.42.
Found: C 32.19, H 6.61, N 8.18, S 7.03.

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