Synthesis of 3′,4′,6′-Trideoxy-6′-fluorokanamycin C, 3′,4′-Dideoxy-6′-*C*-(fluoromethyl)kanamycin B, and Their 1-*N*-[(*S*)-4-Amino-2-hydroxybutanoyl] Derivatives

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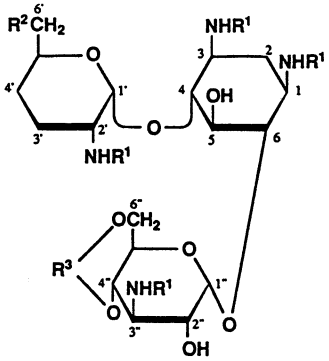
3′,4′,6′-Trideoxy-6′-fluorokanamycin C (**5**) has been prepared by treatment of a protected kanamycin C derivative having the free 6-hydroxyl group with DAST, as the key step. Two 3′,4′-dideoxy-6′-*C*-(fluoromethyl)kanamycin B's (**17a** and **17b**) have been prepared from a 5′-*C*-aldehyde derivative of kanamycin C through a sequence of reactions involving nitromethane condensation and 6′,7′-(*N*-tosylepimino)-ring opening with KHF_2, as the key steps. 1-*N*-[(*S*)-4-Amino-2-hydroxybutanoyl] derivatives of **5** and **17a** were prepared by coupling of the [(*S*)-4-amino-2-hydroxybutanoyl] residue to the 1-amino group of **5** and **17a** by utilizing the zinc acetate-ethyl trifluoroacetate method. Biological effects caused by the introduction of the fluorine atom in these products were discussed based on the chemical character of fluorine.

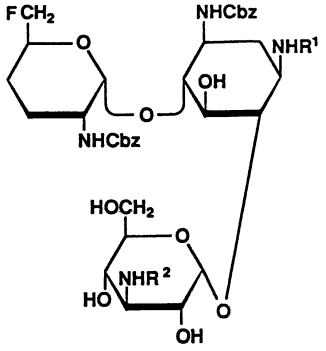
In the course of our synthetic studies on aminoglycoside antibiotics, several fluorine-containing derivatives had been prepared[1-4] by displacement of the hydroxyl groups. One aim of this study is to examine the biological role of the hydroxyl groups replaced by the fluorine in the parent compounds, but another aim is to lower the toxicity of the antibiotics. This latter strategy is based on the conjecture that the strong electron-withdrawing fluorine atom will lower the basicity of the amino groups sterically situated near-by, lowering, in consequence, the toxicity of the antibiotics, because, generally, the basicity and number of the amino groups of aminoglycoside antibiotics are considered to be the main cause of their toxicity.

In this paper we describe the synthesis of 3′,4′,6′-trideoxy-6′-fluorokanamycin C (**5**), its 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative (**9**), two 3′,4′-dideoxy-6′-*C*-(fluoromethyl)kanamycin B's (**17a** and **17b**), and the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative of **17a**, with a brief discussion on the structure-activity relationship. The reason for choice of 3′,4′-dideoxykanamycin C and not kanamycin C as the starting material is to obtain compounds active against resistant bacteria producing 3′- and 4′-modifying enzymes.

Treatment of 3′,4′-dideoxykanamycin C[5] (**1**) with benzyl chloroformate gave the *N*-protected derivative (**2**). Partial protection of the hydroxyl groups of **2** with 1,1-dimethoxycyclohexane under an acid catalyst gave the 4″,6″-*O*-cyclohexylidene derivative (**3**) having free 5-, 6′-, and 2″-hydroxyl groups. Fluorination of **3** with (diethylamino)sulfur trifluoride (DAST) in dichloromethane in a short period gave the 6′-deoxy-6′-fluoro derivative (**4**) fairly selectively in 71% yield with the 5- and 2″-hydroxyl groups remained intact. Presence of the fluorine atom at C-6′ was proved by the splitting pattern of the ^{19}F NMR spectrum, which showed a set of double triplets with large $J_{6'a,F}$ and $J_{6'b,F}$ values, indicating that the fluorine atom was attached by replacing the primary hydroxyl group. Deprotection of **4** gave 3′,4′,6′-trideoxy-6′-fluorokanamycin C (**5**).

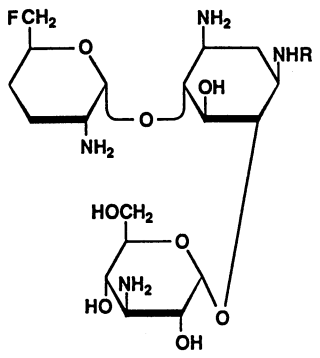


	R^1	R^2	R^3
1	H	OH	H,H
2	Cbz	OH	H,H
3	Cbz	OH	$C(CH_2)_5$
4	Cbz	F	$C(CH_2)_5$



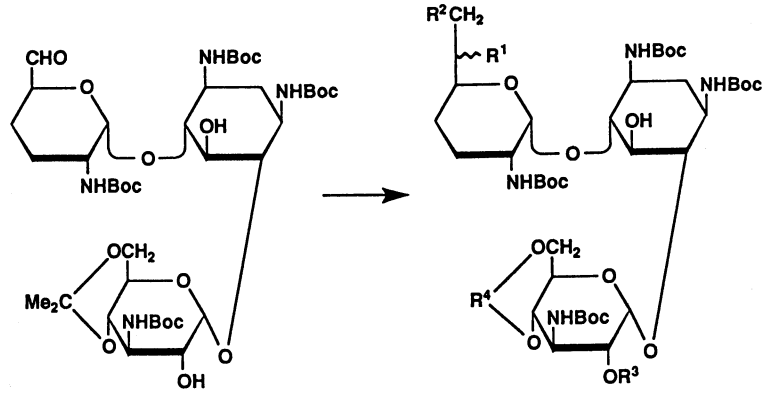
	R^1	R^2
6	H	H
7	H	CF_3CO
8	$COCH(OH)CH_2CH_2NHCbz$ (*S*)	CF_3CO

Cbz: $CO_2CH_2C_6H_5$



5 R = H
9 R = COCH(OH)$CH_2CH_2NH_2$ (*S*)

As 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative of kanamycin A (amikacin)[6] has enhanced antibacterial activity in comparison with that of kanamycin A, the same derivation procedure was applied to **5**. Partial 3,2′-amino protection of **5** was performed according to our established method[7] of zinc acetate-*N*-(benzyloxycarbonyloxy)succinimide in dimethyl sulfoxide, and the 3,2′-bis(*N*-benzyloxycarbonyl) derivative (**6**) was obtained in 77% yield. Definitive proof of the positions of the amino groups thus protected (at 3 and 2′) is rather difficult, but judging from the many successful examples[8-11] with no exception, it is clear that zinc chelations were formed in both the 1- and 3′-amino groups[7] and some hydroxyl groups situated near-by, and protects the amino groups from the attack by succinimide reagent and let the remaining 3- and 2′-amino groups react with the reagent. Further protection[7] of the 3″-amino group of **6** with ethyl trifluoroacetate in *N*,*N*-dimethylformamide (DMF) gave the 3″-*N*-(trifluoroacetyl)-1-amino-free derivative (**7**). This selective trifluoroacetamidation is characteristic for the fragment having an amino and a



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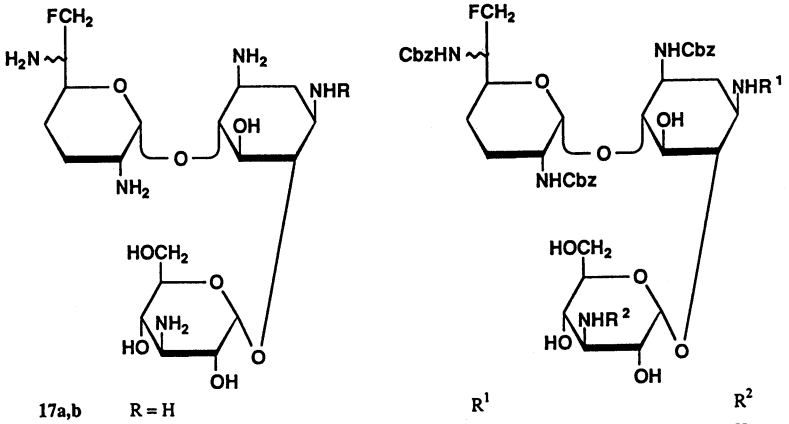
	R^1	R^2	R^3	R^4
11a,b	OH	NO_2	H	H,H
12a,b	OH	NH_2	H	H,H
13a,b	OH	NHTs	H	H,H
14a,b	OH	NHTs	H	$C(CH_2)_5$
15a,b	OBes	NHTs	Bes	$C(CH_2)_5$
16a,b	NHTs	F	Bes	$C(CH_2)_5$



17a,b R = H
21 R = COCH(OH)$CH_2CH_2NH_2$ (*S*)

	R^1	R^2
18	H	H
19	H	CF_3CO
20	COCH(OH)CH_2CH_2NHCbz (*S*)	CF_3CO

Bes : $SO_2CH_2C_6H_5$, Boc : CO_2CMe_3, Cbz : $CO_2CH_2C_6H_5$

hydroxyl groups vicinally situated. Coupling of the (*S*)-4-(benzyloxycarbonylamino)-2-hydroxybutanoyl residue to **7** by using the active ester gave the corresponding 1-urethane (**8**), and deprotection followed gave the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative (**9**) of **5**.

6′-*C*-Fluoromethyl derivative of kanamycin B is next described. 1,3,2′,3″-Terakis(*N*-*t*-butoxycarbonyl)-3′,4′-6′-trideoxy-4″,6″-*O*-isopropylidene-6′-oxokanamycin C[12] (**10**) was condensed with nitromethane, whereupon two nitro alcohols **11a** and **11b** were produced in 92% total yield, the faster-moving product **11a** preponderating. The products were separated by column chromatography, and the structures were confirmed by the ^{1}H NMR spectra, in which the C-7′ methylenes were clearly discerned. Study on absolute configuration at C-6′, however, was not pursued. Catalytic reduction of the nitro groups of **11a** and **11b** gave the ninhydrin-positive derivatives **12a** and **12b**, respectively, and subsequent tosylation gave the 7′-*N*-tosyl derivatives (**13a** and **13b**). After usual cyclohexylidenation (to give **14a** and **14b**), the products were benzylsulfonylated with phenylmethanesulfonyl chloride to give the 6′,2″-bis(*O*-benzylsulfonyl) derivatives (**15a** and **15b**), respectively. Fluorinations of **15a** and **15b** were performed by using potassium hydrogenfluoride (KHF_2) in DMF at 120 °C, when the 7′-fluoro derivatives (**16a** and **16b**) were produced in moderate yields, respectively, with the 2″-benzylsulfonyloxy groups remained intact. These reactions were considered to proceed by first formation of the 6′,7′-(*N*-tosylepimino) ring by removal of the 6′-benzylsulfonyloxy group under the catalysis of the fluoride ion, operating as a base, followed by fluorinative-ring-opening of the epimino ring by the fluoride ion. The presence of the electron-withdrawing *N*-tosyl group will facilitate[13] this reaction. The fluorine atom could also be introduced at C-6′, but the degree may be smaller than that at C-7′, and the 6′-fluorine compound, if formed, will be minor. However the following reactions have also been observed[13] that methyl 2,3-anhydro-2,3-(*N*-tosylepimino)-α-D-allopyranoside was converted, under the similar conditions, into 2-deoxy-2-fluoro-3-tosylamino-α-D-altropyranoside by epimino-ring opening and the latter further converted into the more-stable 3-deoxy-3-fluoro-2-tosylamino-α-D-glucopyranoside via the *N*-tosylepimino ring. Judging from this observation, initially formed 6′-fluoro-7′-(tosylamino) compound could also be converted into the 7′-fluoro-6′-(tosylamino) compound (**16a** and **16b**). Thus real reaction pathway remained unresolved. Detosylation of **16a** and **16b** with sodium in liquid ammonia followed by de(*t*-butoxycarbonyl) and de(cyclohexyliden)ations with trifluoroacetic acid gave 3′,4′-dideoxy-6′-*C*-(fluoromethyl)kanamycin B's, **17a** and **17b**, respectively. The presence of the terminal fluorine at C-7′ in the respective compound was confirmed by the ^{1}H and ^{19}F NMR spectra.

Finally, 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivative (**21**) of **17a** was prepared in a similar way as described for the preparation of **9** from **5**. After selective 3,2′,6′-tris(*N*-benzyloxycarbonyl)ation utilizing the zinc chelation method (to give **18**), 3″-amino group was selectively trifluoroacetylated and the product **19** was condensed with the active ester as described for **8** to give **20**. Deblocking gave the final product, **21**.

Antibacterial activities of the synthetic products **5**, **9**, **17a**, **17b**, and **21** were compared with those of 3′,4′-dideoxykanamycin B[14] (DKB) and its 1-*N*-[(*S*)-4-

Table 1. Minimal Inhibitory Concentration (μg ml^{-1}) of **5**, **9**, **17a**, **17b**, and **21** with DKB and HBK[a]

	5	**9**	**17a**	**17b**	**21**	**DKB**	**HBK**
S. a. FDA 209P	100	6.25	1.56	3.12	0.78	0.39	<0.2
S. a. Smith	25	3.12	1.56	3.12	0.2	0.39	<0.2
S. a. Ap01 (A)	>100	25	12.5	12.5	6.25	1.56	0.39
B. s. PCI 219	25	6.25	0.78	1.56	0.39	<0.2	<0.2
E. c. K-12	50	12.5	3.12	3.12	0.78	0.39	0.39
E. c. K-12 ML 1629 (B)	>100	50	6.25	3.12	3.12	0.78	1.56
E. c. W677	50	25	3.12	3.12	0.78	0.39	<0.2
E. c. JR66/W677 (C, D)	>100	25	100	>100	3.12	25	0.78
K. p. PCI 602	100	12.5	6.25	6.25	1.56	1.56	0.78
P. r. GN 311	50	12.5	3.12	3.12	0.78	0.78	0.78
S. m.	>100	12.5	25	25	12.5	25	12.5
Pr. sp. Pv 16 (E)	>100	>100	25	100	1.56	25	1.56
P. a. A3	100	12.5	1.56	3.12	0.78	<0.2	<0.2
P. a. TI 13 (B)	>100	100	12.5	25	6.25	1.56	0.78
P. a. GN 315 (F)	100	>100	25	100	6.25	100	3.12

a) Abbreviations of bacteria: S. a. *Staphylococcus aureus*, B. s. *Bacillus subtilis*, E. c. *Escherichia coli*, K. p. *Klebsiella pneumoniae*, P. r. *Proteus rettgeri*, S. m. *Serratia marcescens*, Pr. *Providencia*, P. a. *Pseudomonas aeruginosa*. The letters in parenthesis show the resistant bacteria producing 4′-adenylyltransferase (A), 3′-phosphotransferase-I (B), 3′-phosphotransferase-II (C), 2″-adenylyltransferase (D), 2′-acetyltransferase (E), and 6′-acetyltransferase (F).

amino-2-hydroxybutanoyl] derivative[15] (HBK) (see Table 1). Both 6′-deoxy-6′-fluoro compounds **5** and **9** exhibited only weak activities. This result suggests that the 6′-hydroxyl group of kanamycin C (and possibly the protonated 6′-amino group of kanamycin A and B also) is essential for the antibacterial activity, and may operate as a hydrogen donor in binding to the active site of bacteria. In the case of 2′-hydroxyl (in kanamycin A) or 2′-amino groups (in kanamycin B), replacement of the group with fluorine (to give 2′-deoxy-2′-fluorokanamycin A[3]) did not weaken the intrinsic activity, suggesting that the 2′-groups may play a minor role as a hydrogen donor or play as an acceptor. Synthetic 6′-*C*-(fluoromethyl) compounds **17a**, **17b**, and **21** exhibited moderate activities, but they were much weaker than those for the parent compounds (DKB for **17a** and **17b**, and HBK for **21**); this antibacterial character could be assigned to the presence of the 6′-*C*-(fluoromethyl) group. However, judging from the fact that, gentamicin C_{1a} and its 6′-*C*-methyl analog[16,17] (gentamicin C_2), both of which resemble to kanamycin, structurally, have almost equal antibacterial activity, the decreased activity of **17a**, **17b**, and **21** would not immediately be ascribed to the bulk of the fluoromethyl group, because CH_3 (in gentamicin C_2) and CH_2F have a similar size. The decreased activity may be ascribed to the strong electron-withdrawing character of fluorine, which lowers the basicity of the 6′-amino group, and, as the result, would weaken the action of the amino group (in the form of 6-NH_3^+) as a hydrogen donor. Toxicity studies on **17a**, **17b**, and **21** will be described elsewhere.

Experimental

General. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. ^1H and ^{19}F NMR spectra were recorded with a Bruker WM 250 spectrometer. Chemical shifts (δ) of ^1H and ^{19}F were measured, respectively, downfield from internal Me_4Si or Freon 11 ($CFCl_3$). Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck), and column chromatography, on Wakogel C-200, unless otherwise stated.

1,3,2′,3″-Tetrakis(*N*-benzyloxycarbonyl)-3′,4′-dideoxykanamycin C (2). To an ice-cold suspension of **1** (312 mg) and anhydrous sodium carbonate (300 mg) in 1,4-dioxane-water (1 : 1, 10 ml) was added benzyl chloroformate (0.5 ml) and the mixture was stirred for 2 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=10 : 1 : 0.1) of the solution showed a single spot at R_f 0.5. Concentration followed by addition of water gave precipitates, which were filtered, and washed thoroughly with water and ether to give, after drying, a solid of **2**, 660 mg (97%), $[\alpha]_D^{20}$ +69° (*c* 1, pyridine); ^1H NMR (pyridine-d_5) δ=5.1—5.3 (8H, $CO_2CH_2Ph\times4$), 5.37 (1H, d, *J*=4 Hz, H-1′ or H-1″), 5.47 (1H, d, *J*=3.5 Hz, H-1″ or H-1′).

Found: C, 60.55; H, 5.89; N, 5.60%. Calcd for $C_{50}H_{60}N_4O_{17}$: C, 60.72; H, 6.11; N, 5.66%.

1,3,2′,3″-Tetreakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidene-3′,4′-dideoxykanamycin C (3). A solution of **2** (252 mg), 1,1-dimethoxycyclohexane (0.2 ml) and *p*-toluenesulfonic acid monohydrate (15 mg) in DMF (3.5 ml) was kept for 16 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=10 : 1 : 0.1) of the solution showed a single spot at R_f 0.65. Triethylamine (1 ml) was added and the solution was concentrated. The residue was shaken with aqueous sodium hydrogencarbonate (5%, 10 ml) and the precipitates were filtered and washed thoroughly with water to give, after drying, a solid of **3**, 258 mg (95%), $[\alpha]_D^{20}$ +56° (*c* 1, pyridine); ^1H NMR (pyridine-d_5) δ=1.3—2.0 (16H, H-2a, 2b, 3′a, 3′b, 4′a, 4′b and CH_2 of cyclohexylidene), 5.1—5.4 (8H, $CO_2CH_2Ph\times4$), 5.43 (1H, d, *J*=4 Hz, H-1′ or -1″), 5.48 (1H, d, *J*=4 Hz, H-1″ or -1′).

Found: C, 62.77; H, 6.60; N, 5.11%. Calcd for $C_{56}H_{68}N_4O_{17}$: C, 62.91; H, 6.41; N, 5.24%.

1,3,2′,3″-Tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidene-3′,4′,6′-trideoxy-6′-fluorokanamycin C (4). To a cold (−60—−50 °C) solution of **3** (543 mg, 0.51 mmol) in dry dichloromethane (15 ml) was added DAST (0.26 ml, 2.07 mmol) and the solution was gradually warmed to room temperature (1.5 h in total). TLC (chloroform-methanol=25 : 1) of the solution showed two spots of R_f 0.65 (a reaction intermediate?) and 0.3 (**4**) (cf. **3**: R_f 0.2). The solution was washed with 5% aqueous potassium hydrogensulfite, 5% aqueous sodium hydrogencarbonate, and water. The solution without containing the product of R_f 0.65 was dried ($MgSO_4$) and concentrated. The residue was chromatograped on a short column with chloroform-methanol (50 : 1) to give a solid of **4**, 385 mg (71%), $[\alpha]_D^{20}$ +49° (*c* 1, pyridine); ^1H NMR (pyridine-d_5) δ=5.53 (1H, d, *J*=4 Hz, H-1′ or -1″), 5.47 (1H, d, *J*=4 Hz, H-1″ or -1′); ^{19}F NMR (pyridin-d_5) δ=−231.0 (dt, $J_{5',F}$=25, $J_{6'a,F}$=$J_{6'b,F}$=50 Hz).

Found: C, 62.48; H, 6.22; N, 5.22%. Calcd for $C_{56}H_{67}FN_4O_{16}$: C, 62.79; H, 6.31; N, 5.23%.

3′,4′,6′-Trideoxy-6′-fluorokanamycin C (5). A solution of **4** (346 mg) in 80% aqueous acetic acid (10 ml) was kept for 1 h at 90 °C (decyclohexylidenation). Concentration gave a residue, that was dissolved in a mixture of 1,4-dioxane-water-acetic acid (10 : 1 : 0.5, 2 ml). The solution was hydrogenated in the presence of palladium black for 1 h under atmospheric pressure of hydrogen. The product obtained was chromatographed on a column of CM-Sephadex C-25 (NH_4^+ form, 20 ml) with aqueous ammonia (0→0.15 M, gradually changed) to give a solid of **5**, 124 mg (78%), $[\alpha]_D^{20}$ +122° (*c* 1.2, water); ^1H NMR (20% ND_3 in D_2O) δ=1.25 (1H, q, $J_{1,2ax}$=$J_{2ax,2eq}$=$J_{2ax,3}$=9 Hz, H-2ax), 1.5—1.9 (4H, H-3′a, 3′b, 4′a, 4′b), 1.98 (1H, dt, $J_{1,2eq}$=$J_{2eq,3}$=4 Hz, H-2eq), 4.43 (1H, ddd, $J_{5',6'a}$=6, $J_{6'a,6'b}$=10, $J_{6'a,F}$=48 Hz, H-6′), 4.56 (1H, ddd, $J_{5'6'b}$=3, $J_{6'b,F}$=48 Hz, H-6′b) (H-6′a and 6′b were confirmed by decoupling of ^{19}F), 5.04 (1H, d, *J*=3.5 Hz, H-1′ or -1″), 5.11 (1H, d, *J*=3.5 Hz, H-1″ or -1′); ^{19}F NMR (20% ND_3 in D_2O) δ=−228.1 (dt, $J_{5',F}$=22, $J_{6'a,F}$=$J_{6'b,F}$=48 Hz).

Found: C, 45.21; H, 7.47; H, 11.08; F, 3.91%. Calcd for $C_{18}H_{35}FN_4O_8\cdot1/2H_2O\cdot1/2H_2CO_3$: C, 44.93; H, 7.54; N, 11.33; F, 3.84%.

3,2′-Bis(*N*-benzyloxycarbonyl)-3′,4′,6′-trideoxy-6′-fluorokanamycin C (6). A mixture of **5** (30.0 mg, 0.061 mmol) and zinc acetate dihydrate (62.1 mg, 0.28 mmol) in dry dimethyl sulfoxide (0.6 ml) was stirred for 1 h at room temperature. To the resulting clear solution was added *N*-

(benzyloxycarbonyloxy)succinimide (33.0 mg, 0.13 mmol) and the solution was kept for 2 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=9:4:1) of the solution showed a single spot at R_f 0.35 (cf. **5**: R_f 0.1). The solution was poured into a column of CM-Sephadex C-25 (NH_4^+ form, 2 ml), and, after washing the column with 50% aqueous 1,4-dioxane (5 ml), the product was developed with the same solvent mixture but containing ammonia (content of NH_3 in the solvent being 0→0.2 M, gradually changed), and the ninhydrin-positive fractions were collected to give a solid of **6**, 36.8 mg (77%), $[\alpha]_D^{20}$ +88° (*c* 1, dimethyl sulfoxide); ^{1}H NMR (pyridine-d_5) δ=5.15—5.35 (5H, CO_2CH_2Ph×2 and H-1′ or -1″), 5.44 (1H, d, H-1″ or -1′, *J*=3 Hz), 7.0—7.5 (10H, CO_2CH_2Ph×2).

Found: C, 53.78; H, 6.23; N, 6.94%. Calcd for $C_{34}H_{47}FN_4O_{12}\cdot H_2CO_3$: C, 53.57; H, 6.29; N, 7.14%.

3,2′-Bis(*N*-benzyloxycarbonyl)-3′,4′,6′-trideoxy-6′-fluoro-3″-*N*-(trifluoroacetyl)kanamycin C (7). To a solution of **6** (36.8 mg) in DMF (0.8 ml) was added ethyl trifluoroacetate (0.1 ml) and the solution was kept for 1 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=3:1:0.1) of the solution showed a single spot at R_f 0.5 (cf. **7**: R_f 0.15). Concentration of the solution gave a ninhydrin-positive solid of **7**, 38.2 mg (quant.), $[\alpha]_D^{20}$ +80° (*c* 1, dimethyl sulfoxide).

3,2′-Bis(*N*-benzyloxycarbonyl)-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyl]-3′,4′,6′-trideoxy-6′-fluoro-3″-*N*-(trifluoroacetyl)kanamycin C (8). To a mixture of **7** (38 mg) and anhydrous sodium carbonate (20 mg) in 20% aqueous 1,4-dioxane (1.2 ml) was added *N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyloxy]succinimide (27.2 mg), and the solution was kept for 2 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=5:1:0.1) of the solution showed a single spot at R_f 0.45 (cf. **7**: R_f 0.2). Concentration gave a syrupy residue, that was thoroughly washed with water and ether to give a ninhydrin-negative solid of **8**, 44.2 mg (90%), $[\alpha]_D^{20}$ +61° (*c* 1, DMF).

1-*N*-[(*S*)-4-Amino-2-hydroxybutanoyl]-3′,4′,6′-trideoxy-6′-fluorokanamycin C (9). A solution of **8** (44.2 mg) in 1,4-dioxane-28% aq NH_3 (2:1, 1.5 ml) was kept for 16 h at room temperature [de(trifluoroacetyl)ation]. TLC (chloroform-methanol-28% aq NH_3=5:1:0.1) of the solution showed a single spot at R_f 0.1. Concentration gave a residue, that was dissolved in 1,4-dioxan-water-acetic acid (6:3:1, 1 ml), and the solution was hydrogenated for 3 h in the presence of palladium black under atmospheric pressure of hydrogen. After filtration, the solution was concentrated to give a residue, that was chromatographed on a column of CM-Sephadex C-25 (NH_4^+ form, 4 ml) with aqueous ammonia (0→0.4 M ammonia, gradually changed) to give a solid of **9**, 15.8 mg (63%), $[\alpha]_D^{20}$ +80° (*c* 0.7, water); ^{1}H NMR (20% ND_3 in D_2O) δ=1.3—2.0 (8H, H-2a, 2b, 3′a, 3′b, 4′a, 4′b, 3‴a, 3‴b), 5.09 (1H, d, *J*=3 Hz, H-1′ or -1″), 5.17 (1H, d, *J*=3 Hz, H-1″ or -1′); ^{19}F NMR (20% ND_3 in D_2O) δ=−227.9 (dt, $J_{5',F}$=22, $J_{6'a,F}=J_{6'b,F}$=48 Hz).

Found: C, 45.50; N, 7.31; N, 11.61; F, 2.98%. Calcd for $C_{22}H_{42}FN_5O_{10}\cdot 1/2H_2O\cdot 1/2H_2CO_3$: C, 45.37; H, 7.45; N, 11.76; F, 3.19%.

1,3,2′,3″-Tetrakis(*N*-*t*-butoxycarbonyl)-3′,4′-dideoxy-6′-*C*-(nitromethyl)kanamycin C (11a, 11b). To a solution of **10** (320 mg) in methanol (6 ml) were added nitromethane (0.2 ml) and 28% sodium methoxide in methanol (0.5 ml), and the solution was kept for 1 h at room temperature TLC (chloroform-methanol-28% aq NH_3=10:1:0.1) of the solution showed two spots at R_f 0.6 and 0.55 (cf. **10**: R_f 0.5). The solution was made acidic to pH 2 with 4 M aqueous hydrochloric acid and kept for 30 min at room temperature to give a mixture of two products of **11a** (R_f 0.27) and **11b** (0.22). The solution was neutralized with 5% aqueous sodium hydrogencarbonate and, after concentration, the residue was poured into water. The resulting precipitates were chromatographed with chloroform-MeOH-28% aq NH_3 (50:1:0.1→10:1:0.1) to give a solid of **11a**, 185 mg (56%) and a solid of **11b**, 108 mg (36%).

11a: $[\alpha]_D^{20}$ +54° (*c* 1, pyridine); IR (KBr) 1370 and 1560 cm^{-1} (NO_2); ^{1}H NMR (pyridine-d_5) δ=1.43, 1.48, 1.50, and 1.58 (each 9H, s, Boc), 4.88 and 4.99 (each 1H, dd, $J_{6',7'a}$=9, $J_{6',7'b}$=3, $J_{7'a,7'b}$=12.5 Hz, H-7′a and -7′b), 5.33 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1″), 5.59 (1H, d, $J_{1',2'}$=3 Hz, H-1′).

Found: C, 51.21; H, 7.66; N, 7.49%. Calcd for $C_{39}H_{69}N_5O_{17}$: C, 51.36; H, 7.63; N, 7.68%.

11b: $[\alpha]_D^{20}$ +47° (*c* 1, pyridine); IR (KBr) 1370 and 1560 cm^{-1} (NO_2); ^{1}H NMR (pyridine-d_5) δ=1.43, 1.48, 150, and 1.53 (each 9H, s, Boc), 4.85 and 4.93 (each 1H, dd, $J_{6',7'a}$=9, $J_{6',7'b}$=3.5, $J_{7'a,7'b}$=12 Hz, H-7′a and -7′b), 5.33 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1″), 5.58 (1H, d, $J_{1',2'}$=3 Hz, H-1′).

Found: C, 51.23; N, 7.61; N, 7.55%. Calcd for $C_{39}H_{69}N_5O_{17}$: C, 51.36; H, 7.63; N, 7.68%.

6′-*C*-Aminomethyl-1,3,2′,3″-tetrakis(*N*-*t*-butoxycarbonyl)-3′,4′-dideoxykanamycin C (12a and 12b). A solution of **11a** (2.01 g) in methanol-water-acetic acid (25:5:1, 30 ml) was hydrogenated over PtO_2 for 5 d under 4 kg cm^{-2} hydrogen pressure at room temperature. TLC (chloroform-methanol-28% aq NH_3=5:1:0.1) of the reaction mixture showed a single spot at R_f 0.15 (cf. **11a**: R_f 0.75). The mixture was filtered and concentrated. The residue was chromatographed with chloroform-methanol-28% aq NH_3 (5:1:0.1) to give a solid of **12a**, 1.62 g (84%), $[\alpha]_D^{20}$ +73° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.43 (9H), 1.50 (18H), and 1.54 (9H) (each s, Boc), 5.33 (1H, d, $J_{1'',2''}$=3 Hz, H-1″), 5.68 (1H, d, $J_{1',2'}$=3 Hz, H-1′).

Found: C, 52.57; H, 8.15; N, 7.52%. Calcd for $C_{39}H_{71}N_5O_{17}\cdot 1/2H_2O$: C, 52.57; H, 8.14; N, 7.86%.

Compound **11b** (1.45 g) was treated similarly as described above to give a solid of **12b**, 1.12 g (80%), $[\alpha]_D^{20}$ +70° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.43 (9H), 1.49 (18H), and 1.52 (9H) (each s, Boc), 5.33 (1H, d, $J_{1'',2''}$=3 Hz, H-1″), 5.65 (1H, d, $J_{1',2'}$=3 Hz, H-1′).

Found: C, 53.07; H, 8.17; N, 7.91%. Calcd for $C_{39}H_{71}N_5O_{17}$: C, 53.10; H, 8.11; N, 7.94%.

1,3,2′,3″-Tetrakis(*N*-*t*-butoxycarbonyl)-3′,4′-dideoxy-6′-*C*-(tosylaminomethyl)kanamycin C (13a and 13b). To an ice cold mixture of **12a** (500 mg) and anhydrous sodium carbonate (150 mg) in 1,4-dioxane-water (2:1, 15 ml) was added *p*-toluenesulfonyl chloride (150 mg) and the solution was kept for 1 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=5:1:0.1) of the solution showed a single spot at R_f 0.85 (cf. **12a**: R_f 0.15). The solution, after concentration, was poured into water and the precipitates were filtered, washed with water and ether, to give a solid of **13a**, 552 mg (94%), $[\alpha]_D^{20}$ +49° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.43, 1.48, 1.49, and 1.52 (each 9H, s, Boc), 2.22 [3H, s, Ts(CH_3)], 5.31 (1H, d, $J_{1'',2''}$=3 Hz, H-1″), 5.52 (1H, d, $J_{1',2'}$=3 Hz, H-1′).

Found: C, 53.40; H, 7.65; N, 6.49%. Calcd for $C_{46}H_{77}N_5O_{19}S$: C, 53.31; H, 7.49; N, 6.76%.

Compound **12b** (460 mg) was treated similarly as described above to give a solid of **13a**, 502 mg (93%), $[\alpha]_D^{20}$ +48° (*c* 1, pyridine-d_5) δ=1.43, 1.48, 1.49, and 1.51 (each 9H, s, Boc), 2.23 [3H, s, Ts(CH_3)], 5.31 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1''), 5.54 (1H, d, $J_{1',2'}$=3 Hz, H-1').

Found: C, 53.50; H, 7.75; N, 6.90%. Calcd for $C_{46}H_{77}N_5O_{19}S$: C, 53.31; H, 7.49; N, 6.76%.

1,3,2',3''-Tetrakis(*N*-*t*-butoxycarbonyl)-4'',6''-*O*-cyclohexylidene-3',4'-dideoxy-6'-*C*-(tosylaminomethyl)kanamycin C (14a and 14b). A solution of **13a** (1.51 g), 1,1-dimethoxycyclohexane (1.1 ml), and *p*-toluenesulfonic acid monohydrate (80 mg) in DMF (20 ml) was kept for 13 h at room temperature. TLC (chloroform-methanol-28% aq NH_3=10:1:0.1) of the solution showed a single spot at R_f 0.5 (cf. **13a**: R_f 0.2). Triethylamine (1 ml) was added, and the solution was concentrated. The residue was poured into 5% aqueous sodium hydrogencarbonate and the precipitates were filtered, washed with water, and dried to give a solid of **14a**, 1.62 g (quant.), $[\alpha]_D^{20}$ +45° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.46, 1.48, 1.50, and 1.52 (each 9H, s, Boc), 2.23 [3H, s, Ts(CH_3)], 5.38 (1H, d, $J_{1'',2''}$=3 Hz, H-1''), 5.66 (1H, d, $J_{1',2'}$=3 Hz, H-1').

Found: C, 55.55; H, 7.47; N, 6.33%. Calcd for $C_{52}H_{85}N_5O_{19}S\cdot 1/2H_2O$: C, 55.49; H, 7.70; N, 6.22%.

Compound **13b** (1.22 g) was treated similarly as described above to give a solid of **14b**, 1.30 g (99%), $[\alpha]_D^{20}$ +40° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.46 (9H), 1.48 (18H), and 1.50 (9H) (each s, Boc), 2.23 [(3H, s, Ts(CH_3)], 5.39 (1H, d, $J_{1'',2''}$=3 Hz, H-1''), 5.57 (1H, d, $J_{1',2'}$=3 Hz, H-1').

Found: C, 55.64; H, 7.49; N, 6.39%. Calcd for $C_{52}H_{85}N_5O_{19}S$: C, 55.94; H, 7.67; N, 6.27%.

6',2''-Bis(*O*-benzylsulfonyl)-1,3,2',3''-tetrakis(*N*-*t*-butoxycarbonyl)-4'',6''-*O*-cyclohexylidene-3',4'-dideoxy-6'-*C*-(tosylaminomethyl)kanamycin C (15a and 15b). A solution of **14a** (210 mg) and phenylmethanesulfonyl chloride (100 mg) in pyridine (3 ml) was kept for 1 h at 0—5 °C. TLC [toluene-methyl ethyl ketone (MEK)=3:1] of the solution showed a single spot at R_f 0.45 (cf. **14a**: R_f 0.05). Water (0.1 ml) was added and the solution was concentrated. The residue was chromatographed with toluene-MEK (10:1) to give a solid of **15a**, 224 mg (84%), $[\alpha]_D^{20}$ +41° (*c* 1.3, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.2—1.8 (10H, cyclohexylidene), 1.49 (9H), 1.50 (18H), and 1.52 (9H) (each a, Boc), 2.23 [3H,s, Ts(CH_3)], 4.75 and 4.87 [each 2H, s, PhCH_2SO_2], 5.32 (1H, dd, $J_{1'',2''}$=3.5 and $J_{2'',3''}$=10.5 Hz, H-2''), 5.68 (1H, d, $J_{1',2'}$=3 Hz, H-1'), 5.76 (1H, d, H-1'').

Found: C, 55.91; H, 6.82; N, 4.85%. Calcd for $C_{66}H_{97}N_5O_{23}S_3$: C, 55.64; H, 6.86; N, 4.92%.

Compound **14b** (340 mg) was treated similarly as described above to give a solid of **15b**, 375 mg (87%), $[\alpha]_D^{20}$ +42° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.2—1.8 (10H, cyclohexylidene), 1.49 (9H), 1.50 (18H), and 1.51 (9H) (each s, Boc), 2.23 [3H, s, Ts(CH_3)], 4.75 and 4.80 (each 2H, s, PhCH_2SO_2], 5.35 (1H, d, $J_{1',2'}$=3.5, $J_{2'',3''}$=10.5 Hz, H-2''), 5.75 (1H, d, *J*=3.5 Hz, H-1''), 5.78 (1H, d, $J_{1',2'}$= 3 Hz, H-1').

Found: C, 55.88; H, 6.79; N, 4.77%. Calcd for $C_{66}H_{97}N_5O_{23}S_3$: C, 55.64; H, 6.86; N, 4.92%.

2''-*O*-Benzylsulfonyl-1,3,2',3''-tetrakis(*N*-*t*-butoxycarbonyl)-4'',6''-*O*-cyclohexylidene-3',4'-dideoxy-6'-*C*-(fluoromethyl)-6'-*N*-tosylkanamycin B (16a and 16b). A mixture of **15a** (750 mg) and KHF_2 (300 mg, dried in vacuo for 8 h at 100 °C) in DMF (11 ml, dried over molecular sieves 4A, and then distilled in vacuo) was heated in a sealed tube for 4 h at 120 °C. TLC (toluene-MEK=3:1) of the solution showed a main spot (**16a**) at R_f 0.55 together with several minor spots. Chloroform (60 ml) was added and the organic solution was washed with 5% aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. The residue was chromatographed with toluene-MEK (10:1) to give a solid of **16a**, 360 mg (53%), $[\alpha]_D^{20}$ +30° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=1.2—1.8 (46H, Boc×4 and cyclohexylidene), 2.23 [3H, s, Ts(CH_3)], 4.5—4.8 (2H, m, H-7'a, -7'b), 4.86 (2H,s, PHCH_2SO_2), 5.36 (1H, dd, $J_{1'',2''}$=3.5 and $J_{2'',3''}$=10 Hz, H-2''), 5.53 (1H, d, $J_{1',2'}$=3 Hz, H-1'), 5.78 (1H, d, H-1''); ^{19}F NMR (pyridin-d_5) δ=−224.4 (dt, $J_{6',F}$=15, $J_{7'a,F}$=$J_{7'b,F}$=45 Hz).

Found: C, 55.95; H, 7.18; N, 5.47%. Calcd for $C_{59}H_{91}FN_5O_{20}S_2$: C, 55.64; H, 7.20; N, 5.50%.

Compound **15b** (260 mg) was treated similarly as described above to give a solid of **16b**, 72.1 mg (31%) and **15b** recovered 52.8 mg (20%).

16b: $[\alpha]_D^{20}$ +44° (*c* 1, pylidine); ^{1}H NMR (pyridine-d_5) δ=1.2—2.0 (46H, Boc×4 and cyclohexylidene), 2.23 [3H, s, Ts(CH_3)], 4.85 (2H, s, PhCH_2SO_2), 5.36 (1H, dd, $J_{1'',2''}$=3.5 and $J_{2'',3''}$=10 Hz, H-2''), 5.53 (1H, br s, H-1'), 5.78 (1H, d, H-1''); ^{19}F NMR (pyridine-d_5) δ=−226.8 (dt, $J_{6',F}$=22, $J_{7'a,F}$=$J_{7'b,F}$=48 Hz).

Found: C, 55.88; H, 7.05; N, 5.38%. Calcd for $C_{59}H_{91}FN_5O_{20}S_2$: C, 55.64; H, 7.203, N, 5.50%.

3',4'-Dideoxy-6'-*C*-(fluoromethyl)kanamycin B (17a and 17b). To a solution of **16a** (250 mg) in liquid ammonia (30 ml) at −50—−60 °C was added sodium (ca. 300 mg), and the deep-blue solution was kept for 5 min at the temperature [detosyl- and de(benzylsulfonyl)ations]. Addition of methanol until the solution became colorless, followed by gradual warming to room temperature, and finally evaporation under diminished pressure gave a residue. A solution of the residue in methanol (500 ml) was neutralized with 4 M aqueous hydrochloric acid (M=mol dm^{-3}) and concentrated. The residue was dissolved in trifluoroacetic acid (1 ml) and the solution was kept for 30 min at room temperature. TLC (1-butanol-ethanol-chloroform-17% aq NH_3=4:5:2:5) of the solution showed a single spot at R_f 0.3. Concentration in vacuo gave a residue, that was chromatographed on a column of CM-Sephadex C-25 (NH_4^+ form, 30 ml) with aqueous ammonia (0→0.1 M) to give a ninhydrin-positive solid of **17a**, 82.3 mg (80%), $[\alpha]_D^{20}$ +82° (*c* 1, water); ^{1}H NMR (20% ND_3 in D_2O) δ=3.54 (1H, dd, $J_{1'',2''}$=3.5, $J_{2'',3''}$=10 Hz, H-2''), 4.53 (2H, a set of same-shaped m separated by 48 Hz, H-7a' and -7b'), 5.09 (1H, d, H-1''), 5.23 (1H, d, $J_{1',2'}$=3 Hz, H-1'); ^{19}F NMR (20% ND_3 in D_2O) δ=−228.2 (dt, $J_{6',F}$=19, $J_{7'a,F}$=$J_{7'b,F}$=48 Hz).

Found: C, 45.76; H, 7.55; N, 13.63; F, 3.61%. Calcd for $C_{19}H_{38}FN_5O_8\cdot 1/2H_2CO_3$: C, 45.51; H, 7.64; N, 13.61; F, 3.69%.

Compound **16b** (68.8 mg) was treated similarly as described above to give a solid of **17b**, 18.5 mg (65%), $[\alpha]_D^{20}$ +104° (*c* 1, water); ^{1}H NMR (20% ND_3 in D_2O) δ=3.48 (1H, dd, $J_{1'',2''}$=3.5, $J_{2'',3''}$=10 Hz, H-2''), 4.53 (2H, a set of unresolved d separated by 48 Hz, H-7'a and -7'b), 5.02 (1H, d, H-1''), 5.13 (1H, d, $J_{1',2'}$=3 Hz, H-1'); ^{19}F NMR (20% ND_3 in D_2O) δ=−232.9 (dt, $J_{6',F}$=25, $J_{7'a,F}$=$J_{7'b,F}$=48 Hz).

Found: C, 45.33; H, 7.36; N, 13.58; F, 3.44%. Calcd for $C_{19}H_{38}FN_5O_8\cdot 1/2H_2CO_3$: C, 45.51; H, 7.64; N, 13.61; F, 3.69%.

3,2',6'-Tris(*N*-benzyloxycarbonyl)-3',4'-dideoxy-6'-*C*

(fluoromethyl)kanamycin B (18). A mixture of **17a** (60.0 mg, 0.10 mmol) and zinc acetate dihydrate (130 mg, 0.59 mmol) in dry dimethyl sulfoxide (1.2 ml) was stirred for 1 h at room temperature. To the resulting clear solution was added *N*-(benzyloxycarbonyloxy)succinimide (100 mg, 0.40 mmol) and the mixture was stirred for 1 h at room temperature. TLC (chloroform–MeOH–28% aq NH_3=9:4:1) of the solution showed a single spot at R_f 0.4 (cf. **17**: R_f 0.05). After dilution with 50% aqueous 1,4-dioxane (4 ml), the solution was poured into a column of CM-Sephadex C-25 (NH_4^+ form, 4 ml) and the column was washed with 50% aqueous 1,4-dioxane (10 ml). Development of the product with 1,4-dioxane–water–28% aqueous NH_3 (1:1:0→1:1:0.5, gradually changed) gave a solid of **18**, 65.5 mg (68%), $[\alpha]_D^{20}$ +40° (*c* 1, pyridine); ^{1}H NMR (pyridine-d_5) δ=5.1—5.4 (7H, PhCH_2CO$_2$×3 and H-1′), 5.66 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1″).

Found: C, 55.88; H, 6.47; N, 7.22%. Calcd for $C_{43}H_{56}FN_5O_{14}\cdot H_2CO_3$: C, 55.75; H, 6.17; N, 7.39%.

3,2′,6′-Tris(*N*-benzyloxycarbonyl)-3′,4′-dideoxy-6′-*C*-(fluoromethyl)-3″-*N*-(trifluoroacetyl)kanamycin B (19). To a solution of **18** (54.0 mg) in DMF (1 ml) was added ethyl trifluoroacetate (0.15 ml) and the solution was treated similarly as described for **7** to give a solid of **19**, 57.5 mg, $[\alpha]_D^{20}$ +58° (*c* 1, pyridine).

3,2′,6′-Tris(*N*-benzyloxycarbonyl)-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyl]-3′,4′-dideoxy-6′-*C*-(fluoromethyl)-3″-*N*-(trifluoroacetyl)kanamycin B (20). A mixture of **19** (57.5 mg) and anhydrous sodium carbonate (30 mg) in 20% aqueous 1,4-dioxane (1.8 ml) was treated with *N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyloxy]succinimide (40.0 mg) as described for **8** to give a solid of **20**, 65.8 mg (95% based on **18**), $[\alpha]_D^{20}$ +55° (*c* 1, pyridine).

1-*N*-[(*S*)-4-Amino-2-hydroxybutanoyl]-3′,4′-dideoxy-6′-*C*-(fluoromethyl)kanamycin B (21). Compound **20** (65.8 mg) was treated similarly as described for **9** to give a solid of **21**, 25.3 mg (75%), $[\alpha]_D^{20}$ +64° (*c* 1, water); ^{1}H NMR (20% ND_3 in D_2O) δ=1.9—2.7 (8H, H-2a, 2b, 3′a, 3′b, 4′a, 4′b, 3‴a, 3‴b), 4.9—5.2 (2H, m, H-7′a and -7′b), 5.69 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1″), 5.78 (1H, d, $J_{1',2'}$=3 Hz, H-1′); ^{19}F NMR (20% ND_3 in D_2O) δ=−228.4 (dt, $J_{6',F}$=19, $J_{7'a,F}$=$J_{7'b,F}$=48 Hz).

Found: C, 44.98; H, 7.32; N, 13.51; F, 2.78%. Calcd for $C_{23}H_{45}FN_6O_{10}\cdot 1/2H_2O\cdot 1/2H_2CO_3$: C, 45.18; H, 7.58; N, 13.45; F, 3.04%.

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