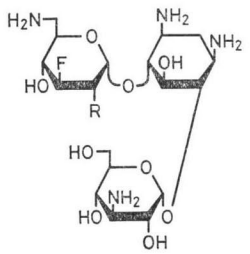
SYNTHESIS OF 3′-DEOXY-3′-FLUOROKANAMYCINS A AND B ACTIVE AGAINST RESISTANT BACTERIA*

Sir:

Kanamycins are inactivated by enzymes of resistant bacteria phosphorylating or adenylylating the 3′, 4′ or 2″-hydroxyl groups[1,2], among which the 3′-hydroxyl group is most frequently modified. Syntheses of 3′-deoxykanamycin A[3], 3′,4′-dideoxykanamycin B[4] (dibekacine), and other deoxy derivatives of kanamycins and related aminoglycoside antibiotics were the major solutions[1,2] in order to overcome the inactivation by resistant bacteria. In this communication we describe the synthesis of useful derivatives by replacing the 3′-hydroxyl group with a fluorine atom to avoid the above modifications by resistant bacteria**. For the first approach, we prepared 3′-deoxy-3′-fluorokanamycins A (**1**) and B (**2**).

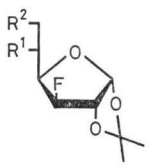
3′-Deoxy-3′-fluorokanamycin A was prepared by condensation of 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-3-fluoro-α-D-glucopyranosyl bromide (**10**) and a protected derivative (**11**) of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD). 3-Deoxy-3-fluoro-1,2;5,6-di-*O*-isopropylidene-D-glucofuranose[14] (**3**) was selec-

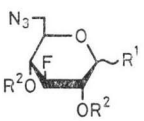
Fig. 1.



1 R=OH
2 R=NH_2

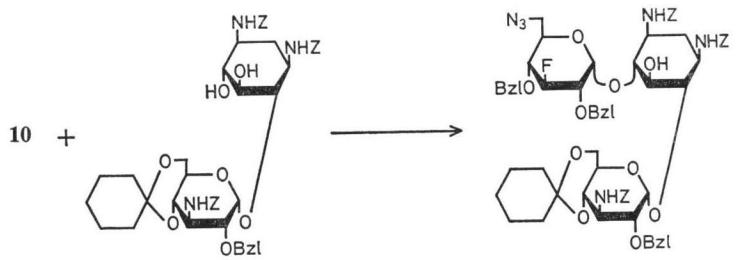
Fig. 2.





	R^1	R^2
3	Me_2CO(O)	
4	HO	HO
5	HO	TsO
6	HO	N_3

	R^1	R^2
7	OMe	H
8	OMe	Bzl
9	OAc	Bzl
10	Br(α)	Bzl



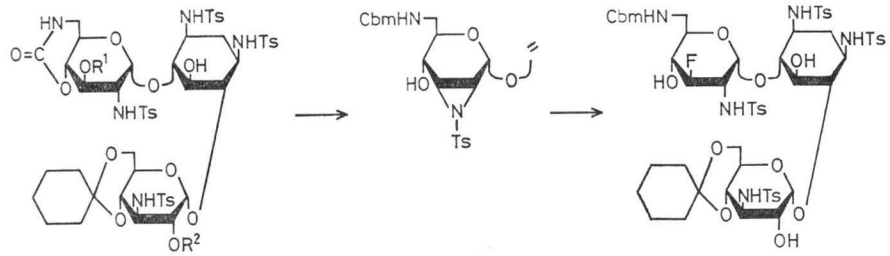
11 **12**

Bzl: $CH_2C_6H_5$, Z: $CO_2CH_2C_6H_5$.

* The contents of this article were read at the 1984 Intl. Chem. Congr. of Pacific Basin Soc., Honolulu, Hawaii, U.S.A., Dec. 17, 1984.

** Fluorination of aminoglycoside antibiotics at sites remote from bacterial enzymatic inactivation have been reported; 5-deoxy-5-epifluorosisomicin[5], 3-epifluoro-, 3-fluoro-, and 3,3-difluoro-3-demethoxysporaricins A[6,7], 5-deoxy-5-fluorosporaricin A[8], 5″-deoxy-5″-fluorolividomycin B[9], 4″-deoxy-4″-fluoro-[10] and 4″-deoxy-4″-epifluorokanamycins A[11], several analogs[12] of the latter, and 6″-deoxy-6″-fluorokanamycin A[13].

Fig. 3.



	R^1	R^2
13	H	H
14	H	Ac
15	$SO_2CH_2C_6H_5$	Ac

16 **17**

Table 1. Minimal inhibitory concentration (μg/ml) of 3′-deoxy-3′-fluorokanamycin A (**1**), 3′-deoxy-3′-fluorokanamycin B (**2**), and related compounds [kanamycin A (KMA), kanamycin B (KMB), 3′-deoxy-kanamycin A (3′DKMA), and dibekacin (DKB)].

	KMA	3′DKMA	**1**	KMB	DKB	**2**
Staphylococcus aureus FDA 209P	1.56	3.13	1.56	0.78	0.78	0.78
S. aureus Smith	1.56	1.56	0.78	<0.20	<0.20	<0.20
S. aureus Ap01[a]	25	50	50	6.25	3.13	25
S. epidermidis 109[a]	50	50	100	50	3.13	>100
Micrococcus flavus FDA 16	12.5	25	12.5	3.13	25	3.13
Bacillus subtilis PCI 219	0.39	0.78	0.39	0.39	0.39	0.39
Corynebacterium bovis 1810	12.5	25	50	1.56	12.5	3.13
Escherichia coli K-12	0.78	3.13	1.56	0.39	0.78	0.78
E. coli K-12 R5[b]	100	100	100	12.5	100	25
E. coli K-12 ML1629[c]	100	6.25	3.13	>100	1.56	1.56
E. coli K-12 ML1410	6.25	12.5	6.25	1.56	3.13	3.13
E. coli K-12 ML1410 R81[c]	100	6.25	6.25	>100	1.56	1.56
E. coli K-12 LA290 R55[d]	50	100	100	50	100	100
E. coli W677	1.56	3.13	1.56	0.78	0.78	0.78
E. coli JR66/W677[d,e]	100	100	50	>100	50	25
E. coli K-12 C600 R135[f]	1.56	3.13	1.56	0.39	0.78	0.78
Mycobacterium smegmatis ATCC 607	0.78	1.56	0.78	1.56	0.78	0.78
Klebsiella pneumoniae PCI602	3.13	3.13	3.13	0.78	1.56	0.78
K. pneumoniae 22#3038[d,e]	100	100	100	>100	50	50
Shigella dysenteriae JS11910	6.25	12.5	6.25	1.56	3.13	1.56
Proteus rettgeri GN311	0.78	1.56	0.78	0.78	0.78	0.39
Serratia marcescens	3.13	25	12.5	3.13	25	12.5
Providencia sp. Pv16[g]	6.25	6.25	6.25	12.5	25	25
Providencia sp. 2991[g]	50	50	12.5	25	100	100
Pseudomonas aeruginosa A3	3.13	0.39	0.39	1.56	<0.20	<0.20
P. aeruginosa No. 12	25	3.13	3.13	12.5	1.56	3.13
P. aeruginosa H-9[e]	100	6.25	3.13	>100	3.13	3.13
P. aeruginosa TI-13[e]	25	6.25	3.13	25	1.56	3.13
P. aeruginosa 99[f]	100	6.25	6.25	50	3.13	6.25
P. aeruginosa GN315[b]	100	100	100	100	100	>100

Resistance mechanism: [a] AAD(4′), [b] AAC(6′), [c] APH(3′)-I, [d] AAD(2″), [e] APH(3′)-II, [f] AAC(3), [g] AAC(2′).

tively deacetonated to give **4** quantitatively. 6-*O*-Tosylation gave **5** (76%) which was treated with sodium azide in DMF to give the 6-azido derivative (**6**, quantitatively) as a syrup, $[\alpha]_D^{20}$ −27° (*c* 1, $CHCl_3$), IR (neat) 2110 cm^{-1}.

Methanolysis of **6** in the presence of Amberlite CG-120 (H^+ form) gave the 6-azido-3-fluoro-glucopyranoside derivative (**7**, quantitatively), which, after benzylation of the 2- and 4-hydroxyl groups to give **8**, was treated with acetic anhydride - sulfuric acid (100: 1) affording the 1-*O*-acetyl derivative (**9**, 75% from **7**). Treatment of **9** with $TiBr_4$ in dichloromethane - EtOAc (10: 1) (room temp, 40 hours) gave the corresponding 1-bromide (**10**) as a syrup (71%), $[\alpha]_D^{25}$ +193° (*c* 1, $CHCl_3$); IR (neat) 2110 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.99 (1H, dt, H-3), 6.31 (1H, t, H-1); $J_{1,2}={}^4J_{H-1,F}$=4 Hz, $^3J_{H-2,F}$=12 Hz, $^2J_{H-3,F}$=53 Hz, and $^3J_{H-4,F}$=14 Hz.

2′-*O*-Benzyl-1,3,3′-tris(*N*-benzyloxycarbonyl)-4′,6′-*O*-cyclohexylidene derivative (**11**) of 3AD was prepared by cyclohexylidenation of the 4′,6′-unprotected precursor[15]. Condensation of **10** and **11** was carried out in dichloromethane in the presence of mercury (II) cyanide and Drierite ($CaSO_4$) and the product (**12**) purified by column chromatography (30% based on **11**). Treatment with aqueous acetic acid to remove the cyclohexylidene group, followed by hydrogen in the presence of palladium black to reduce the azido and remove the benzyl and benzyloxycarbonyl groups gave, after purification by CM-Sephadex C-25 column chromatography (0~0.15 M aqueous ammonia), the desired product (**1**, 60%) as the monocarbonate, $[\alpha]_D^{25}$ +116° (*c* 0.5, H_2O); ^1H NMR (250 MHz, 20% ND_3 in D_2O) δ 4.58 (1H, dt, H-3′), 5.02 (1H, d, H-1″), 5.38 (1H, t, H-1′); $J_{1',2'}={}^4J_{H-1',F}$=4 Hz, $J_{2',3'}=J_{3',4'}$=9 Hz, $^2J_{H-3',F}$=54 Hz, $J_{1'',2''}$=3.8 Hz.

3′-Deoxy-3′-fluorokanamycin B (**2**) was prepared from kanamycin B as follows: 4′,6′-*O*,*N*-Carbonyl-4″,6″-*O*-cyclohexylidene-1,3,2′,3″-tetrakis(*N*-tosyl)kanamycin B[16] (**13**) was selectively acetylated with *N*-acetylimidazole in DMSO-pyridine to give the 2″-*O*-acetyl derivative (**14**, quantitatively); ^1H NMR (250 MHz, pyridine-d_5) δ 5.51 (dd, $J_{1'',2''}$=3.7 Hz, $J_{2'',3''}$= 10.5 Hz, H-2″). Sulfonylation of **14** with benzylsulfonyl chloride in pyridine gave the 3′-*O*-benzylsulfonyl derivative (**15**, quantitatively); ^1H NMR (250 MHz, pyridine-d_5) δ 5.41 (t, J= 10.3 Hz, H-3′). Treatment of **15** with 0.5 M methanolic sodium hydroxide gave the *N*-tosyl-2′,3′-aziridine (**16**, 95%) with concomitant cleavage of the 4′,6′-cyclic carbamate to give the 6′-*N*-methoxycarbonyl group; ^1H NMR (250 MHz, pyridine-d_5) δ 3.57 (dd, H-2′) and 3.73 (dd, H-3′); $J_{1',2'}$=~4 Hz, $J_{2',3'}$=7 Hz, and $J_{3',4'}$=3 Hz. Ring opening of the *N*-tosylaziridine with KHF_2 in DMF (150°C, 2 hours) gave the 3′-fluoro-2′-*N*-tosyl derivative (**17**) in 60% yield; ^1H NMR (250 MHz, pyridine-d_5) δ 5.31 (dt, $J_{2',3'}=J_{3',4'}$= 10 Hz, $^2J_{H-3',F}$=54 Hz, H-3′). Removal of the protecting groups of **17** with sodium in liquid ammonia to remove the *N*-Ts groups followed by treatments with aqueous sodium hydroxide and Dowex 50WX2 (H^+ form) gave, after purification by CM-Sephadex C-25 column (developed by 0.05~0.15 M aqueous ammonia), the final product (**2**, 30%), $[\alpha]_D^{22}$ +127° (*c* 0.6, H_2O); ^1H NMR (250 MHz, 20% ND_3 in D_2O) δ 4.47 (1H, dt, H-3′), 5.03 (1H, d, H-1″), 5.34 (1H, t, H-1′); $J_{1',2'}={}^4J_{H-1',F}$=3.8 Hz, $^3J_{H-2',F}=$ $^3J_{H-4',F}$=10 Hz, $^2J_{H-3',F}$=54 Hz, $J_{1'',2''}$=3.8 Hz.

3′-Deoxy-3′-fluorokanamycin A and 3′-deoxy-3′-fluorokanamycin B showed strong activities (Table 1) in inhibiting the growth of sensitive and resistant bacteria. It should be stressed that these are the first examples in which a fluorine atom has been substituted for a hydroxyl group at a site of bacterial enzymatic inactivation.

TSUTOMU TSUCHIYA
YOSHIAKI TAKAHASHI
YOSHIHIKO KOBAYASHI
SUMIO UMEZAWA

Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki 211, Japan

HAMAO UMEZAWA

Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received April 30, 1985)

References

1) UMEZAWA, S.: Structures and syntheses of aminoglycoside antibiotics. Adv. Carbohydr. Chem. Biochem. 30: 111~182, 1974
2) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics. Adv.

Carbohydr. Chem. Biochem. 30: 183~225, 1974

3) UMEZAWA, S.; T. TSUCHIYA, R. MUTO, Y. NISHIMURA & H. UMEZAWA: Synthesis of 3'-deoxykanamycin effective against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 274~275, 1971
4) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3',4'-Dideoxy-kanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485~487, 1971
5) DANIELS, P. J. L.; D. F. RANE, S. W. MCCOMBIE, R. T. TESTA, J. J. WRIGHT & T. L. NAGABHUSHAN: Chemical and biological modification of antibiotics of the gentamicin group. *In* Aminocyclitol Antibiotics. *Eds.*, K.L. RINEHART, Jr. & T. SUAMI, pp. 371~392, ACS Symposium Series, Publ. ACS, Honolulu, 1980
6) TSUCHIYA, T.; T. TORII, S. UMEZAWA & H. UMEZAWA: Syntheses of 3-fluoro derivatives of sporaricin A, 3-epifluoro-, 3-fluoro, and 3,3-difluoro-3-demethoxysporaricin A. J. Antibiotics 35: 1245~1247, 1982
7) TSUCHIYA, T.; T. TORII, Y. SUZUKI & S. UMEZAWA: Syntheses of 3-fluoro-, 3-epi-3-fluoro-, and 3,3-difluoro-3-de(methoxy)sporaricin A. Carbohydr. Res. 116: 277~287, 1983
8) TORII, T.; T. TSUCHIYA & S. UMEZAWA: Synthesis of 5-deoxy-5-fluoro sporaricin A. Carbohydr. Res. 116: 289~294, 1983
9) TORII, T.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 5''-deoxy-5''-fluorolividomycin B. Bull. Chem. Soc. Jpn. 56: 1522~1526, 1983
10) ALBERT, R.; K. DAX & A. E. STÜTZ: Carbohydrate triflates: Introduction of substituents by double inversion at C-4'' of kanamycin A. Carbohydr. Res. 132: 162~167, 1984
11) ALBERT, R.; K. DAX, A. E. STÜTZ & H. WEIDMANN: Chemical modification of kanamycin A. II. Nucleophilic displacement reactions of kanamycin-A-4''-sulfonates. J. Carbohydr. Chem. 1: 289~299, 1982-1983
12) ALBERT, R.; K. DAX & A. E. STÜTZ: Synthese fluorierter Kanamycin-A-derivate, modifizierung der Positionen 4'' und 6''. J. Carbohydr. Chem. 3: 267~278, 1984
13) ALBERT, R.; K. DAX & A. E. STÜTZ: Synthesis of fluorinated kanamycin A derivatives. Tetrahedron Lett. 24: 1763~1766, 1983
14) TEWSON, T. J. & M. J. WELCH: New approaches to the synthesis of 3-deoxy-3-fluoro-D-glucose. J. Org. Chem. 43: 1090~1092, 1978
15) UMEZAWA, S.; K. TATSUTA & S. KOTO: The total synthesis of kanamycin A. Bull. Chem. Soc. Jpn. 42: 533~537, 1969
16) MIYAKE, T.; Y. TAKAHASHI, T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: A synthesis of 3'-deoxykanamycin B (tobramycin). Nippon Kagaku Kaishi 1982: 1706~1712, 1982