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Synthesis of 5-deoxy-5-fluoro- and 5-deoxy-5,5-difluoro-netilmicin

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ABSTRACT

5-Deoxy-5-fluoro- (**9**) and 5-deoxy-5,5-difluoro-netilmicin (**27**) have been prepared from the corresponding 5-epi and 5-oxo derivatives of netilmicin by treatment with DAST. Structures of the fluorinated by-products (**10**, **11**, and **12**) obtained in one of the synthesis of **9** were determined. 5-Epinetilmicin (**13**) and 5-epi-6′-*N*-methylnetilmicin (**21**) have also been prepared.

INTRODUCTION

In a previous paper[1] we reported the synthesis of 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of kanamycin B, 3′-deoxykanamycin B (tobramycin), and 3′,4′-dideoxykanamycin B (dibekacin), and noted that these fluorinated compounds showed markedly reduced toxicity in comparison to the parent antibiotics without reducing the antibacterial activity. The present paper describes mainly the synthesis of 5-deoxy-5-fluoro- (**9**) and 5-deoxy-5,5-difluoro-netilmicin (**27**), and this is an extension of the previous study. Before our study, Daniels et al.[2] prepared structurally related fluorinated compounds, 5-deoxy-5-fluoro- and 5-deoxy-5-epifluoro-sisomicin, by treatment of protected 5-episisomicin and sisomicin derivatives with diethylaminosulfur trifluoride (DAST). Here we have prepared the 5-deoxy-5-fluoro derivatives of netilmicin (netilmicin[3] corresponds to 1-*N*-ethylsisomicin) to clarify the relationship between the deoxyfluorination and toxicity of the fluorinated compounds.

RESULTS AND DISCUSSION

The amino groups of netilmicin were fully tosylated and the penta-*N*-tosyl derivative (**1**) was subjected to the Mitsunobu reaction to invert the C-5 substituent

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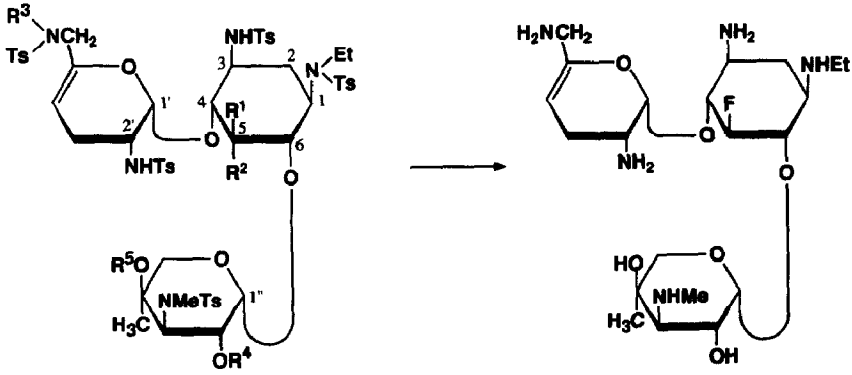


9

	R^1	R^2	R^3	R^4	R^5
1	OH	H	H	H	H
2	OH	H	Bz	Bz	H
3	OMs	H	Bz	Bz	H
4	OMs	H	Bz	Bz	Ms
5	H	OAc	Bz	Bz	H
6	H	OH	H	H	H
7	H	OH	Bz	Bz	H
8	F	H	Bz	Bz	H

by applying the same reaction conditions as described[1] for the penta-*N*-tosyl derivatives of 3′,4′-dideoxykanamycin B and related compounds. In this instance, no corresponding 5-*O*-benzoyl-5-epi derivative was obtained. Thus, **1** was benzoylated, and the 6′-*N*,2″-*O*-dibenzoyl derivative **2** was subjected to the Mitsunobu reaction, but again the desired compound was not obtained. The failure of this reaction is attributable to a slight change in the steric environment around C-5 of **1** and **2** from that for the compounds reacting successfully[1]. The *N*,*O*-dibenzoyl structure of **2** was confirmed by the ^{1}H NMR spectrum, in which the resonances of H-6′ and -2″ shifted downfield by 0.85 and 1.83 ppm, respectively, compared to those for **1**, whereas those for H-3, -5, -2′ and CH_3-4″ showed no appreciable shifts (0.03–0.12 ppm). To effect the reaction, the procedure of Daniels et al.[2] was applied; the 5-*O*-mesyl derivative **3** was treated with sodium acetate in *N*,*N*-dimethylformamide (DMF) and the resulting 5-*O*-acetyl-5-epi derivative **5** was deacylated to give the 5-epihydroxyl derivative **6**. Treatment of **6** with DAST, however, gave no fluorinated product. The OH-2″ of **6** was then protected by benzoylation, and the resulting 6′-*N*,2″-*O*-dibenzoyl derivative **7** was treated with DAST. This time, the desired 5-deoxy-5-fluoro derivative **8** was obtained in 25% yield along with a mixture of other products. The structure of **8** was confirmed by the ^{1}H (see Table I) and ^{19}F NMR spectra; the large $J_{4,5}$ and $J_{5,6}$ values (9 Hz, respectively) indicated the presence of an equatorial F-5. Debenzoylation of **8** followed by detosylation with sodium in liquid ammonia gave 5-deoxy-5-fluoronetilmicin (**9**).

TABLE I

Selected ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (Hz) of netilmicin (NT), **1–10**, and **13–27** [a] measured in pyridine-d_5 at 70°C

Splitting (→)	NCH_2CH_3 t	H-5	H-1′ d	H-4′	H-1″ d	H-2″ dd	H-3″ d	H-5″a d	H-5″b d	NCH_3 s	CH_3-4″ s	$J_{4,5} = J_{5,6}$	$J_{3'a,4'}$	$J_{3'b,4'}$	$J_{2'',3''}$
NT	1.14	3.62 t	5.40	4.92 m	5.01	3.85	2.59	3.38	4.07	2.59	1.27	9	~1.5	6	10
1	1.06	3.65 t	5.62	4.98 dd	5.48	4.55	4.61	3.68	4.14	3.23	1.57		1.5	6	11
2	0.68	~3.60	5.68	5.02 dd	6.23	6.38	5.18	3.72	4.70	3.12	1.45		1.5	6	11
3	0.83	5.25 t	5.55	4.90 t	6.46	6.38	5.13	3.78	4.50	3.27	1.54	8	3.5	3.5	9
4	0.83	5.21 t	5.60	4.88 t	6.47	6.12	5.30	3.75	4.75	3.21	1.99	8	3.5	3.5	9
5	0.63	6.20 t	5.40	4.93 br d	6.22	6.36	5.06	3.72	4.43	3.19	1.41	4			10
6	1.03	5.14 br s	5.52	4.95 br d	5.50					3.28	1.51	2			
7	0.75	5.05 br s	5.43	4.90 br d	5.99	6.33		3.88	4.64	3.20	1.48	2			11
8	0.65	4.15 dt	5.42	4.99 br d	5.99	5.81	5.50			2.81	1.58	9			9
9	1.08	4.57 dt	5.26	4.88 dd	4.92	3.80	2.54	3.32	3.91	2.52	1.20	9	2	5	10.5
10	0.98	3.99 dt	5.38		5.00		4.28 dd	3.57		3.00	1.51 d	9			9
13	1.08	4.37 br s	5.14	4.88 br d	4.99	3.77	2.62	3.37	3.90	2.53	1.21	~1.5	1.5	6	10
14	1.06	4.92 br s	5.49	4.77 dd	5.39		5.10	3.52	4.35	3.39	1.16		1.5	6	12
15	1.04	4.85 br s	5.48	4.80 br d	4.97			3.69	4.19	2.86	1.30				
16	0.94	4.85 br s	5.46	4.78 br d	5.40	5.56		3.86	4.32	2.88	1.32				
17	0.98	4.40 dt	5.60	4.77 dd		5.66		3.86	4.20	2.88	1.36	9	1.5	6	4
18	0.80	5.20 t	5.55	4.90 m	6.40	6.34	5.14	3.75	4.45	3.26	1.57				11
19	0.85	5.25	5.64	4.90 t	6.42	6.35	5.13			3.27	1.54	9			11
20	1.03	5.20 br s	5.56	4.95 m	5.55	4.64				3.30	1.52				11
21	1.12	4.40 br s	5.17	4.92 m	5.01	3.80	2.65	3.41	3.95	2.56	1.26	~2			10
22	1.04		5.83	4.82 dd	5.32		5.12			3.36	1.25		2	6	12
23	1.05		5.84	4.81 m	4.90				4.22	2.83	1.34				
24	0.95		5.79	4.80 dd		5.69			4.25	2.89	1.37		2	6	4
25	0.95					5.73				2.85	1.38				4
26	0.95					5.72				2.87	1.34				4
27	1.09		5.28	4.91 dd	4.96	3.82	2.59	3.38	3.92	2.52	1.22		2	5	10.5

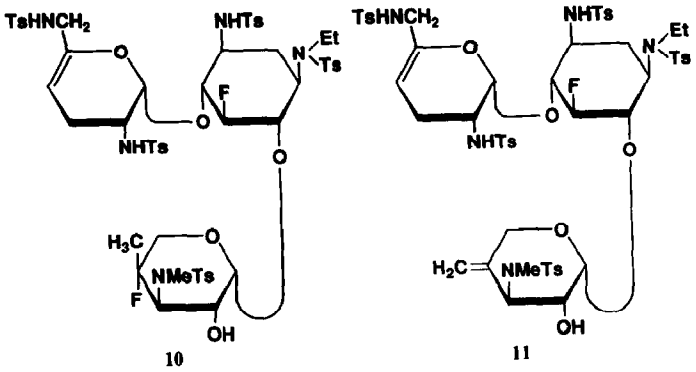
[a] The data for netilmicin, **9**, **13** and **27** were obtained with a Bruker AM X-500 spectrometer. [b] The data for netilmicin, **9**, **13**, **21**, and **27** were obtained in 20% ND_3 in D_2O at room temperature.

TABLE II

^{13}C NMR chemical shifts (δ) and coupling constants ($J_{C,F}$, Hz, in parentheses) for netilmicin, **13**, **21**, **9**, and **27** in 20% ND_3 in D_2O

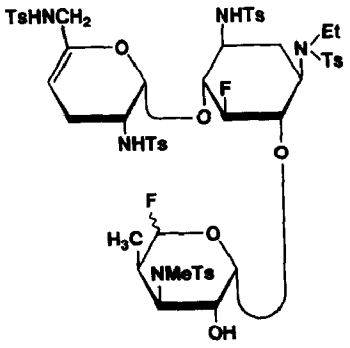
	Netilmicin	13	21	9	27
C-1	58.1	54.6	54.7	57.2 d (9.1)	56.6 d (7.7)
C-2	32.9	33.2	33.3	32.5	32.0
C-3	50.3	47.0	47.2	49.4 d (9.4)	49.0 d (7.4)
C-4	86.7	84.0	84.1	82.7 d (15.3)	80.6 t (17.5)
C-5	75.4	69.5	69.7	96.5 d (180)	121.1 t (250)
C-6	85.5	80.7	80.9	84.2 d (17.0)	82.6 t (18.2)
NCH_2CH_3	14.7	14.8	14.9	14.7	14.7
NCH_2CH_3	41.2	40.9	41.0	41.2	41.1
C-1′	100.9	96.9	99.1	100.2	100.5
C-2′	47.5	47.2	47.3	47.4	47.3
C-3′	25.5	25.7	25.9	25.3	25.2
C-4′	96.6	96.8	97.1	96.9	97.0
C-5′	150.5	150.3	147.5	150.2	150.1
C-6′	43.4	43.4	52.8	43.4	43.3
NCH_3-6′			34.9		
C-1″	102.3	102.9	103.0	102.5	103.3
C-2″	70.0	70.0	70.1	69.9	70.0
C-3″	64.3	63.9	64.0	64.2	64.1
C-4″	73.1	73.1	73.2	73.1	73.0
C-5″	68.7	68.5	68.6	68.6	68.8
CH_3-4″	22.7	22.6	22.7	22.6	22.6
NCH_3-3″	37.7	37.7	37.8	37.7	37.7

The structure was confirmed by the ^1H, ^{19}F, and ^{13}C NMR spectra (Table I and II), as well as by the 2D ^1H–^{13}C shift-correlated spectrum. The products obtained along with **8** were also pursued. Deacylation of the mixture followed by chromatographic separation gave a difluoro product **10** (31% based on **7**) and a mixture of two products. The structure of **10** was determined by the ^1H and ^{19}F NMR spectra; one of the fluorines of **10** demonstrated the same pattern as **8** and therefore indicated F-5 to be equatorial; the other fluorine had $J_{CH_3,F}$ 25 Hz and small $J_{3'',F}$, $J_{5''a,F}$, and $J_{5''b,F}$ values (each $\sim$ 7 Hz), indicating F-4″ to be equatorial. Compound **10** is evidently formed by a set of S_N2 reactions occurring both at C-5 and C-4″. The other two products obtained along with **10** were difficult to separate by standard chromatography, and were isolated by HPLC. One of the products, obtained in 6% yield, was determined to be the 5-deoxy-5-fluoro-4″-methylene derivative **11**. This compound is presumably formed by loss of NEt_2SF_2OH (the H atom arising from one of the CH_3-4″ hydrogens) from the reaction intermediate having a 4″-O-SF_2NEt_2 fragment. The fourth product was determined to be the 5,4″-dideoxy-5,5″-difluoro derivative **12** having the CH_3-4″ group up, and the structure was confirmed by the mass and NMR spectra. The axial orientation of CH_3-4″ in **12** was concluded from the small $J_{3'',4''}$ and $J_{4'',5''}$ values, and the presence of a fluorine at C-5″ was established from the connectivity check of the signals, as well as by the large $J_{5'',F}$ value. The striking feature in



10

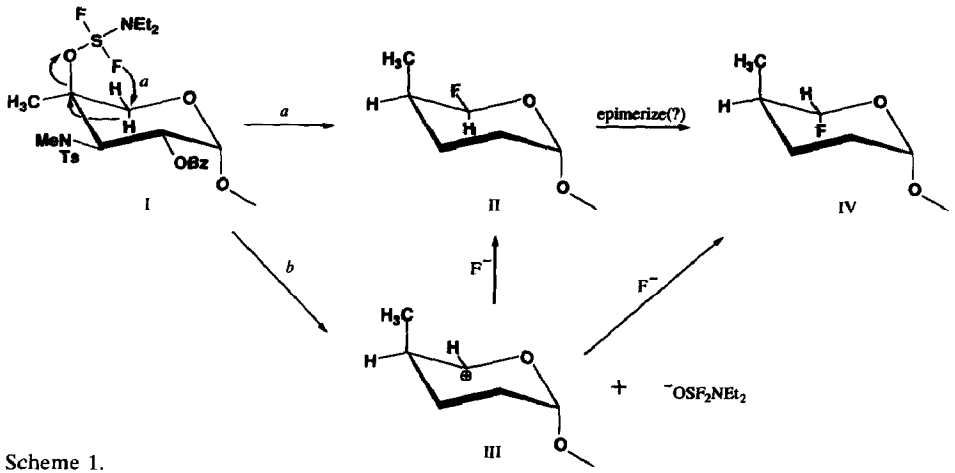
11



12

the NMR spectrum of this compound was the presence of the F-5–F-5″ coupling (11 Hz), confirmed by the decoupling method. As it is difficult to imagine that this coupling is transmitted through bonds, it presumably occurs through space. Inspection of a molecular model (W. Büchi's stereomodel) suggests that the two fluorines come close to each other (see Scheme 2) more readily when the F-5″ is down (axial) than when it is up (equatorial); however, the magnitudes of $J_{4'',F}$ (11 Hz) and $J_{5'',F}$ (54 Hz) suggest * that F-5″ should be up. Compound **12** is formed through either route *a* or *b*, as shown in Scheme 1. The hydride shift (from C-5″ to C-4″) in this reaction will be facilitated by the diaxial relationship of *C*-OSF_2NEt_2 and H-5″ as shown in I. Formation of IV (rather than II) through II or III may be explained based on the anomeric effect. However, electrostatic repulsion between O-1″–F-5″ suggests the priority of II (rather than IV). The absolute configuration at C-5″ cannot yet be determined unequivocally to be *R* (form II). Molecular mechanics calculations [MM2′ (77)] of A and B as model compounds for the

* In structurally related α- and β-D-mannopyranosyl fluorides, the $J_{1,F}$ values were reported to be 48–50 and 51–53 Hz, respectively, and those for $J_{2,F}$ were 0.5–2 and 13–15 Hz (see ref. 4).



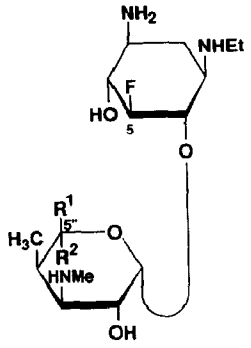
Scheme 1.

2,5-dideoxy-1-*N*-ethyl-5-fluoro-6-*O*-glycosyl portion of **12** showed that, in the conformation of minimum energy, A and B had 2.84 and 3.72 Å for the F-5–F-5″ (center-to-center) distances, respectively. The stereoscopic view of A (prepared by M of T Systems Technology Ltd.) is shown in Scheme 2.

As 5-deoxy-5-fluoronetilmicin (**9**) was not obtained in good yield, an alternative method using benzyloxycarbonyl as the *N*-protecting group was tried. Detosylation of **6** with sodium in liquid ammonia gave 5-epinetilmicin[2] (**13**). Treatment of **13** with benzyl chloroformate gave the pentakis(*N*-benzyloxycarbonyl) derivative, which, on treatment with sodium hydride in DMF *, gave the 3″,4″-cyclic carbamate **15**. Fluorination of **15** with DAST, however, gave no fluorinated product. Then, after benzoylation, the 2″-*O*-benzoyl-5-ol **16** was treated with DAST, whereupon the 5-deoxy-5-fluoro derivative **17** was obtained in high yield. This result suggests that the protection of both OH-2″ and -4″ is necessary to afford the 5-deoxy-5-fluoro derivative in high yield, and for that purpose, the 3″-*N*-benzyloxycarbonylation followed by cyclic carbamate formation[5] is convenient. This pathway had been utilized[2] in the preparation of sisomicin derivatives. Removal of the *N*-protecting groups from **17** by treatment with sodium in liquid ammonia, followed by cleavage of the cyclic carbamate by heating the product in an alkaline medium gave 5-deoxy-5-fluoronetilmicin (**9**) in 70% yield.

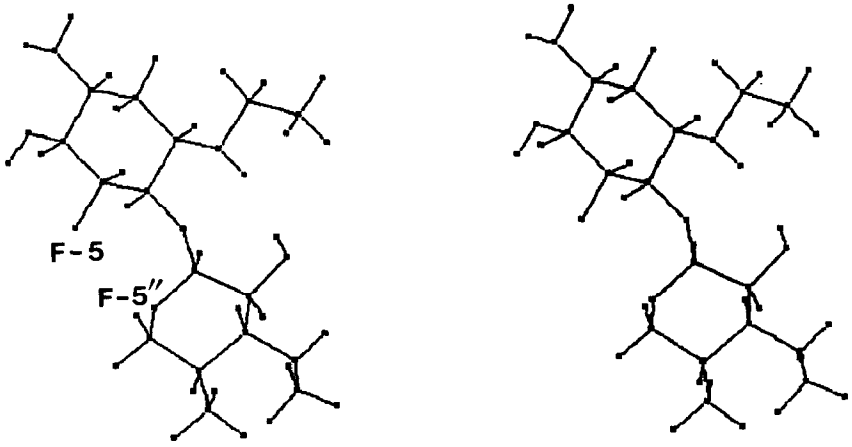
As 6′-*N*-methylation is believed to restrict the enzymic action by resistant bacteria acetylating the NH_2-6′ group [AAC(6′)], 5-epi-6′-*N*-methylnetilmicin (**21**) was prepared. The 6′-*N*-benzoyl group of **3** was selectively removed by mild treatment with sodium methoxide, and the product **18** was methylated with methyl iodide–silver oxide to give the 6′-*N*-methyl derivative **19**. Inversion at C-5 of **19** (to give **20**) followed by deprotection gave 5-epi-6′-*N*-methylnetilmicin (**21**). The presence of the CH_3N-6′ group was proved by the ^{13}C NMR spectrum of **21**, in that

* Cyclic carbamate formation by this method was first reported in our laboratory (see ref. 5).



A $R^1 = H$, $R^2 = F$

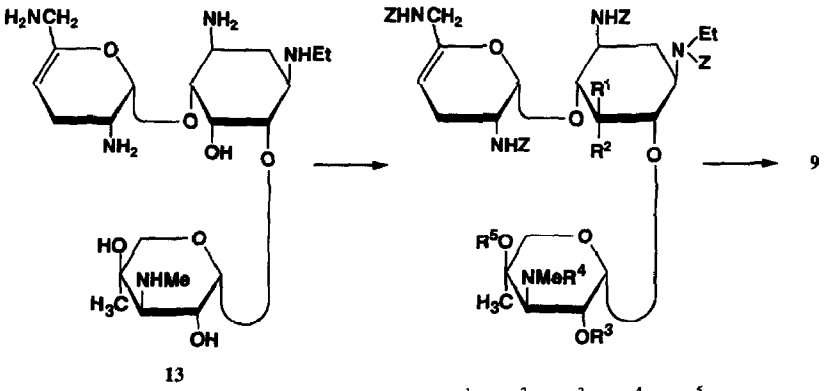
B $R^1 = F$, $R^2 = H$



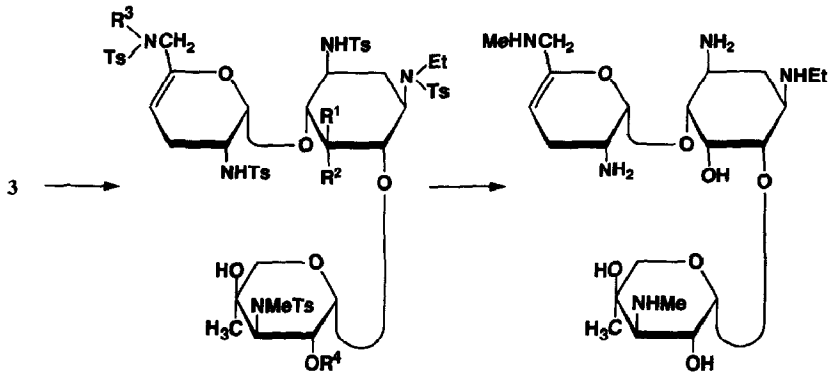
Scheme 2.

the resonance of C-6′ shifted downfield by 9.4 ppm and that of C-5′ shifted upfield (2.8 ppm) from the corresponding resonances for 5-epinetilmicin, respectively (see Table II).

5-Deoxy-5,5-difluoronetilmicin (**27**) has been prepared through a similar reaction pathway as reported[1] previously. Pentakis(*N*-benzyloxycarbonyl)netilmicin (**22**) was transformed into the 3″,4″-cyclic carbamate (**23**) by treatment with sodium hydride in DMF, and after benzoylation, the 2″-*O*-benzoyl-5-ol (**24**) was oxidized with dimethyl sulfoxide–acetic anhydride to give the 5-oxo derivative **25**. Treatment of **25** with DAST gave the 5-deoxy-5,5-difluoro derivative **26**, and removal of the protecting groups from **26** gave 5-deoxy-5,5-difluoronetilmicin (**27**). It is noteworthy that the difluoro group was stable during the treatment with sodium in liquid ammonia. The structure of **27** was confirmed by the ^1H, ^{19}F, and ^{13}C NMR spectra (Table II), as well as by the 2D ^1H–^{13}C shift-correlated spectrum.

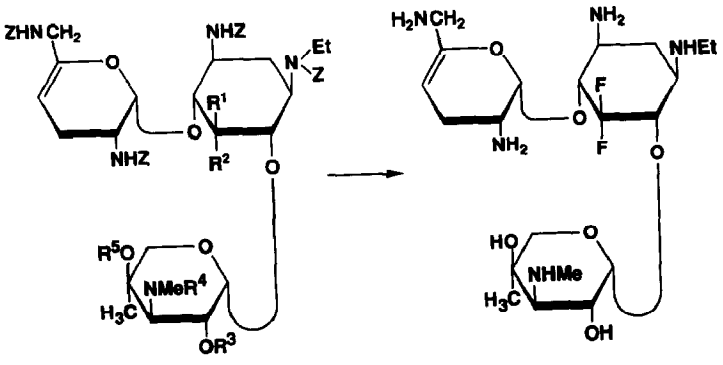


	R^1	R^2	R^3	R^4	R^5
14	H	OH	H	Z	H
15	H	OH	H	C=O	
16	H	OH	Bz	C=O	
17	F	H	Bz	C=O	



	R^1	R^2	R^3	R^4
18	OMs	H	H	Bz
19	OMs	H	Me	Bz
20	H	OH	Me	H

The antibacterial spectra of 5-deoxy-5-fluoronetilmicin (**9**), 5-deoxy-5,5-difluoronetilmicin (**27**), 5-epinetilmicin (**13**), and 5-epi-6′-*N*-methylnetilmicin (**21**) were determined together with that of netilmicin (see Experimental); it was found that **9**, **13**, and netilmicin showed similar antibacterial activities and **27** slightly less, and **21** was clearly less active than netilmicin. Compound **21**, however, showed activity against a resistant strain acetylating the NH_2-6′ group. In terms of toxicity, preliminary measurement (intravenous injection in mice) showed that 5-deoxy-5-fluoronetilmicin (**9**) had 1/2 to 1/3 of the acute toxicity of netilmicin (LD_{50} ~ 30



27

	R^1	R^2	R^3	R^4	R^5
22	OH	H	H	Z	H
23	OH	H	H	C=O	
24	OH	H	Bz	C=O	
25	=O		Bz	C=O	
26	F	F	Bz	C=O	

Z: $CO_2CH_2C_6H_5$

mg/kg mouse). These results, together with those reported[1], suggest that 5-deoxy-5-fluorination (and 5-deoxy-5,5-difluorination) gives rise to compounds of decreased toxicity in comparison to the parent compounds, without diminishing (sometimes enhancing) or slightly decreasing the antibacterial activity.

EXPERIMENTAL

General methods.—Optical rotations were determined with a Perkin–Elmer 241 polarimeter. Mass spectra were determined by the field-desorption method with a Jeol SX-102 spectrometer; data are reported as *m/z*. NMR spectra (^1H at 250 MHz, ^{13}C at 62.9 MHz, and ^{19}F at 235.3 MHz) were recorded with a Bruker WM 250 spectrometer unless otherwise stated. Chemical shifts (δ) of ^1H, ^{13}C, and ^{19}F spectra were measured downfield from internal Me_4Si (for ^1H), internal 1,4-dioxane (for ^{13}C, $\delta = \delta^{dioxane} + 67.4$), or internal Freon 11 (for ^{19}F), unless otherwise stated, and confirmed, in most cases, by shift-correlated 2D spectra. Most of the prominent ^1H NMR signals commonly appeared in the synthetic compounds are listed in Table I, and the signals specific for individual compounds are given in the respective sections. TLC was performed on Kieselgel 60 F_{254} (Merck), and column chromatography on Wakogel C-200. Analytical samples of **2**, **5**, **6**, **7**, **16**, **18**, **20**, and **24** were prepared by purifying the crude products, obtained after final concentration, by column chromatography (10:1 $CHCl_3$–MeOH).

1,3,2',6',3''-Penta-N-*tosylnetilmicin* (**1**).—To an ice-cold suspension of netilmicin base (200 mg, 0.42 mmol) and anhyd Na_2CO_3 (200 mg) in 1:1 1,4-dioxane–H_2O (10 mL) was added *p*-toluenesulfonyl chloride (500 mg, 2.6 mmol) and the mixture was stirred for 3 h at room temperature. TLC (10:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) of the mixture showed a single spot at R_f 0.4. Concentration gave a residue that was extracted with $CHCl_3$. The solution was washed with water, dried (Na_2SO_4), and concentrated. The residue was washed with diethyl ether and dried to give **1** as a solid, 518 mg, 99%); $[\alpha]_D^{26}$ +132° (*c* 1.2, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 2.17, 2.23, 2.25, 2.26 and 2.33 [each s, 3 H, Ts(*Me*) × 5], ~3.37 (2 H, H-1, 3), 3.75 (m, 1 H, H-2'), and 4.00 (slightly br s, 2 H, H-6'a,6'b). Anal. Calcd for $C_{56}H_{71}N_5O_{17}S_5$: C, 53.96; H, 5.74; N, 5.61. Found: C, 53.80; H, 6.01; N, 5.90.

6'-N,*2''*-O-*Dibenzoyl-1,3,2',6',3''-penta*-N-*tosylnetilmicin* (**2**).—To an ice-cold solution of **1** (200 mg, 0.16 mmol) in dry pyridine (4 mL) was added benzoyl chloride (0.1 mL, 0.86 mmol) and the solution was kept for 3 h at room temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.7 (cf. **2**: 0.2). Addition of water (0.2 mL) followed by concentration gave a residue that was dissolved in $CHCl_3$ and the solution was washed with aq $NaHCO_3$ (satd), dried (Na_2SO_4), and concentrated to give **2** as a solid (225 mg, 96%); $[\alpha]_D^{26}$ +113° (*c* 0.6, $CHCl_3$); ^1H NMR (pyridine-d_5): δ ~3.27 (2 H, H-1 and 3), 3.72 (m, 1 H, H-2') and 4.85 (slightly br s, 2 H, H-6'a,6'b). Anal. Calcd for $C_{70}H_{79}N_5O_{19}S_5 \cdot 0.5\ H_2O$: C, 57.44; H, 5.51; N, 4.78. Found: C, 57.34; H, 5.42; N, 4.62.

6'-N,*2''*-O-*Dibenzoyl-5*-O-*mesyl-* (**3**) *and -5,4''-di*-O-*mesyl-1,2,2',6',3''-penta*-N-*tosylnetilmicin* (**4**).—To an ice-cold solution of **2** (87.5 mg, 0.06 mmol) in dry pyridine (1.5 mL) was added methanesulfonyl chloride (0.05 mL, 0.65 mmol) and the solution was kept for 24 h in the cold (0–5°C). TLC (25:1 $CHCl_3$–MeOH), showed two spots at R_f 0.65 (**3**) and 0.75 (**4**) (cf. **2**: R_f 0.7). Conventional work-up as described for **2** gave a crude solid that was chromatographed with 100:1 $CHCl_3$–MeOH to give **3** as a solid (65.7 mg, 71%) and **4** also as a solid (14.5 mg, 15%).

Compound **3** had: $[\alpha]_D^{26}$ +82° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 3.51 (s, 3 H, Ms) and 4.01 (m, 1 H, H-2'). Anal. Calcd for $C_{71}H_{81}N_5O_{21}S_6$: C, 55.63; H, 5.33; N, 4.57. Found: C, 55.31; H, 5.21; N, 4.47.

Compound **4** had: $[\alpha]_D^{25}$ +75° (*c* 1.2, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 3.13 and 3.43 (each s, 3 H, Ms × 2), and 4.00 (m, 1 H, H-2'). Anal. Calcd for $C_{72}H_{83}N_5O_{23}S_7$: C, 53.68; H, 5.19; N, 4.35. Found: C, 53.68; H, 5.04; N, 4.44.

5-O-*Acetyl-6'*-N,*2''*-O-*dibenzoyl-5-epi-1,3,2',6',3''-penta*-N-*tosylnetilmicin* (**5**).—A mixture of **3** (180 mg, 0.12 mmol) and anhyd NaOAc (180 mg) in DMF (2.7 mL) was heated for 4 h at 90°C. TLC (25:1 $CHCl_3$–MeOH), showed a single spot at R_f 0.6 (cf. **3**: R_f 0.7). Concentration gave a residue that was extracted with $CHCl_3$. The solution was washed with water, dried (Na_2SO_4), and concentrated to give **5** as a solid (155 mg, 88%); $[\alpha]_D^{26}$ +77° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{36}H_{71}N_5O_{17}S_5 \cdot H_2O$: C, 57.09; H, 5.52; N, 4.62. Found: C, 56.97; H, 5.38; N, 4.39.

5-Epi-1,3,2',6',3''-penta-N-*tosylnetilmicin* (**6**).—To a solution of **5** (120 mg, 0.08 mmol) in $CHCl_3$ (2.5 mL) was added 28% NaOMe in MeOH (0.5 mL) and the solution was kept for 1 h at room temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.15. After neutralization with aq HCl, the solution was washed with water, dried (Na_2SO_4), and concentrated to give **6** as a solid (98.5 mg, 99%); $[\alpha]_D^{26}$ +115° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{56}H_{71}N_5O_{17}S_5$: C, 53.96; H, 5.74; N, 5.61. Found: C, 53.91; H, 5.35; N, 5.38.

6'-N,*2''*-O-*Dibenzoyl-5-epi-1,3,2',6',3''-penta*-N-*tosylnetilmicin* (**7**).—Compound **6** (250 mg, 0.2 mmol) was treated with benzoyl chloride (0.12 mL, 1.0 mmol) as described for **2** to give **7** as a solid (273 mg, 93%); $[\alpha]_D^{24}$ +105° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{70}H_{79}N_5O_{19}S_5 \cdot 0.5\ H_2O$: C, 57.44; H, 5.51; N, 4.78. Found: C, 57.56; H, 5.46; N, 4.88.

6'-N,*2''*-O-*Dibenzoyl-5-deoxy-5-fluoro-1,3,2',6',3''-penta*-N-*tosylnetilmicin* (**8**).—To an ice-cold solution of **7** (140 mg, 0.1 mmol) in dry CH_2Cl_2 (3 mL) was added DAST (0.1 mL, 0.8 mmol), and the solution was kept for 30 min at room temperature. TLC (15:1 $CHCl_3$–acetone) showed two spots at R_f 0.75 (**8**) and 0.72 (cf. **7**: R_f 0.15). After washing with aq $NaHCO_3$ (satd), the solution was dried ($MgSO_4$), and concentrated. The residue was chromatographed with 100:1 $CHCl_3$–acetone to give **8** as a solid (34.5 mg, 25%) and a mixture of products (R_f 0.72), 83.6 mg.

Compound **8** had: $[\alpha]_D^{25}$ +75° (*c* 1, $CHCl_3$); ^{19}F NMR (pyridine-d_5): δ −188.0 (br d); $J_{5,F}$ 48 Hz. Anal. Calcd for $C_{70}H_{78}FN_5O_{18}S_2$: C, 57.72; H, 5.40; N, 4.81. Found: C, 57.39; H, 5.42; N, 5.04.

5-Deoxy-5-fluoronetilmicin (**9**).—*From* **8**. To a solution of **8** (34.2 mg, 0.03 mmol) in $CHCl_3$ (0.8 mL) was added 28% NaOMe in MeOH (0.2 mL) and the solution was kept for 1 h at room temperature (debenzoylation). TLC (7:1 $CHCl_3$–acetone) showed a single spot at R_f 0.3 (cf. **8**: R_f 0.8). After neutralization with aq HCl, the $CHCl_3$ solution was washed with water, dried ($MgSO_4$), and concentrated. To the residue dissolved in liquid NH_3 ($\approx$ 2 mL) at −60°C was added Na ($\sim$ 30 mg) and the deep-blue solution was kept for 2 min (detosylation). Ammonium chloride was added until the solution became colorless, ammonia was evaporated (under diminished pressure), and the residue was packed onto a column of CM-Sephadex C-25 (NH_4^+ form, 5 mL). After washing the column with water thoroughly, the product was eluted with aq NH_3 (0 → 0.15 M) to give **9** as the solid carbonate, 8.1 mg (64%), $R_{f\ netilmicin}$ 1.1 (TLC, 9:4:1 $CHCl_3$–MeOH–aq 28% NH_4); $[\alpha]_D^{25}$ +167° (*c* 1, H_2O); ^1H NMR (20% ND_3 in D_2O; at 500 MHz with a Bruker AM X-500 spectrometer): δ 1.14 (q, 1 H, H-2*ax*), 1.98 (with small splittings, 1 H, H-3'a), 2.16 (dt, 1 H, H-2*eq*), 2.18 (dt, 1 H, H-3'b), 2.47 (dq, 1 H, $J_{Ha,Hb}$ 10.5, J_{CH,CH_3} 7 Hz, NC*Ha*HbCH_3), 2.71 (dq, 1 H, NCHa*Hb*CH_3), 2.77 (dt, 2 H, H-1, 3), 3.02 (dd, 1 H, H-2'), 3.14 (s, 2 H, H-6'a,6'b), 3.56 (dt, 1 H, H-6), 3.72 (d, 1 H, H-4); $J_{1,2ax} = J_{1,6} = J_{2ax,3} = J_{3,4}$ 11, $J_{1,2eq} = J_{2eq,3}$ 4, $J_{2ax,2eq}$ 12.5, $J_{4,F} = J_{6,F}$ 12, $J_{5,F}$ 51.5, $J_{1',2'}$ 2.5, $J_{2',3'a}$ 10.5, $J_{2',3'b}$ and $J_{3'a,3'b}$ 16.5 Hz. ^{19}F NMR (20% ND_3 in D_2O): δ −193.6 (dt, F-5).

Anal. Calcd for $C_{21}H_{40}FN_5O_6 \cdot H_2CO_3$: C, 48.97; H, 7.85; F, 3.52; N, 12.98. Found: C, 48.91; H, 7.73; F, 3.35; N, 12.92.

From **17**. A solution of **17** (120 mg, 0.1 mmol) in $CHCl_3$ (3 mL) was treated with 28% NaOMe in MeOH (0.2 mL) as already described. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.25 (cf. **17**: R_f 0.5). The product obtained was then treated with Na (~50 mg) in liquid NH_3 (~10 mL) at −60°C to give the de(benzyloxycarbonyl)ated product. The product, dissolved in aq 0.7 M NaOH (3 mL), was heated for 4 h at 90° [de(cyclic carbamate)]. TLC (9:4:1 $CHCl_3$–MeOH–aq 28% NH_3) showed a single spot at R_f 0.25 (cf. R_f 0.4 before the treatment). After neutralization with aq HCl, the solution was poured into a column of CM-Sephadex C-25 (NH_4^+ form, 32 mL) and worked-up as already described to give the carbonate of **9** as a solid (37.2 mg, 66%).

5,4″-Dideoxy-5,4″-difluoro-4″-epi-1,3,2′,6′,3″-penta-N-*tosylnetilmicin* (**10**).—To a solution (2 mL) of the product mixture of R_f 0.72 (80 mg) described in **8** was added 28% NaOMe in MeOH (0.5 mL), and the solution was kept for 1 h at room temperature. After neutralization with aq HCl, the $CHCl_3$ layer was washed with water, dried ($MgSO_4$), and concentrated. TLC (7:1 $CHCl_3$–acetone) of the residue showed two spots at R_f 0.2 (**10**) and 0.3. Column chromatography of the residue with 30:1 $CHCl_3$–acetone gave **10** as a solid (35.2 mg, 31% based on **7**) and a product mixture (R_f 0.3, 13.6 mg).

Compound **10** had: $[\alpha]_D^{25}$ +123° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5): $J_{4,F\text{-}5} = J_{6,F\text{-}5}$ 12.5, $J_{5,F\text{-}5}$ 48, $J_{3'',F\text{-}4''}$ 7, and $J_{CH_3,F\text{-}4''}$ 25 Hz. ^{19}F NMR (pyridine-d_5): δ −144.9 (br s, 1 F, F-4″) and −186.9 (dt, 1 F, F-5). Anal. Calcd for $C_{56}H_{69}F_2N_5O_{15}S_5$: C, 53.80; H, 5.56; N, 5.60. Found: C, 53.96; H, 5.60; N, 5.62.

4″-Dimethyl-5,4″-dideoxy-5-fluoro-4″-C-methylene-1,3,2′,6′,3″-penta-N-*tosylnetilmicin* (**11**) *and 5,4″-dideoxy-5,5″-difluoro-4″-epi-1,3,2′,6′,3″-penta*-N-*tosylnetilmicin* (**12**).—The product mixture (R_f 0.3, 30 mg) obtained with **10** was subjected to HPLC (SSC-Silicaget 842, Senshu Sci. Co. Ltd., 30 × 250 mm, with 8:1 $CHCl_3$–CH_3CN) to give **11** as a solid, (15.7 mg, 6.2% based on **7**), **12** as a solid (3.8 mg, 1.6% based on **7**), and the solid debenzoyl derivative (8.3 mg) of **8**.

Compound **11** had: $[\alpha]_D^{24}$ +111° (*c* 1, $CHCl_3$); ^{1}H NMR (pyridine-d_5): δ 0.96 (t, 3 H, CH_3CH$_2$), 2.16 (3 H), 2.25 (6 H), 2.28 (3 H), and 2.32 (3 H) [each s, Ts(*Me*) × 5], 2.90 (s, 3 H, NMe), 3.98 (dt, 1 H, $J_{4,5} = J_{5,6} = 9$, $J_{5,F}$ 48 Hz, H-5), 4.70 and 5.37 (each d together forming an ABq system, 1 H, *J* 12 Hz, H-5″a,5″b), 5.00 (m, 1 H, H-4′), 5.06 and 5.16 (each s, 1 H, C=CH$_2$), 5.40 (d, 1 H, $J_{1',2'}$ 2.5 Hz, H-1′), and 5.57 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″). ^{19}F NMR (pyridine-d_5): δ −186.6 (dt, $J_{4,F} = J_{6,F}$ 14, and $J_{5,F}$ 48 Hz, F-5). Mass spectrum: *m/z* 1230 $(M)^+$.

Compound **12** had: $[\alpha]_D^{24}$ +116° (*c* 0.4, $CHCl_3$); ^{1}H NMR (pyridine-d_5): δ 0.96 (t, 3 H, CH_3CH$_2$), 1.12 (d, 3 H, J_{4'',CH_3} 7 Hz, Me-4″), 2.20, 2.27, 2.28, 2.30, and 2.35 [each s, 3 H, Ts(*Me*) × 5], 2.98 (m, 1 H, H-4″), 3.09 (s, 3 H, NMe), 4.19 (dd, 1 H, $J_{1'',2''}$ 4, $J_{2'',3''}$ 11 Hz, H-2″), 4.52 (dt, 1 H, $J_{4,5} = J_{5,6} = 8.5$, $J_{5,F\text{-}5}$ 50 Hz, H-5), 5.02 (m, 1 H, H-4′), 5.06 (dd, 1 H, $J_{3'',4''}$ 5 Hz, H-3″), 5.44 (d, 1 H, $J_{1',2'}$ 2.5 Hz, H-1′), 5.50 (d, 1 H, H-1″), and 5.58 (dd, 1 H, $J_{4'',5''}$ 2, $J_{5'',F\text{-}5''}$ 54 Hz, H-5″). ^{19}F

NMR (pyridine-d_5): δ −115.1 (dt, $J_{4'',F} = J_{F\text{-}5,F\text{-}5''} = 11$ Hz, F-5″) and −183.0 (br d, J 50 Hz, F-5); irradiation of F-5 collapsed the dt of F-5″ to dd (J 11 and 50 Hz). Mass spectrum: m/z 1251 $(M + H)^+$.

5-Epinetilmicin (**13**).—To a solution of **6** (355 mg, 0.29 mmol) in liquid NH_3 (~ 30 mL) at −60°C was added Na (~ 0.4 g) and the deep-blue solution was kept for 2 min. Post-treatment as described for **9** gave **13** as the hemihydrate · hemicarbonate (105 mg, 72%); $R_{f\,\text{netilmicin}}$ 0.85 (TLC, 9:4:1 $CHCl_3$–MeOH–aq 28% NH_3); $[\alpha]_D^{23}$ +138° (*c* 1.2, H_2O). Anal. Calcd for $C_{21}H_{41}N_5O_7 \cdot 0.5H_2O \cdot 0.5H_2CO_3$: C, 51.25; H, 8.40; N, 13.58. Found: C, 50.97; H, 8.16; N, 13.90.

5-Epi-1,3,2′,6′,3″-pentakis(N-*benzyloxycarbonyl*)*netilmicin* (**14**).—A mixture of **13** (110 mg, 0.23 mmol), benzyl chloroformate (0.2 mL, 1.4 mmol), and anhyd Na_2CO_3 (100 mg) in 1:1 1,4-dioxane–H_2O (5 mL) was stirred for 1 h at room temperature. Concentration gave a syrup, that was thoroughly washed with diethyl ether, and dissolved in $CHCl_3$. TLC (10:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) showed a single spot at R_f 0.55. The solution was washed with water, dried ($MgSO_4$), and concentrated to give **14** as a solid (232 mg, 95%); $[\alpha]_D^{26}$ +95° (*c* 1, $CHCl_3$); IR (KBr): 1690 (urethane C=O) and 1530 cm^{-1} (amide II). Anal. Calcd for $C_{61}H_{71}N_5O_{17}$: C, 63.92; H, 6.24; N, 6.11. Found: C, 63.83; H, 6.27; N, 5.88.

3″-N:*4″*-O-*Carbonyl-5-epi-1,3,2′,6′-tetrakis*(N-*benzyloxycarbonyl*)*netilmicin* (**15**).—To a solution of **14** (220 mg, 0.19 mmol) in dry DMF (8 mL) was added 50% (in oil) NaH (80 mg), and the mixture was vigorously stirred for 30 min under an atmosphere of N_2 at room temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.25 (cf. **14**: R_f 0.15). After addition of AcOH (0.2 mL), the solution was concentrated in vacuo, and the residue dissolved in $CHCl_3$ was washed thoroughly with water, dried ($MgSO_4$), and concentrated to give **15** as a solid 170 mg 85%); $[\alpha]_D^{23}$ +77° (*c* 1, $CHCl_3$); IR (KBr): 1760 (cyclic carbamate), 1700 (urethane C=O), and 1530 cm^{-1} (amide II). Anal. Calcd for $C_{54}H_{63}N_5O_{16}$: C, 62.48; H, 6.12; N, 6.75. Found: C, 62.12; H, 6.14; N, 6.52.

2″-O-*Benzoyl-3″*-N : *4″*-O-*carbonyl-5-epi-1,3,2′,6′-tetrakis*(N-*benzyloxycarbonyl*)*netilmicin* (**16**).—To an ice-cold solution of **15** (200 mg, 0.19 mmol) in pyridine (4 mL) was added BzCl (0.1 mL, 0.86 mmol) and the solution was kept for 1 h at room temperature. Addition of water (0.3 mL) followed by work-up as described for **2** gave **16** as a solid (212 mg, 96%); $[\alpha]_D^{26}$ +92° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{61}H_{67}N_5O_{17} \cdot 0.5H_2O$: C, 63.64; H, 6.04; N, 6.08. Found: C, 63.77; H, 5.92; N, 5.70.

2″-O-*Benzoyl-3″*-N : *4″*-O-*carbonyl-5-deoxy-5-fluoro-1,3,2′,6′-tetrakis*(N-*benzyloxycarbonyl*)*netilmicin* (**17**).—To an ice-cold solution of **16** (212 mg, 0.2 mmol) in dry (3 mL) was added DAST (0.09 mL, 0.72 mmol), and the solution was kept for 30 min at room temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.5 (cf. **16**: R_f 0.4). After conventional post-treatment, the product was chromatographed with 100:1 $CHCl_3$–MeOH to give **17** as a solid (180 mg, 85%); $[\alpha]_D^{26}$ +99° (*c* 0.3, $CHCl_3$). Anal. Calcd for $C_{61}H_{40}FN_5O_6 \cdot 0.5H_2O$: C, 63.53; H, 5.82; N, 6.07; F, 1.64. Found: C, 63.57; H, 5.86; N, 5.78; F, 2.01.

2''-O-Benzoyl-5-O-mesyl-1,3,2',6',3''-penta-N-tosylnetilmicin (**18**).—To an ice-cold solution of **3** (105 mg, 0.07 mmol) in $CHCl_3$ (2 mL) was added 28% NaOMe in MeOH (0.1 mL) and the solution was kept for 30 min at the same temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.4 (cf. **3**: R_f 0.65). After neutralization with aq HCl, the solution was washed with water, dried ($MgSO_4$), and concentrated to give **18** as a solid (93.5 mg, 96%; $[\alpha]_D^{20}$ +86° (*c* 1, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 3.55 (s, 3 H, Ms). Anal. Calcd for $C_{64}H_{77}N_5O_{20}S_6$: C, 53.80; H, 5.43; N, 4.90. Found: C, 53.47; H, 5.26; N, 4.77.

2''-O-Benzoyl-5-O-mesyl-6'-N-methyl-1,3,2',6',3''-penta-N-tosylnetilmicin (**19**).—A mixture of **18** (490 mg, 0.34 mmol), MeI (0.4 mL, 6.4 mmol), and Ag_2O (300 mg) in MeCN (10 mL) was stirred for 2 h at room temperature. TLC (35:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.45 (cf. **18**: R_f 0.4). After filtration, the solution was concentrated, and the residue was chromatographed with 100:1 $CHCl_3$–MeOH to give **19** as a solid (430 mg, 87%); $[\alpha]_D^{20}$ +79° (*c* 1.2, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 2.80 (s, 3 H, NCH_3-6') and 3.50 (s, 3 H, Ms). Anal. Calcd for $C_{65}H_{79}N_5O_{20}S_6$: C, 54.11; H, 5.52; N, 4.85. Found: C, 54.43; H, 5.19; N, 4.57.

5-Epi-6'-N-methyl-1,3,2',6',3''-penta-N-tosylnetilmicin (**20**).—A mixture of **19** (163 mg, 0.11 mmol) and anhyd NaOAc (150 mg) in DMF (2.5 mL) was heated for 4 h at 90°C. Concentration gave a residue that was dissolved in $CHCl_3$ (5 mL). The solution was washed with water, and dried ($MgSO_4$) overnight. To the solution was added 28% NaOMe in MeOH (0.3 mL) and the mixture was kept for 1 h at room temperature. TLC (25:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.2. After neutralization with aq HCl, the solution was washed with water, dried ($MgSO_4$), and concentrated to give **20** as a solid (126 mg, 87%); $[\alpha]_D^{22}$ +104° (*c* 1.3, $CHCl_3$); ^1H NMR (pyridine-d_5): δ 2.87 (s, 3 H, NCH_3-6'). Anal. Calcd for $C_{57}H_{73}N_5O_{17}S_5 \cdot H_2O$: C, 53.55; H, 5.91; N, 5.48. Found: C, 53.78; H, 6.11; N, 5.33.

5-Epi-6'-N-methylnetilmicin (**21**).—To a solution of **20** (96.5 mg, 0.08 mmol) in liquid NH_3 (~ 10 mL) at −60°C was added Na (~ 0.1 g) and the deep-blue solution was kept for 2 min. Similar post-treatment as described for **12** gave **21** as a solid hydrate (28.7 mg, 75%); $R_{f\,\text{netilmicin}}$ 1 (TLC, with 9:4:1 $CHCl_3$–MeOH–aq 28% NH_3); $[\alpha]_D^{20}$ +155° (*c* 1.1, H_2O); ^1H NMR (20% ND_3 in D_2O): δ 2.32 (s, 3 H, NCH_3-6'). Anal. Calcd for $C_{22}H_{43}N_5O_7 \cdot H_2O$: C, 52.05; H, 8.94; N, 13.80. Found: C, 52.34; H, 8.76; N, 13.61.

1,3,2',6',3''-Pentakis(N-benzyloxycarbonyl)netilmicin (**22**).—Netilmicin base (420 mg, 0.88 mmol) was treated with benzyl chloroformate (1.0 mL, 7 mmol) as described for **14** to give **22** as a solid (947 mg, 94%); $[\alpha]_D^{24}$ +83° (*c* 0.3, $CHCl_3$). Anal. Calcd for $C_{61}H_{71}N_5O_{17}$: C, 63.92; H, 6.24; N, 6.11. Found: C, 63.74; H, 6.15; N, 6.18.

3'-N : 4''-O-Carbonyl-1,3,2',6'-tetrakis(N-benzyloxycarbonyl)netilmicin (**23**).—A mixture of **22** (1.09 g, 0.95 mmol) and 50% NaH (in oil, 110 mg) in DMF (10 mL) was treated as described for **15** to give **23** as a solid (931 mg, 94%); $[\alpha]_D^{24}$ +75° (*c* 0.3, $CHCl_3$). Anal. Calcd for $C_{54}H_{63}N_5O_{16}$: C, 62.48; H, 6.12; N, 6.75. Found: C, 62.16; H, 5.99; N, 6.56.

2″-O-Benzoyl-3″-N:4″-O-carbonyl-1,3,2′,6′-tetrakis(N-benzyloxycarbonyl)netilmicin (**24**).—Compound **23** (891 mg, 0.8 mmol) was treated with benzoyl chloride (0.5 mL, 4.3 mmol) as described for **16** to give **24** as a solid (902 mg, 92%); $[\alpha]_D^{24}$ +94° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{61}H_{67}N_5O_{17} \cdot 0.5H_2O$: C, 63.64; H, 6.04; N, 6.08. Found: C, 63.61; H, 5.81; N, 6.22.

2″-O-Benzoyl-2″-N:4″-O-carbonyl-5-deoxy-5-oxo-1,3,2′,6′-tetrakis(N-benzyloxycarbonyl)netilmicin (**25**).—To a solution of **24** (641 mg, 0.56 mmol) in dry Me_2SO (1.5 mL) was added Ac_2O (0.5 mL) and the solution was kept for 16 h at room temperature. After addition of $CHCl_3$ (50 mL), the solution was washed with aq $NaHCO_3$ (satd) and dried ($MgSO_4$). TLC (50:1 $CHCl_3$–MeOH) showed a single spot at R_f 0.32 (cf. **24**: R_f 0.25). Concentration gave a residue that was chromatographed (100:1 $CHCl_3$–MeOH) to give **25** as a solid (608 mg, 95%); $[\alpha]_D^{24}$ +101° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{61}H_{65}N_5O_{17}$: C, 64.14; H, 5.74; N, 6.13. Found: C, 63.95; H, 5.50; N, 6.14.

2″-O-Benzoyl-3″-N:4″-O-carbonyl-5-deoxy-5,5-difluoro-1,3,2′,6′-tetrakis(N-benzyloxycarbonyl)netilmicin (**26**).—To an ice-cold solution of **25** (511 mg, 0.45 mmol) in CH_2Cl_2 (8 mL) was added DAST (0.6 mL, 4.8 mmol) and the solution was kept for 6 h at room temperature. TLC (50:1 $CHCl_3$–MeOH) showed a main spot at R_f 0.5. After addition of aq $NaHCO_3$ (satd, 15 mL), the mixture was shaken for 30 min and the organic layer separated and concentrated. The residue was chromatographed with 100:1 $CHCl_3$–MeOH to give **26** as a solid (325 mg, 62%); $[\alpha]_D^{24}$ +90° (*c* 0.3, $CHCl_3$). Anal. Calcd for $C_{61}H_{65}F_2N_5O_{16}$: C, 63.04; H, 5.64; N, 6.03. Found: C, 62.82; H, 5.48; N, 6.10.

5-Deoxy-5,5-difluoronetilmicin (**27**).—To a suspension of **26** (245 mg, 0.21 mmol) in liquid NH_3 (~ 25 mL) at −60°C was added Na (~ 300 mg) and the mixture was stirred for 10 min at the temperature [de(benzyloxycarbonyl)ation]. After gradual addition of MeOH (1 mL), the clear solution was concentrated, and the residue was dissolved in water (5 mL). TLC (9:4:1 $CHCl_3$–MeOH–aq 28% NH_3) showed a single spot at R_f 0.4. The solution was heated for 3 h at 80°. TLC (the same solvent mixture as already described was used) showed a main spot at R_f 0.3. After neutralization with aq HCl, the solution was concentrated, and the residue was packed on a column of CM-Sephadex C-25 (NH_4^+ form, 50 mL). After washing the column with water, the product was eluted with aq NH_3 (0 → 0.2 M). The ninhydrin-positive fractions were collected, and further chromatographed on silica gel with 9:4:1 $CHCl_3$–MeOH–aq 28% NH_3 to give **27** as the solid hemihydrate · hemicarbonate (54.4 mg, 49%); $[\alpha]_D^{24}$ +148° (*c* 1, H_2O); ^1H NMR (20% ND_3 in D_2O; at 500 MHz with a Bruker AM X-500 spectrometer): δ 1.17 (q, 1 H, H-2*ax*), 2.00 (q with small splittings, 1 H, H-3′a), 2.20 (dt, 1 H, H-3′b), 2.23 (dt, 1 H, H-2*eq*), 2.50 (dq, 1 H, $J_{Ha,Hb}$ 10.5, J_{CH,CH_3} 7 Hz, NC*Ha*HbCH_3), 2.72 (dq, 1 H, NCHaC*Hb*CH_3), 2.87 (m, 1 H, H-3), 2.90 (m, 1 H, H-1), 3.04 (ddd, 1 H, H-2′), 3.14 (s, 2 H, H-6′a,6′b), 3.72 (br ddd, 1 H, H-6), 3.90 (br dddd, 1 H, H-4); $J_{1,2ax} = J_{1,6} = J_{2ax,3} = J_{3,4} = 11$, $J_{1,2eq} = J_{2eq,3} = 4$, $J_{2ax,2eq}$ 12.5, $J_{4,F\text{-}5ax} = J_{6,F\text{-}5ax} = \sim 20$, $J_{4,Feq} = \sim 3$, $J_{1',2'}$ 2.5, $J_{2',3'a}$ 6, and $J_{3'a,3'b}$ 16.5 Hz. ^{19}F NMR (20% ND_3 in

D_2O): δ −128.7 (dt, 1 F, F-5*ax*) and −113.5 (br d, 1 F, F-5*eq*); $J_{4,F\text{-}5ax} = J_{6,F\text{-}5ax} =$ 20.5, and $J_{F\text{-}5ax,F\text{-}5eq}$ 245 Hz. Anal. Calcd for $C_{21}H_{39}F_2N_5O_6 \cdot 0.5\,H_2O \cdot 0.5H_2CO_3$: C, 48.21; H, 7.72; F, 7.09; N, 13.08. Found: C, 48.07; H, 7.64; F, 7.30; N, 13.22.

Minimal inhibitory concentration (μg/mL) of netilmicin, 5-deoxy-5-fluoronetilmicin (**9**), *5-epinetilmicin* (**13**), *5-epi-6′-N-methylnetilmicin* (**21**), *and 5-deoxy-5,5-difluoronetilmicin* (**27**).—Performed on Mueller–Hinton agar for 18 h at 37°C. *Staphylococcus aureus* FDA 209P: < 0.2, 0.39, 0.39, 0.78, and 3.12, in the foregoing order; *S. aureus* Smith: < 0.2, < 0.2, < 0.2, 0.39, and 0.78; *S. aureus* Ap 01 [AAD(4′)]: 0.78, 1.56, 0.78, 1.56, and 12.5; *S. aureus* MS 15009 (gentamicin resistant): 6.25, 1.56, 0.39, 6.25, and 3.12; *Micrococcus luteus* FDA 16: 6.25, 3.12, 6.25, 25, and 6.25; *Corynebacterium bovis* 1810: 1.56, 0.39, 1.56, 12.5, and 1.56; *Escherichia coli* N1HJ: < 0.2, < 0.2, < 0.2, 0.78, and < 0.2; *E. coli* K-12: < 0.2, < 0.2, < 0.2, 0.78, and 0.39; *E. coli* K-12 R 5 [ACC(6′)]: 50, 100, 50, 6.25, and > 100; *E. coli* K-12 ML 1629 [APH(3′)-I]: 0.39, 0.39, 0.78, 1.56, and 0.78; *E. coli* K-12 ML 1410 R 81 [APH(3′)-I]: 0.39, 0.39, 0.39, 1.56, and 0.78; *E. coli* W 677: < 0.2, < 0.2, < 0.2, 0.78, and < 0.2; *E. coli* JR 66/W 677 [AAD(2″) and APH(3′)-II]: 0.39, 0.78, 0.39, 3.12, and 0.78; *Klebsiella pneumoniae* PCI 602: 0.39, 0.78, 0.39, 1.56, and 1.56; *Shigella sonnei* JS 11746: 0.39, 0.78, 0.78, 3.12, and 0.78; *Salmonella enteritidis* 1891: 0.39, 0.78, 0.78, 1.56, and 1.56; *Proteus rettgeri* GN 311: 0.39, 0.78, 0.78, a.56, and 1.56; *Serratia marcescens*: 6.25, 12.5, 1.56, 3.12, and 6.25; *Providencia* sp. Pv16 [AAC(2′)]: 6.25, 6.25, 0.78, 6.25, and 12.5; *Pseudomonas aeruginosa* A 3: < 0.2, 0.39, < 0.2, < 0.2, and 1.56; *P. aeruginosa* H 9 [APH(3′)-II]: 3.12, 6.25, 1.56, 6.25, and 12.5.

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