CHEMICAL MODIFICATION OF FORTIMICIN A

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Chlorination of antibiotic fortimicin A with triphenylphosphine and carbon tetrachloride has been attempted, and 2-chloro-, 2,5-dichloro-, and 2-chloro-4-ene derivatives have been obtained. Successive dehalogenation of the chlorinated fortimicins A with tributylstannane gave the corresponding deoxyfortimicins A. Among five deoxyfortimicins A, 2-deoxyfortimicin A exhibits improved antimicrobial activity, compared to the parent fortimicin A.

Aminocyclitol antibiotics fortimicin A and fortimicin B were found in a fermentation broth of *Micromonospora olivoasterospora*.[1] Their structures have been established as a pseudodisaccharide composed of a diamino-sugar (6-*epi*-purpurosamine B) and a 1,4-diaminocyclitol (fortamine). Fortimicin A (**1**) differs from fortimicin B (**2**) only by an existence of a glycyl group on the methylamino group in the fortamine moiety.[2]

Subsequently, a number of fortimicin analogs have been discovered in the fermentation broth as a by-product, and these are fortimicins C, D, KE[3] and E.[4] Also, other strains of *Streptomyces* produced fortimicin analogs, such as sporaricins A (**3**), B (**4**),[5,6,7] istamycins A (**5**), B (**6**),[8] sannamycin B (**7**),[9] SF-2052 (**8**),[10] and SF-1854 (**9**).[11]

A common structural feature of fortimicin family members is a pseudodisaccharide consisting

Fig. 1—(1).

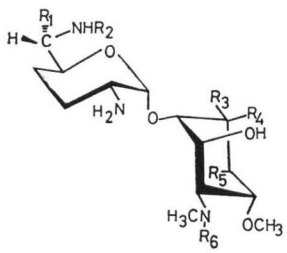
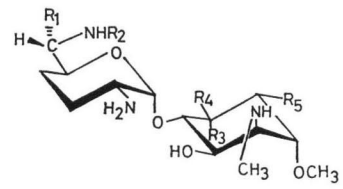


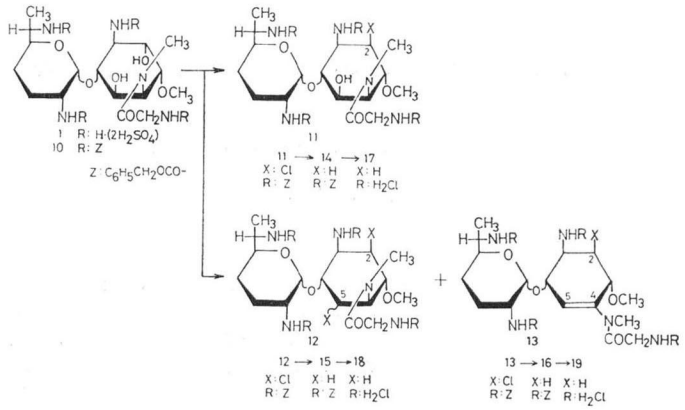
Fig. 1—(2).



	R_1	R_2	R_3	R_4	R_5	R_6
Fortimicin A **1**	CH_3	H	H	NH_2	OH	$COCH_2NH_2$
Sporaricin A **3**	CH_3	H	NH_2	H	H	$COCH_2NH_2$
Istamycin A **5** (Sannamycin A)	H	CH_3	H	NH_2	H	$COCH_2NH_2$
Istamycin B **6**	H	CH_3	NH_2	H	H	$COCH_2NH_2$
SF-2052 **8**	CH_3	H	H	NH_2	OH	$COCH_2NH$-CH=NH
SF-1854 **9**	CH_3	H	H	NH_2	OH	$COCH_2NH$-CHO

	R_1	R_2	R_3	R_4	R_5
Fortimicin B **2**	CH_3	H	H	NH_2	OH
Sporaricin B **4**	CH_3	H	NH_2	H	H
Sannamycin B **7**	H	CH_3	H	NH_2	H

Scheme 1.



of a purpurosamine sugar attached to the O-6 of an inosadiamine-1,4 derivative *via* α-D-glycosidic linkage. A relationship between structure of the antibiotic and the antimicrobial activity known at the present time is as follows. (1) An introduction of an aminoacyl group or an aminoalkyl group, especially a glycyl group into a methylamino group at the C-4 position greatly enhances the antimicrobial activity.[12,23] (2) A configurational inversion of the amino group at the C-1 is not detrimental for the activity. (3) Deoxygenation of the hydroxyl group on the C-2 is not injurious to the activity.

To establish a detailed relationship between a variation of the structure of the aminocyclitol moiety and the antimicrobial activity of the corresponding antibiotic, chemical modifications of fortimicin A have been attempted.

Table 1. Assignment of ^{13}C NMR spectrum of **17**, **18**, **19**, **20** and **21**.

	Fortimicin A **1**			**17**			**18**			**19**			**20**			**21**		
	sulfate	free base	Δ	pD 3.0	pD 12.5	Δ	pD 3.5	pD 12.5	Δ	pD 3.4	pD 13.0	Δ	pD 4.0	pD 12.0	Δ	pD 4.0	pD 12.0	Δ
C-1′	95.4	100.1	4.7	95.3	100.4	5.1	93.9	98.7	4.8	91.3	97.8	6.5	90.3	96.3	6.0	95.2	100.4	5.2
C-2′	51.7	50.5		50.8	50.5		51.0	50.8		49.1	50.2		50.4	50.1		51.6	50.4	
C-3′	21.6	26.9	5.3	21.6	27.0	5.4	21.7	27.1	5.4	21.4	27.2	5.8	21.6	27.0	5.4	21.5	26.9	5.4
C-4′	26.3	27.3		26.4	27.4		26.4	27.4		26.2	27.0		26.2	27.4		26.4	27.4	
C-5′	70.9	74.9	4.0	70.8	75.1	4.3	70.7	75.0	4.3	70.8	75.0	4.2	70.7	75.0	4.3	71.0	75.4	4.4
C-6′	49.4	50.1		49.5	50.3		49.4	50.3		48.5	47.6		49.0	49.5		49.4	50.2	
6′-CH_3	15.0	18.4	3.4	15.3	18.6	3.3	15.3	18.5	3.2	15.3	18.7	3.4	15.3	18.6	3.3	15.4	18.7	3.3
C-1	54.1	52.5		51.7	51.3		51.7	53.6		51.8	50.5		51.8	50.5		55.4	57.3	
C-2	66.3	71.1	4.8	29.2	33.1	3.9	29.0	32.3	3.3	28.9	32.5	3.6	27.9	33.6	5.7	59.9	64.5	4.6
C-3	72.4	72.9		71.3	73.8		71.6	74.2		71.4	75.0		77.7	79.7		75.3	75.0	
C-4	51.8	55.4		56.7	55.8		53.0	56.2		141.0	140.3		53.5	54.1		56.4	57.8	
C-5	71.6	73.7		69.8	71.0		27.0	27.4		127.5	129.5		26.9	28.0		70.8	73.3	
C-6	74.5	78.4	3.9	73.3	77.6	4.3	72.9	76.2	3.3	73.7	77.9	4.2	72.9	78.8	5.9	73.4	76.8	3.4
O-CH_3	55.8	56.4		56.7	57.6		57.0	57.4		58.9	58.6		57.8	57.8		59.1	57.8	
N-CH_3	32.0	32.2		32.0	32.1		29.6	29.3		35.6	35.5		31.9	31.5		32.6	32.6	
Gly-CH_2	41.3	43.3		41.3	43.3		41.3	43.3		41.2	43.0		41.4	43.3		41.4	43.4	
Gly-CO	168.8	176.2		168.5	176.3		168.4	176.0		167.5	175.8		167.8	175.6		169.0	176.6	

When 1,4,2′,6′-tetra-N-benzyloxycarbonylfortimicin A[13] (**10**) was treated with triphenylphosphine in carbon tetrachloride and acetonitrile[14] at 50°C for 72 hours, a chloro-deoxy derivative (**11**) was obtained in 23% yield by a chromatographic fractionation as a single product.

The same chlorination under a more drastic condition (80~85°C for 24 hours) afforded a dichlorodideoxy derivative (**12**) and another chloro-deoxy derivative (**13**) in 13% and 17% yield, respectively. Structures of **11**, **12** and **13** were deduced from structures of successive reaction products.

Dechlorination of **11**, **12** and **13** with tributylstannane in the presence of α,α'-azo-bis-isobutylonitrile gave corresponding deoxy derivatives, **14**, **15** and **16** in 73%, 82% and 86% yield, respectively.

Catalytic hydrogenolysis of **14**, **15** and **16** in ethanol containing 0.1 M HCl in the presence of palladium on carbon afforded deoxyfortimicins A tetrahydrochloride, **17**,[15] **18** and **19**, in almost quantitative yields. Structures of **17**, **18** and **19** were established by mass, PMR, CMR spectra and elemental analyses.

Mass spectra of **17**, **18** and **19** revealed the corresponding molecular ions (M^+) and characteristic fragmentation patterns of 6-*epi*-purpurosamine B at *m/z* 143, 2-deoxy-4-N-glycylfortamine at *m/z* 230, 2,5-dideoxy-4-N-glycylfortamine at *m/z* 214 and 2,5-dideoxy-4-ene-4-N-glycylfortamine at *m/z* 212.

^{13}C NMR spectrum (Table 1) of **17** revealed a disappearance of one methine carbon signal at δ 66.3, which had been observed in the spectrum of fortimicin A as the signal of C-2,[2] and an appearance of a new methylene carbon signal at δ 29.2, which showed a large upfield shift by the N-protonation effect.[16,17] Since the effect of N-protonation appears strongly on a β-carbon and weakly on a γ-carbon,[17] the deoxygenation must occur on the C-2 of the fortamine moiety.

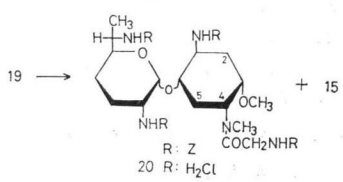
Furthermore, this has been demonstrated by ^1H NMR spectroscopy as follows. ^1H NMR spectrum of **17** showed a same signal pattern (a double doublet) of H-4 ($J_{3,4}=11.1$ Hz, $J_{4,5}=2.3$ Hz) as that of H-4 of fortimicin A ($J_{3,4}=10.0$ Hz, $J_{4,5}=2.0$ Hz).[2] Therefore, a hydroxyl group on C-5 is not deoxygenated and a structure of **17** is assigned as 2-deoxyfortimicin A.

Structure of **18** has been established by ^{13}C NMR spectroscopy. The spectrum of **18** revealed four methylene carbon signals at δ 21.7, 26.4, 27.0 and 29.2 ppm. This fact indicated that two hydroxyl groups have been deoxygenated, and **18** is assigned as 2,5-dideoxyfortimicin A.

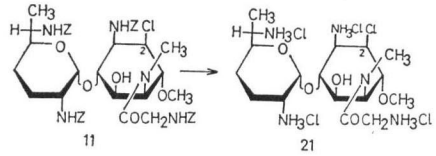
Structure of **19** has been established by ^{13}C and ^1H NMR spectroscopies as follows. The ^{13}C NMR spectrum of **19** showed newly introduced one methylene carbon signal at δ 32.5 ppm and two double-bonded carbons at δ 129.5 and 140.3 ppm. The signal at δ 32.5 ppm is assigned as that of C-2 by the position of the chemical shift value, and this has been supported by a large upfield shift by a N-protonation effect.[16,17] A position of the double bond in **19** has been determined by ^1H NMR spectroscopy as follows. In the ^1H NMR spectrum of **19**, one vinyl proton was observed at δ 6.01, and a disappearance of a H-4 on fortimicin A suggested an existence of the double bond between C-4 and 5. Therefore, **19** is assigned as 2,5-dideoxyfortimicin A 4-ene.

Catalytic hydrogenation of **19** in the presence of platinum oxide in an acidic ethanol solution gave a mixture of two products. After converting to tetra-N-benzyloxycarbonyl derivatives, the mixture was isolated by a column chromatography. One of the two products was identical with **15**, and another component was the corresponding 2,5-dideoxy-4-*epi*-fortimicin A deri-

Scheme 2.



Scheme 3.



11 21

vative. Catalytic hydrogenolysis of the latter compound afforded 2,5-dideoxy-4-*epi*-fortimicin A (**20**).

The structure of **20** has been established by ^{13}C, ^{1}H, and mass spectroscopies. That is, ^{13}C NMR spectrum of **20** revealed two newly introduced methylene carbon signals at δ 26.9 and 27.9 ppm. In the ^{1}H NMR spectrum, a H-4 signal appeared at δ 4.45, indicating an axial conformation of the H-4 proton.

The fifth fortimicin A analog, 2-chloro-2-deoxy-2-*epi*-fortimicin A tetrahydrochloride (**21**), was prepared by catalytic hydrogenolysis of **11** in the presence of palladium on carbon in an ethanol solution containing hydrochloric acid. The structure of **21** was established analogously as described above

Table 2. Antibacterial spectra (MIC mcg/ml) of **17**.

Test organisms	Marker	**17**	FM-A H_2SO_4*
Staphylococcus aureus FDA 209P	G (+)	0.39	0.78
Staphylococcus aureus Smith	〃	0.2	0.78
Staphylococcus epidermidis	〃	0.2	0.78
Streptococcus faecalis ATCC 10541	〃	25	50
Sarcina lutea PCI 1001	〃	0.78	1.56
Bacillus subtilis ATCC 6633	〃	0.2	0.39
Escherichia coli NIHJC-2	G (−)	1.56	6.25
Escherichia coli GN 2411-5	〃	3.13	6.25
Klebsiella pneumoniae 8045	〃	0.2	0.39
Klebsiella pneumoniae KY 4274	〃	1.56	3.13
Pseudomonas aeruginosa #1	〃	6.25	25
Pseudomonas aeruginosa 145	〃	50	50
Pseudomonas cepacia ATCC 25610	〃	6.25	6.25
Serratia marcescens T-55	〃	3.13	3.13
Proteus mirabilis 1287	〃	6.25	6.25
Proteus vulgaris ATCC 6897	〃	6.25	12.5
Proteus inconstans 39	〃	3.13	12.5
Escherichia coli KY 8349	APH (3′)-I	0.87	1.56
Pseudomonas aeruginosa KY Z 445	AAC (6′)-3	25	25
Pseudomonas aeruginosa KY 8510	AAC (6′)-4	25	50
Serratia marcescens 1065	〃	3.13	6.25
Providencia 164	AAC (2′)	6.25	6.25
Escherichia coli KY 8348	AAC (3)-I	>100	>100
Pseudomonas aeruginosa KY 8563	AAC (3)-II	100	100
Pseudomonas aeruginosa KY 8565	AAC (3)-III	>100	>100
Escherichia coli 57 R/W 677	AAD (2″)	12.5	25
Staphylococcus aureus KY 8970	AAD (4′)	0.39	0.78

* Fortimicin A sulfate.

Table 3. Antibacterial spectra (MIC mcg/ml) of **18**.

Test organisms	Marker	**18**	FM-A H_2SO_4*
Staphylococcus aureus FDA 209P	G(+)	100	0.39
Staphylococcus aureus Smith	〃	25	0.39
Staphylococcus epidermidis	〃	25	0.39
Streptococcus faecalis ATCC 10541	〃	>100	100
Sarcina lutea PCI 1001	〃	>100	0.78
Bacillus subtilis ATCC 6633	〃	6.25	0.39
Escherichia coli NIHJC-2	G(−)	>100	3.13
Escherichia coli GN 2411-5	〃	>100	3.13
Klebsiella pneumoniae 8045	〃	12.5	0.1
Klebsiella pneumoniae KY 4274	〃	100	1.56
Pseudomonas aeruginosa #1	〃	>100	6.25
Pseudomonas aeruginosa 145	〃	>100	25
Pseudomonas putida 264	〃	>100	6.25
Serratia marcescens T-55	〃	>100	3.13
Proteus mirabilis 1287	〃	>100	6.25
Proteus vulgaris ATCC 6897	〃	>100	6.25
Proteus inconstans 39	〃	>100	6.25
Escherichia coli KY 8349	APH (3′)-I	50	3.13
Pseudomonas aeruginosa KY Z 445	AAC (6′)-3	>100	25
Pseudomonas aeruginosa KY 8510	AAC (6′)-4	>100	25
Serratia marcescens 1065	〃	>100	3.13
Providencia 164	AAC (2′)	>100	3.13
Escherichia coli KY 8348	AAC (3)-I	>100	>100
Pseudomonas aeruginosa KY 8563	AAC (3)-II	>100	50
Pseudomonas aeruginosa KY 8565	AAC (3)-III	>100	100
Escherichia coli 57 R/W 677	AAD (2″)	>100	12.5
Staphylococcus aureus KY 8970	AAD (4′)	50	0.39

* Fortimicin A sulfate.

Table 4. Antibacterial spectra (MIC mcg/ml) of **19**.

Test organisms	**19**	FM-A H_2SO_4*
Staphylococcus aureus ATCC 6838P	40	0.08
Escherichia coli ATCC 26	<40	0.63
Bacillus subtilis	40	>0.02
Proteus vulgaris ATCC 6897	<40	1.25
Shigella sonnei ATCC 9290	<40	2.5
Salmonella typhosa ATCC 9992	<40	0.16
Klebsiella pneumoniae ATCC 10031	<40	0.08

* Fortimicin A sulfate.

Table 5. Antibacterial spectra (MIC mcg/ml) of **20**.

Test organisms	**20**	FM-A H_2SO_4*
Staphylococcus aureus ATCC 6838P	20	0.16
Escherichia coli ATCC 26	—	0.63
Bacillus subtilis	10	0.08
Proteus vulgaris ATCC 6897	—	1.25
Shigella sonnei ATCC 9290	—	2.5
Salmonella typhosa ATCC 9992	>40	0.32
Klebsiella pneumoniae ATCC 10031	40	0.16

* Fortimicin A sulfate.

by spectroscopy. Mass spectrum of **21** showed a characteristic fragmentation pattern of a chlorine containing compound: a molecular ion peak appeared at *m/z* 425 and 423, a chloro-deoxy-4-N-glycyl-fortamine at *m/z* 264 and a 6-*epi*-purpurosamine B at *m/z* 143. The ^{13}C NMR spectrum revealed a newly

Table 6. Antibacterial spectra (MIC mcg/ml) of **21**.

Test organisms	Marker	**21**	FM-A H_2SO_4*
Staphylococcus aureus FDA 209P	G (+)	6.25	0.78
Staphylococcus aureus Smith	〃	1.56	0.39
Staphylococcus epidermidis	〃	0.78	0.39
Streptococcus faecalis ATCC 10541	〃	50	50
Sarcina lutea ATCC 9341	〃	12.5	1.56
Bacillus subtilis ATCC 6633	〃	0.78	0.39
Escherichia coli NIHJC-2	G (−)	6.25	3.13
Escherichia coli GN 2411-5	〃	6.25	3.13
Klebsiella pneumoniae 8045	〃	1.56	0.2
Klebsiella pneumoniae KY 4274	〃	12.5	1.56
Pseudomonas aeruginosa #1	〃	25	3.13
Pseudomonas aeruginosa 145	〃	100	12.5
Pseudomonas putida 264	〃	12.5	12.5
Serratia marcescens T-55	〃	6.25	1.56
Proteus mirabilis 1287	〃	25	12.5
Proteus vulgaris ATCC 6897	〃	12.5	6.25
Proteus inconstans 39	〃	12.5	6.25
Escherichia coli KY 8349	APH (3′)-1	1.56	1.56
Pseudomonas aeruginosa KY Z 445	AAC (6′)-3	100	25
Pseudomonas aeruginosa KY 8510	AAC (6′)-4	100	25
Serratia marcescens 1065	〃	6.25	3.13
Providencia 164	AAC (2′)	25	6.25
Escherichia coli KY 8348	AAC (3)-I	25	>100
Pseudomonas aeruginosa KY 8563	AAC (3)-II	>100	50
Pseudomonas aeruginosa KY 8565	AAC (3)-III	>100	>100
Escherichia coli 57 R/W 677	AAD (2″)	12.5	25
Staphylococcus aureus KY 8970	AAD (4′)	1.56	0.39

* Fortimicin A sulfate.

introduced methine carbon at δ 64.5 ppm, showing a large upfield shift (4.6 ppm) by a N-protonation effect,[17] and the signal was reasonably ascribed to C-2. In the ^{1}H NMR spectrum, a signal of H-2 appeared at δ 4.57 as a double doublet ($J_{1,2}$=3.5 Hz, $J_{2,3}$=9.9 Hz), indicating an axial conformation of H-2. Therefore, the structure of **21** was confirmed.

Antimicrobial activities of **17**, **18**, **19**, **20** and **21** were determined against several microorganisms by the known procedure,[18] and the results are listed on Tables 2~6. Compound **17** exhibits an improved activity against a number of Gram-positive and- negative bacteria, compared with that of the parent fortimicin A. Compounds **18**, **19** and **20** showed a marked decrease of activity against all the microorganisms tested. Compound **21** exhibits a slight decrease of activity against most of the bacteria tested and a slight increase of activity against resistant strains, *Escherichia coli* KY 8348 and 57R/W677.

In addition to the structure activity relationships between fortimicins as mentioned above, the following two points can be added: (1) Deoxygenation of the hydroxyl group on C-5 results in a marked decrease of activity. (2) Displacement of the hydroxyl group on C-2 by a chlorine atom with an inversion of the configuration is not seriously detrimental to activity.

Experimental

General

Melting points were measured on a Mitamura Riken micro mp apparatus and are uncorrected. Optical rotations were determined with a Hitachi model 225 polarimeter. ^{1}H NMR spectra were determined on Varian EM 360 A (60 MHz), Varian EM 390 (90 MHz) and JEOL FX-100 (100 MHz) spectrometers with TMS ($CDCl_3$) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal standards. Chemical shifts are given on the ppm from TMS. Mass spectra were measured on JEOL JMS-01SG-2. Column chromatography was performed on Wako gel C-300 and TLC plates were Merck silica gel F_{254}, and spots were detected by spraying with 10% sulfuric acid and/or ninhydrin (0.25% pyridine solution).

1,2′,6′-Tris(N-benzyloxycarbonyl)-4-N-(2-N-benzyloxycarbonylglycyl)fortimicin B; tetrakis(N-benzyloxycarbonyl)fortimicin A (**10**)

To a suspension of fortimicin A sulfate (commercially 539 μg/mg potency, 9.30 g) in 150 ml of aqueous acetone (1/1 v/v%) containing of 7.84 g of anhydrous sodium carbonate, 13 ml of benzyloxycarbonyl chloride was added under ice cooling with mechanical stirring. The mixture was cooled at 0°C for 1 hour, then at room temperature for 1.5 hours. A disappearance of the starting material (Rf 0.15 on TLC in 28% ammonia - chloroform - IPA, 1 : 1 : 4 solvent system) and a formation of the desired product (Rf 0.20 on TLC in 2-butanone - toluene, 1 : 1) were confirmed. The organic solvent was removed by evaporation, and the residual aqueous suspension was extracted with chloroform (130 ml; 50 ml×1, 40 ml×1, 20 ml×2) and evaporated to afford a pale yellow oily product, which was purified by silica-gel column chromatography (Wako gel C-300 250 g, 2-butanone - toluene, 1 : 1). Fractions showing Rf 0.20 on TLC (2-butanone - toluene, 1 : 1) were combined and evaporated to afford **10** as a white solid (15.3 g, quantitative yield). Mp 87~88°C. $[\alpha]_D^{20}$ +52.3° (*c* 1.0, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.15 (m, 3H, CH_3-6′), 1.43~2.20 (m, 4H, CH_2-3′,4′), 2.69~3.13 (m, 3H, N-CH_3), 3.30 (s, 3H, O-CH_3), 4.80 (d, 1H, J=3.0 Hz, H-1′), 5.07 (s, 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.34 (s, 20H, 4×$CO_2$-$CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{59}N_5O_{14}$: C, 62.48; H, 6.31; N, 7.43%
Found: C, 62.25; H, 6.34; N, 7.51%

1,2′,6′-Tris(N-benzyloxycarbonyl)-4-N-(2-N-benzyloxycarbonylglycyl)-2-chloro-2-deoxyfortimicin B; tetrakis(N-benzyloxycarbonyl)-2-chloro-2-deoxyfortimicin A (**11**)

Compound **10** (1.00 g, 1.06 mmol) was dissolved in a mixture of dry carbon tetrachloride (9 ml) and dry acetonitrile (3 ml), then 0.278 g (1.06 mmol) of triphenylphosphine was added. The reaction was carried out at 50°C under stirring for 72 hours with further addition of each 0.15 g of triphenylphosphine after 2.5 and 24 hours. TLC (2-butanone - toluene, 1 : 1) showed a disappearance of the starting material (Rf 0.20) and a formation of several compounds (Rf 0.32, 0.35, 0.41, 0.46, 0.53, 0.62 and 0.65). The mixture was evaporated to afford an oily residue which was purified a repeated (3 times) silica gel column chromatography. (1) Wako gel C-300 100 g, 2-butanone - toluene, 1 : 1. Fractions showing Rf 0.41~0.78 on the same solvent were combined and evaporated to afford 461 mg of an oil. (2) Wako gel C-300 40 g, 2-butanone - toluene, 1 : 2. Fractions showing Rf 0.41~0.62 (2-butanone - toluene, 1 : 2) were combined and evaporated to afford 358 mg of an oil. (3) Wako gel C-300 25 g, 2-butanone - toluene, 2 : 5. Fractions showing Rf 0.46 (2-butanone - toluene, 2 : 5) were combined and evaporated to afford 231 mg (23%) of **11** as a colorless oil. Analytical sample was obtained by solidification from *n*-hexane, mp 96~97°C. $[\alpha]_D^{20}$ +40.1° (*c* 1.0, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.16 (m, 3H, CH_3-6′), 1.43~2.00 (m, 4H, CH_2-3′,4′), 2.85 (s, 3H, N-CH_3), 3.45 (s, 3H, O-CH_3), 4.82 (d, 1H, J=3.0 Hz, H-1′), 5.06, 5.10 (each s, total 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.30, 7.32, 7.34 (each s, total 20H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{55}N_5O_{13}Cl$: C, 61.28; H, 6.09; N, 7.29; Cl, 3.69%
Found: C, 61.53; H, 6.14; N, 7.04; Cl, 3.35%

1,2′,6′-Tris(N-benzyloxycarbonyl)-4-N-(2-N-benzyloxycarbonylglycyl)-2,5-dichloro-2,5-dideoxyfortimicin B; tetrakis(N-benzyloxycarbonyl)-2,5-dichloro-2,5-dideoxyfortimicin A (**12**) and 1,2′,6′-tris(N-benzyloxycarbonyl)-4-N-(2-N-benzyloxycarbonylglycyl)-2-chloro-2,5-dideoxyfortimicin B-4-ene;

tetrakis(N-benzyloxycarbonyl)-2-chloro-2,5-dideoxyfortimicin A-4-ene (**13**)

To a solution of **10** (3.00 g, 3.18 mmol) in a mixture of dry carbon tetrachloride (26 ml) and dry acetonitrile (10 ml), 3.35 g (12.8 mmol) of triphenylphosphine was added. The mixture was heated under reflux for 24 hours with stirring. TLC (2-butanone - toluene, 1: 1) showed a disappearance of **10** (Rf 0.20) and a formation of three products (Rf 0.46, 0.54 and 0.62). The mixture was then evaporated to afford an oily residue which was purified by silica gel column chromatography (Wako gel C-300 250 g, 2-butanone - toluene, 1: 1). Fractions possessing Rf 0.62 (the same solvent) were combined and evaporated to afford 405 mg (13.0%) of **12** as an oil which was solidified from *n*-hexane for analytical sample, mp 91~92°C. $[\alpha]_D^{22.5}$ +22.9° (*c* 1.02, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.22 (m, 3H, CH_3-6′), 1.44~2.50 (m, 4H, CH_2-3′,4′), 2.90 (broad d, 3H, N-CH_3), 3.44 (s, 3H, O-CH_3), 5.07, 5.11 (each s, total 8H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{57}N_5O_{12}Cl_2$: C, 60.12; H, 5.87; N, 7.15; Cl, 7.24%
Found: C, 60.33; H, 5.87; N, 6.97; Cl, 7.43%

Fractions possessing Rf 0.54 were combined and evaporated to afford 499 mg (16.7%) of **13** as an oil which was solidified from *n*-hexane for analytical sample, mp 83~84°C. $[\alpha]_D^{24.5}$ +44.1° (*c* 1.04, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.00 (d, 3H, *J*=6.5 Hz, CH_3-6′), 1.27~1.91 (m, 4H, CH_2-3′,4′), 3.01 (s, 3H, N-CH_3), 3.41 (s, 3H, O-CH_3), 4.82 (d, 1H, *J*=3.8 Hz, H-1′), 5.00, 5.06, 5.08 and 5.14 (each s, total 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.30 and 7.33 (each s, total 20H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{56}N_5O_{12}Cl\cdot H_2O$: C, 61.28; H, 6.09; N, 7.29; Cl, 3.69%
Found: C, 61.56; H, 5.97; N, 7.20; Cl, 3.55%

Dechlorination of **11**, **12** and **13**. 1,2′,6′-Tris(N-benzyloxycarbonyl)-4-(2-N-benzyloxycarbonylglycyl)-2-deoxyfortimicin B; tetrakis(N-benzyloxycarbonyl)-2-deoxyfortimicin A (**14**), 1,2′,6′-tris(N-benzyloxycarbonyl)-4-(2-N-benzyloxycarbonylglycyl)-2,5-dideoxyfortimicin B; tetrakis(N-benzyloxycarbonyl)-2,5-dideoxyfortimicin A (**15**) and 1,2′,6′-tris(N-benzyloxycarbonyl)-4-(N-benzyloxycarbonylglycyl)-2,5-dideoxyfortimicin B-4-ene; tetrakis(N-benzyloxycarbonyl)-2,5-dideoxyfortimicin A-4-ene (**16**)

To a solution of **11** (164 mg, 0.171 mmol) in 1,4-dioxane (7 ml), tributylstannane (1 ml) and α,α'-azo-bis-isobutylonitrile (5 mg) were added. The mixture was heated under reflux with stirring in nitrogen flow for 2 hours. TLC (2-butanone - toluene, 1: 1) showed a disappearance of **11** (Rf 0.46) and a formation of a single product (Rf 0.39). The mixture was evaporated and the residue was washed with *n*-hexane, then purified by silica gel column chromatography (Wako gel C-300 20 g, 2-butanone - toluene, 1: 1). Fractions possessing Rf 0.39 (the same solvent) were combined and evaporated to afford 115.4 mg (73.0%) of **14** as a colorless oil which solidified from *n*-hexane for analytical sample, mp 76~77°C. $[\alpha]_D^{20}$ +56.2° (*c* 1.0, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.18 (m, 3H, CH_3-6′), 1.38~2.62 (m, 6H, CH_2-2,3′,4′), 2.87 (s, 3H, N-CH_3), 3.26 (s, 3H, O-CH_3), 4.81 (d, 1H, *J*=3.6 Hz, H-1′), 5.05 (s, 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.25 (s, 20H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{59}N_5O_{13}$: C, 63.56; H, 6.42; N, 7.56%
Found: C, 63.50; H, 6.58; N, 7.40%

Under the same procedure, **12** (251 mg, 0.256 mmol) was converted into 192.2 mg (82.4% as a colorless oil) of **15** which was solidified from *n*-hexane for analytical sample, mp 75~76°C. Rf 0.36 (2-butanone - toluene, 1: 1). $[\alpha]_D^{20}$ +43.9° (*c* 1.05, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.17 (d, 3H, *J*=7.1 Hz, CH_3-6′), 1.37~2.55 (m, 8H, CH_2-2,5,3′,4′), 2.72 and 2.78 (each s, total 3H, N-CH_3), 3.23 (s, 3H, O-CH_3), 5.00 and 5.09 (each s, total 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.29 (s, 20H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{59}N_5O_{12}$: C, 64.67; H, 6.53; N, 7.70%
Found: C, 64.57; H, 6.57; N, 7.48%

Compound **13** (201.2 mg, 0.214 mmol) was converted into 166.2 mg (85.8% as a colorless oil) of **16** which was solidified from *n*-hexane for analytical sample, mp 67~68°C. Rf 0.41 (2-butanone - toluene, 1: 1). $[\alpha]_D^{19.5}$ +47.5° (*c* 0.97, MeOH). ^{1}H NMR ($CDCl_3$): δ 0.98 (d, 3H, *J*=6.2 Hz, CH_3-6′), 1.22~2.53 (m, 6H, CH_2-2,3′,4′), 2.97 (s, 3H, N-CH_3), 3.28 (s, 3H, O-CH_3), 4.78 (m, 1H, H-1′), 5.00, 5.06 and 5.09 (each s, total 8H, 4×$CO_2\underline{CH_2}C_6H_5$), 7.29 and 7.32 (each s, total 20H, 4×$CO_2CH_2\underline{C_6H_5}$).

Anal. Calcd. for $C_{49}H_{57}N_5O_{12}\cdot H_2O$: C, 63.56; H, 6.42; N, 7.56%

Found: C, 63.85; H, 6.28; N, 7.26%

Hydrogenolysis of **14**, **15** and **16**. 2-Deoxyfortimicin A tetrahydrochloride (**17**), 2,5-dideoxyfortimicin A tetrahydrochloride (**18**) and 2,5-dideoxyfortimicin A-4-ene tetrahydrochloride (**19**)

To a solution of **14** (100 mg, 0.108 mmol) in ethanol (10 ml), a mixture of 0.1 N hydrochloric acid (6 ml) and water (4 ml) was added. The mixture was hydrogenated in the presence of 10% palladium on carbon (20 mg) under 50 psi initial hydrogen atmosphere for 15 hours. TLC (2-butanone - toluene, 1: 1) showed a disappearance of **14** (Rf 0.39) and a formation of a single product (Rf 0.22, ammonia-water - chloroform - IPA, 1: 1: 4). After removal of the catalyst, the filtrate was evaporated (and coevaporated with MeOH) to afford a white solid which was purified by passing through Amberlite IRA-400 (OH^-) (5 ml) column. A ninhydrin positive aqueous eluate was acidified (pH 4) by addition of 0.1 N hydrochrolic acid, and evaporated to afford 60.3 mg (quantitative yield) of **17** as a white hygroscopic solid. $[\alpha]_D^{23}+67.0°$ (*c* 1.08, H_2O). ^1H NMR (D_2O, pD 3.0): δ 1.33 (d, 3H, J=6.6 Hz, CH_3-6′), 1.4~2.8 (m, 6H, CH_2-2,3′,4′), 3.13 (s, 3H, N-CH_3), 3.45 (s, 3H, O-CH_3), 4.06 (s, 2H, glycyl-CH_2), 4.57 (dd, 1H, $J_{3,4}$=11.1 Hz, $J_{4,5}$=2.3 Hz, H-4), 5.35 (d, 1H, J=2.4 Hz, H-1′). ^1H NMR (D_2O pD 12.5): δ 1.02 (d, 3H, J=6.6 Hz, CH_3-6′), 1.1~2.5 (m, 6H, CH_2-2,3′,4′), 3.07 (s, 3H, N-CH_3), 3.39 (s, 3H, O-CH_3), 3.52 (s, 2H, glycyl-CH_2), 4.59 (dd, 1H, $J_{3,4}$=11.4 Hz, $J_{4,5}$=2.8 Hz, H-4), 4.86 (d, 1H, J=2.9 Hz, H-1′). Mass: *m/z* 390 (M^++1, 2.0%), 389 (M^+, 2.0%), 230 ($C_{10}H_{20}N_3O_3$, 2-deoxy-4-N-glycylfortamine, 34.4%), 143 ($C_7H_{15}N_2O$, 6-*epi*-purpurosamine B, 100%). ^{13}C NMR: see Table 1.

Under the same procedure, **15** (52.0 mg, 0.057 mmol) was converted into 31.1 mg (quantitative yield) of **18** as a white hygroscopic solid, Rf 0.22 (ammonia-water - chloroform - IPA, 1: 1: 4). $[\alpha]_D^{21}+75.1°$ (*c* 0.87, H_2O). ^1H NMR (D_2O, pD 1.5): δ 1.33 (d, 3H, J=6.8 Hz, CH_3-6′), 1.4~2.8 (m, 8H, CH_2-2,5,3′,4′), 2.98 (s, 3H, N-CH_3), 3.39 (s, 3H, O-CH_3), 5.34 (d, 1H, J=3.4 Hz, H-1′). ^1H NMR (D_2O, pD 12.5): δ 1.01 (d, 3H, J=6.6 Hz, CH_3-6′), 1.1~2.4 (m, 8H, CH_2-2,5,3′,4′), 2.90 (s, 3H, N-CH_3), 3.33 (s, 3H, O-CH_3), 4.6 (m, 1H, H-4), 4.86 (d, 1H, J=3.4 Hz, H-1′). Mass: *m/z* 373 (M^+, 2.0%), 214 ($C_{10}H_{20}N_3O_2$, 2,5-dideoxy-4-N-glycylfortamine, 28.5%), 143 ($C_7H_{15}N_2O$, 6-*epi*-purpurosamine, 100%). ^{13}C NMR: see Table 1.

Compound **16** (70.0 mg, 0.077 mmol) was converted into 43.6 mg (quantitative yield) of **19** as a white hygroscopic solid, Rf 0.25 (ammonia-water - chloroform - IPA, 1: 1: 4). $[\alpha]_D^{19}+52.9°$ (*c* 0.78, H_2O). ^1H NMR (D_2O, pD 1.3): δ 1.34 (d, 3H, J=6.6 Hz, CH_3-6′), 1.4~2.6 (m, 6H, CH_2-2,3′,4′), 3.12 (s, 3H, N-CH_3), 3 45 (s, 3H, O-CH_3), 5.52 (d, 1H, J=3.4 Hz, H-1′), 6.27~6.41 (broad s, 1H, H-5). ^1H NMR (D_2O, pD 11.9): δ 0.98 (d, 3H, J=6.6 Hz, CH_3-6′), 1.1~2.4 (m, 6H, CH_2-2,3′,4′), 3.07 (s, 3H, N-CH_3), 3.40 (s, 3H, O-CH_3), 4.96 (d, 1H, J=3.7 Hz, H-1′), 6.01 (broad s, 1H, H-5). Mass: *m/z* 372 (M^++1, 2.0%), 212 ($C_{10}H_{18}$-N_3O_2, 2,5-dideoxy-4-N-glycylfortamine 4-ene, 9.3%), 143 ($C_7H_{15}N_2O$, 6-*epi*-purpurosamine B, 100%). ^{13}C NMR: see Table 1.

2,5-Dideoxy-4-*epi*-fortimicin A tetrahydrochloride (**20**)

To a solution of **16** (139 mg, 0.153 mmol) in ethanol (9 ml), a mixture of 0.1 N HCl (9 ml) and water (4 ml) was added. The mixture was hydrogenated in the presence of 10% palladium on carbon (28 mg) under 50 psi initial hydrogen atmosphere for 15 hours. After removal of the catalyst, platinum dioxide (28 mg) was added to the filtrate. The mixture was hydrogenated more 15 hours (50 psi initial hydrogen atmosphere). TLC (ammonia-water - chloroform - IPA, 1: 1: 4) showed a formation of two products (Rf 0.24 and 0.22). After removal of the catalyst, the filtrate was evaporated (and coevaporated with MeOH) to afford a white solid which was purified by passing through Amberlite IRA-400 (OH^-) (8 ml) column. A ninhydrin positive aqueous eluate was acidified (pH 4) by addition of 0.1 N HCl, and evaporated to afford 78 mg of a white solid.

The solid was dissolved in a mixture of acetone (5 ml) and water (5 ml) containing sodium carbonate (352 mg), then benzyloxycarbonyl chloride (0.16 ml) was added to the solution. The mixture was stirred at 0°C for 1 hour, then at room temperature for 1.5 hours. TLC (chloroform - ethanol, 40: 1) showed a formation of three products (Rf 0.28, 0.16 and 0.10). The mixture was evaporated and the residue was suspended in water (30 ml), then extracted with chloroform (30 ml; 20 ml×1, 10 ml×1). The extract was evaporated to afford an oily product which was washed with *n*-hexane and chromatographed on silica gel (Wako gel C-300 30 g, chloroform - ethanol, 40: 1). Fractions showing Rf 0.28

(the same solvent) were combined and evaporated to afford an oily residue which was hydrogenated without further purification. The residue was dissolved in ethanol (4 ml) and a mixture of 0.1 N HCl (2 ml) and water (1 ml) was added. The mixture was hydrogenated in the presence of 10% palladium on carbon (10 mg) under 50 psi initial hydrogen atmosphere for 15 hours. TLC (chloroform - ethanol, 40: 1) showed a disappearance of the starting material (Rf 0.28) and a formation of a single product (Rf 0.24). After removal of the catalyst, the filtrate was evaporated (and coevaporated with MeOH) to afford a white solid which was purified by passing through Amberlite IRA-400 (OH^-) (5 ml) column. A ninhydrin-positive aqueous eluate was acidified (pH 4) by addition of 0.1 N HCl and evaporated to afford 22.6 mg (quantitative yield) of **20** as a white hygroscopic solid. $[\alpha]_D^{23}+98.4°$ (*c* 0.87, MeOH). ^1H NMR (D_2O, pD 4.0): δ 1.35 (d, 3H, J=6.6 Hz, CH_3-6′), 1.5~2.5 (m, 8H, CH_2-2,5,3′,4′), 3.04 (s, 3H, N-CH_3), 3.41 (s, 3H, O-CH_3), 4.06 (s, 2H, glycyl-CH_2), 4.45 (m, 1H, H-4), 5.48 (d, 1H, J=3.7 Hz, H-1′). ^1H NMR (D_2O, pD 12.0): δ 1.02 (d, 3H, J=6.6 Hz, CH_3-6′), 1.1~2.4 (m, 8H, CH_2-2,5,3′,4′), 2.97 (s, 3H, N-CH_3), 3.36 (s, 3H, O-CH_3), 3.50 (s, 2H, glycyl-CH_2), 4.34 (m, 1H, H-4), 4.95 (d, 1H, J= 3.4 Hz, H-1′). Mass: *m/z* 374 (M^++1), 373 (M^+), 214 ($C_{10}H_{20}N_3O_2$, 2,5-dideoxy-4-N-glycylfortamine), 143 ($C_7H_{15}N_2O$, 6-*epi*-purpurosamine B). ^{13}C NMR: see Table 1.

2-Chloro-2-deoxy-2-*epi*-fortimicin A tetrahydrochloride (**21**)

To a solution of **11** (40.1 mg, 0.042 mmol) in ethanol (4 ml), a mixture of 0.1 N hydrochloric acid (2.5 ml) and water (1 ml) was added. The mixture was hydrogenated in the presence of 10% palladium on carbon (8 mg) under 50 psi initial hydrogen atmosphere for 15 hours. TLC (2-butanone - toluene, 1: 1) showed a disappearance of **11** (Rf 0.46) and a formation of a single product (Rf 0.26; ammonia-water - chloroform - IPA, 1: 1: 4). After removal of the catalyst, the filtrate was evaporated (and coevaporated with MeOH) to afford a white solid which was purified by passing though Amberlite IRA-400 (OH^-) (5 ml) column. A ninhydrin-positive aqueous eluate was acidified (pH 4) by addition of 0.1 N HCl and evaporated to afford 25.7 mg (quantitative yield) of **21** as a white hygroscopic solid. $[\alpha]_D^{22.5}+51.7°$ (*c* 1.01, MeOH). ^1H NMR (D_2O, pD 4.0): δ 1.37 (d, 3H, J=6.8 Hz, CH_3-6′), 1.5~2.2 (m, 4H, CH_2-3′,4′), 3.22 (s, 3H, N-CH_3), 3.64 (s, 3H, O-CH_3), 5.37 (d, 1H, J=3.4 Hz, H-1′). ^1H NMR (D_2O, pD 12.0): δ 1.02 (d, 3H, J=6.6 Hz, CH_3-6′), 1.5~1.8 (m, 4H, CH_2-3′,4′), 3.13 (s, 3H, N-CH_3), 3.49 (s, 3H, O-CH_3), 4.57 (dd, 1H, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.9 Hz, H-2), 4.86 (d, 1H, J=3.4 Hz, H-1′). Mass: *m/z* 426 and 424 ($C_{17}H_{35}N_5O_5Cl$, M^++1), 425 and 423 ($C_{17}H_{34}N_5O_5Cl$, M^+), 266 and 264 ($C_{10}H_{19}N_3O_3Cl$, 2-chloro-2-deoxy-4-N-glycylfortamine), 143 ($C_7H_{15}N_2O$, 6-*epi*-purpurosamine B). ^{13}C NMR: see Table 1.

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References

1) NARA, T.; M. YAMAMOTO, I. KAWAMOTO, K. TAKAYAMA, R. OKACHI, S. TAKASAWA, T. SATO & S. SATO: Fortimicins A and B, new aminoglycoside antibiotics. I. Producing organism, fermentation and biological properties of fortimicins. J. Antibiotics 30: 533~540, 1977
2) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DEVAULT, A. C. SINCLAIR, E. E. FAGER & L. A. MITSCHER: Fortimicins A and B, new aminoglycoside antibiotics. III. Structural identification. J. Antibiotics 30: 552~563, 1977
3) SUGIMOTO, M.; S. ISHII, R. OKACHI & T. NARA: Fortimicins C, D and KE, new aminoglycoside antibiotics. J. Antibiotics 32: 868~873, 1979
4) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. R. MARTIN, P. COLLUM & G. G. POST: The isolation and structure of fortimicin E. J. Antibiotics, in preparation.
5) DEUSHI, T.; A. IWASAKI, K. KAMIYA, T. KUNIEDA, T. MIZOGUCHI, M. NAKAYAMA, H. ITOH, T. MORI & T. ODA: A new broadspectrum aminoglycoside antibiotic complex, sporaricin. I. Fermentation, isolation and characterization. J. Antibiotics 32: 174~179, 1979

6) IWASAKI, A.; H. ITOH & T. MORI: A new broad-spectrum aminoglycoside antibiotic complex, sporaricin. II. Taxonomic studies on the sporaricin producing strain *Saccharopolyspora hirsuta* subsp. *kobensis* nov. subsp. J. Antibiotics 32: 180~186, 1979
7) DEUSHI, T.; M. NAKAYAMA, I. WATANABE, T. MORI, H. NAGANAWA & H. UMEZAWA: A new broad-spectrum aminoglycoside antibiotic complex, sporaricin. III. The structures of sporaricins A and B. J. Antibiotics 32: 187~192, 1979
8) OKAMI, Y.; K. HOTTA, M. YOSHIDA, D. IKEDA, S. KONDO & H. UMEZAWA: New aminoglycoside antibiotics, istamycins A and B. J. Antibiotics 32: 964~966, 1979
9) WATANABE, I.; T. DEUSHI, T. YAMAGUCHI, K. KAMIYA, M. NAKAYAMA & T. MORI: The structural elucidation of aminoglycoside antibiotics. Sannamycins A and B. J. Antibiotics 32: 1066~1068, 1979
10) INOUYE, S.; K. OHBA, T. SHOMURA, M. KOJIMA, T. TSURUOKA, J. YOSHIDA, N. KATŌ, M. ITŌ, S. AMANO, S. OMOTO, N. EZAKI, T. ITO, T. NIIDA & K. WATANABE: A novel aminoglycoside antibiotic, substance SF-2052. J. Antibiotics 32: 1354~1356, 1979
11) INOUYE, S.; T. SHOMURA, K. OHBA, H. WATANABE, S. OMOTO, T. TSURUOKA, M. KOJIMA & T. NIIDA: Isolation and identification of N-formylfortimicin A. J. Antibiotics 33: 510~513, 1980
12) SATO, M.; Y. MORI: Chemical modification of fortimicins: Preparation of 4-N-substituted fortimicin B. J. Antibiotics 32: 371~378, 1979
13) TADANIER, J.; J. R. MARTIN, P. KURATH, A. W. GOLDSTEIN & P. JOHNSON: 4-N-Acylfortimicins B and the preparation of fortimicin A from fortimicin B. Carbohydr. Res. 79: 91~102, 1980
14) SZAREK, W. A.: Deoxyhalogeno sugars. Advan. Carbohydr. Chem. & Biochem. 28: 225~306, 1973
15) SUAMI, T.: Modifications of aminocyclitol antibiotics. Jap. J. Antibiotics 32, Suppl.: S91~S102, 1979
16) KOTOWYCZ, G. & R. U. LEMIEUX: Nuclear magnetic resonance in carbohydrate chemistry. Chem. Rev. 73: 669~698, 1973
17) KOCH, K. F.; J. A. RHOADES, E. W. HAGAMAN & E. WENKERT: Carbon-13 nuclear magnetic resonance spectral analysis of tobramycin and related antibiotics. J. Am. Chem. Soc. 96: 3300~3305, 1974
18) WASHINGTON, J. A. & A. L. BARRY: Dilution test procedures. In manual of clinical microbiology, 2nd ed., E. H. LENNETT, E. H. SPAULDING & J. P. TRUANT (*Eds*): pp. 410~417, American Society of Microbiology, 1974