3-*O*-DEMETHYL-2,3-DI-*EPI*-FORTIMICINS AND 3-*O*-DEMETHYL-3-*EPI*-FORTIMICINS

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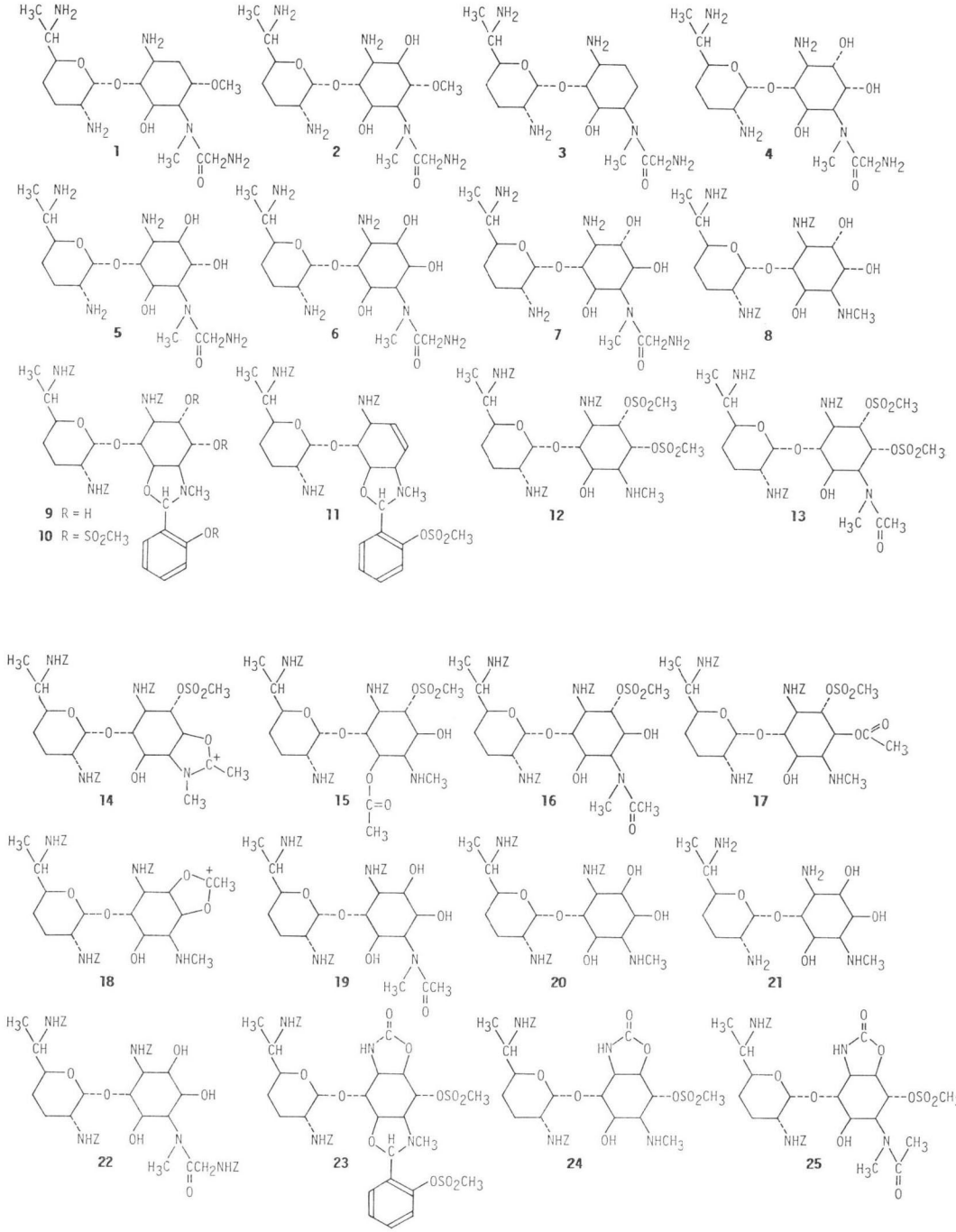
Syntheses of the 3-*O*-demethyl-2,3-di-*epi*-fortimicins A and B and the 3-*O*-demethyl-3-*epi*-fortimicins A and B have been accomplished in processes the key steps of which were solvolyses of 4-*N*-acetyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin derivatives. Antibacterial activities of the new antibiotics are reported.

Among the most active derivatives of fortimicin A are those modified at the C_2 and C_3 positions of the cyclitol ring. Examples of such active derivatives are 2-deoxyfortimicin A (**1**)[1], 2-*epi*-fortimicin A (**2**)[2], 2-deoxy-3-demethoxyfortimicin A (**3**)[3], 3-*O*-demethylfortimicin A (**4**)[4,5] and 3-*O*-demethyl-2-*epi*-fortimicin A (**5**)[6]. The objects of the present work were the preparations of the remaining two diastereomers of 3-*O*-demethylfortimicin A, 3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**6**) and 3-*O*-demethyl-3-*epi*-fortimicin A (**7**).

Our initial synthesis of 3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**6**) was accomplished as follows: 1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**8**)[3,4] was converted to the 4,5-salicylaldehyde oxazolidine **9** and then to the corresponding trimethanesulfonate (**10**) as previously described[3]. The structure of **10** had been previously established by its conversion to the 2,3-anhydrofortimicin derivative **11**[3] and thus confirmed that the oxazolidine derivatives **9** and **10** were the 4,5-*cis* derivatives as had been expected. Acid catalyzed cleavage of the oxazolidine ring of **10** gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*O*-methanesulfonylfortimicin B (**12**). Acylation of **12** with acetic anhydride in chloroform gave a product which showed essentially a single spot on thin-layer chromatography on an Analtech, silica gel GF plate in an ethyl acetate - triethylamine (200 : 1) system. In contrast to the apparent simplicity of the product on thin-layer chromatography, the ^1H NMR spectrum in $CDCl_3$ indicated a three component mixture with peaks characteristic of two $OCOCH_3$ groups, one $NCOCH_3$ group, $NHCH_3$ groups and $N(CH_3)COCH_3$ groups. In addition, the infrared spectrum showed a weak carbonyl absorption characteristic of an $N(CH_3)COCH_3$ group, and an absorption at 1170 cm^{-1} of an intensity which suggested the presence of one OSO_2CH_3 group. These data, together with our previous discovery of significant *O*-acylation which occurs with some 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B derivatives[7], suggested that the acylation product consisted of an interconvertible mixture of the two *O*-acetylated products **15** and **17** and the *N*-acetylated product **16**. Formation of the mixture of **15**, **16** and **17** might be accommodated by initial 4-*N*-acetylation to give **13** followed by intramolecular displacement of the 3-*O*-methanesulfonate group by the 4-*N*-acetyl carbonyl with inversion at C_3 to give the oxazolinium ion **14**. Hydrolysis of the latter during workup would then give a mixture of the three acylated tautomers **15**, **16** and **17**.

Treatment of the acylation product thus obtained with a solution of aqueous methanolic sodium bicarbonate at room temperature for 24 hours gave a two component mixture (~1 : 1 by TLC) the ^1H



NMR and infrared spectra of which showed the absence of a methanesulfonate group (no peak at 1170 cm^{-1}) and were consistent for a mixture of 4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**19**) and 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**). Prolonged treatment of the mixture of **19** and **20** with aqueous methanolic sodium bicarbonate

resulted in complete cleavage of the 4-*N*-acetyl group of **19** to give **20** as essentially the sole product obtained in the sequence starting with **8**. Formation of the mixture of **19** and **20** from the mixture of the interconvertible tautomers **15**, **16** and **17** may be accounted for by a solvolysis *via* participation of the acetyl carbonyl of **17** in the intramolecular displacement of the 2-*O*-methanesulfonate group with inversion at C_2 to give the ion **18** which on hydrolysis may give the mixture of **19** and **20**.

Catalytic hydrogenolysis of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**) gave 3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**21**). Acylation of **20** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide in tetrahydrofuran gave a product which appeared from its infrared spectrum to be *O*-acylated. Treatment of the latter product with triethylamine in aqueous tetrahydrofuran gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**22**). Catalytic hydrogenation of **22** gave 3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**6**).

Chemical proof of the configuration at C_2 of the 3-*O*-demethyl-2,3-di-*epi*-fortimicins prepared as described above was provided by an alternate synthesis starting with 2′,6′-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2-*epi*-3-*O*-methanesulfonyl-4-*N*,5-*O*-(2-*O*-methanesulfonyl)salicylidenefortimicin B (**23**). The latter was obtained as a by-product from the TIPSON-COHEN elimination of the tri-*O*-methanesulfonyl oxazolidine (**10**) which we have reported elsewhere[3] gave the olefin **11**. Formation of the 1,2-carbamate **23** appeared to be favored by use of a lesser amount of zinc in the TIPSON-COHEN reaction.

Mild acid-catalyzed hydrolysis of **23** to cleave the oxazolidine ring, followed by acetylation with acetic anhydride in the solution first made basic with excess sodium bicarbonate gave 4-*N*-acetyl-2′,6′-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**27**). The sequence **23** to **27** was carried out as a one-pot conversion without isolation of the intermediates. Formation of **27** may be accounted for in a sequence which involves initial acid-catalyzed hydrolysis of the oxazolidine ring of **23** to give the hydroxy amine **24**. Acetylation of the latter to give the 4-*N*-acetyl derivative **25** is then followed by solvolysis with intramolecular displacement of the 3-*O*-methanesulfonate group by the acetyl carbonyl with inversion at C_3 followed by hydrolysis of the resulting oxazolinium ion **26** to give **27**. Base-catalyzed hydrolysis of **27** gave 3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**21**) identical with that prepared from 2,3-di-*O*-methanesulfonyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**12**) as described above. In addition the sample of **21** prepared from the 3-*O*-methanesulfonyl-1,2-carbamate (**23**) was converted to 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**) identical with that prepared from the 2,3-di-*O*-methanesulfonate **12**.

In order to prepare 3-*O*-demethyl-3-*epi*-fortimicin A (**7**) it was necessary to protect the C_2-hydroxyl group of a suitable 3-*O*-demethylfortimicin derivative. This was accomplished by converting the 4,5-salicylaldehyde oxazolidine **9** of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B to the 1,2-carbamate **28** with sodium hydride in dimethylformamide. Treatment of **28** with methanesulfonyl chloride in pyridine gave the dimethanesulfonate **29** which was converted to the hydroxy amine **30** by mild acid-catalyzed hydrolysis. Acylation of **30** with acetic anhydride in chloroform gave the 4-*N*-acetyl-1,2-carbamate **31** which rearranged essentially quantitatively to the 1,5-carbamate **32** on treatment with sodium bicarbonate in aqueous methanol. Treatment of **32** with sodium bicarbonate in refluxing methanol gave a mixture of 2′,6′-di-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-1-*N*-methoxycarbonylfortimicin B (**33**) and 2′,6′-di-*N*-benzyloxycarbonyl-1,4-*N*-carbonyl-3-*O*-demethyl-3-*epi*-fortimicin B (**34**) which could be separated by column chromatography. Formations of both **33** and **34** most probably occur in processes which involve intramolecular displacement of the 3-*O*-methanesulfonyl group by the

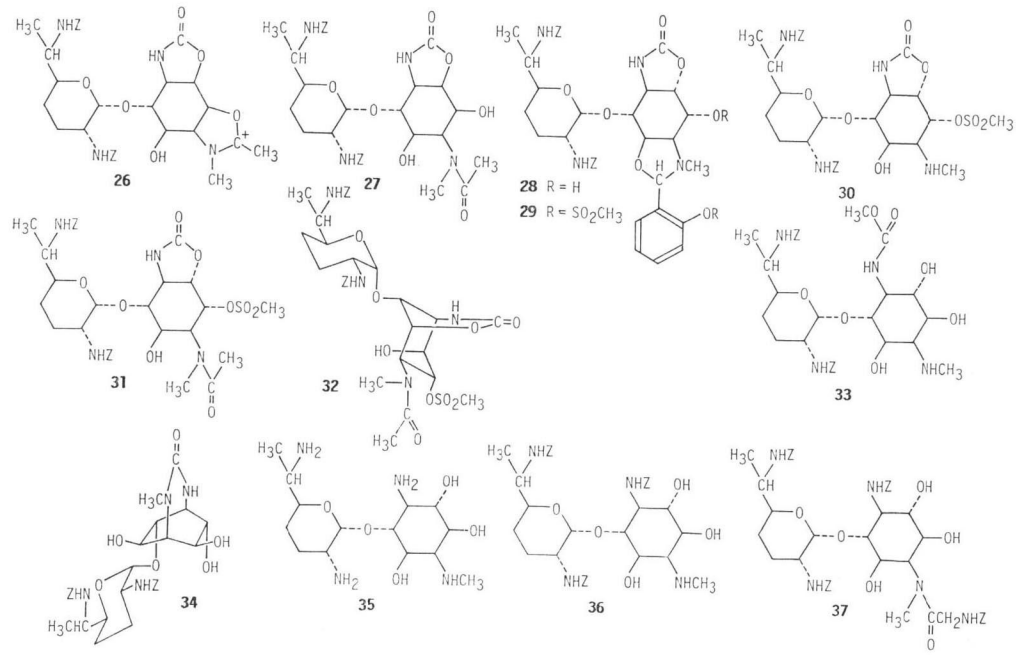


Table 1. *In vitro* antibacterial activities[a] (minimum inhibitory concentrations, μg/ml).

Organism	Fortimicin A	3-*O*-Demethyl-fortimicin A **4**[4,5]	3-*O*-Demethyl-2-*epi*-fortimicin A **5**[6]	3-*O*-Demethyl-3-*epi*-fortimicin A **7**	3-*O*-Demethyl-2,3-di-*epi*-fortimicin A **6**
Staphylococcus aureus Smith	0.78	0.39	0.39	50	3.1
Streptococcus faecalis 10541	50	25	50	>100	100
Enterobacter aerogenes 13048	3.1	1.56	6.2	>100	12.5
Escherichia coli JUHL	6.2	3.1	12.5	>100	12.5
E. coli BL 3676 (Res)	25	12.5	25	>100	50
E. coli 76-2	1.56	1.56	6.2	>100	6.2
Klebsiella pneumoniae 10031	1.56	1.56	3.1	100	6.2
K. pneumoniae KY-4262	6.2	3.1	12.5	>100	25
Proteus inconstans 1577	1.56	0.78	3.1	100	6.2
Pseudomonas aeruginosa BMH #10	1.56	0.78	1.56	100	3.1
P. aeruginosa KY 8512	3.1	1.56	3.1	>100	6.2
P. aeruginosa KY 8516	3.1	1.56	6.2	>100	12.5
P. aeruginosa 209	>100	100	>100	>100	>100
P. aeruginosa 27853	12.5	3.1	12.5	>100	25
Salmonella typhimurium Ed. #9	3.1	1.56	3.1	>100	6.2
Serratia marcescens 4003	1.56	1.56	3.1	>100	6.2
Shigella sonnei 9290	6.2	3.1	12.5	>100	12.5
Proteus rettgeri U6333	12.5	3.1	12.5	>100	25
P. vulgaris JJ	3.1	1.56	3.1	>100	6.2
P. mirabilis Fin. #9	3.1	1.56	3.1	100	6.2

[a] The *in vitro* activities were determined by the serial dilution method using Müeller-Hinton agar with the persulfate salts. Activities are expressed as minimum inhibitory concentrations of free base in μg/ml.

4-*N*-acetyl carbonyl with inversion at C_3. It should be noted that rearrangement of 1-*N*,2-*O*-carbonylfortimicin B to the 1,5-carbamate and formation of 1,4-*N*-carbonylfortimicin B have precedence in our earlier work[7,8].

Base-catalyzed hydrolysis of the mixture of **33** and **34** obtained by solvolysis of **32** gave 3-*O*-demethyl-3-*epi*-fortimicin B (**35**). The latter was converted to the 1,2′,6′-tri-*N*-benzyloxycarbonyl derivative **36** with *N*-benzyloxycarbonyloxysuccinimide. Acylation of the latter with *N*-benzyloxycarbonylglycine anhydride followed by treatment of the resulting product with triethylamine in aqueous tetrahydrofuran gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-fortimicin A (**37**). Catalytic hydrogenolysis of **37** gave 3-*O*-demethyl-3-*epi*-fortimicin A (**7**).

The *in vitro* antibacterial activities of fortimicin A and the four C-2, C-3 diastereomeric 3-*O*-demethylfortimicins A are recorded in Table 1. Whereas reported previously[4~7] 3-*O*-demethylfortimicin A (**4**) has significantly greater antibacterial activity than fortimicin A, and 3-*O*-demethyl-2-*epi*-fortimicin A has slightly less activity than fortimicin A, 3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**6**) has only about 40% the activity of fortimicin A and 3-*O*-demethyl-3-*epi*-fortimicin A (**7**) is almost devoid of activity.

Experimental

General

Optical rotations were determined with a Perkin Elmer Model 241 photoelectric polarimeter. IR spectra were determined using a Perkin Elmer Model 521 grating spectrometer. ^{1}H NMR spectra were determined at 100 MHz with a Varian Associates XL-100 spectrometer. Chemical shifts determined with D_2O solutions are reported from internal sodium trimethylsilylpropionate 2,2,3,3,-d_4 (TSP). Chemical shifts determined with $CDCl_3$ solutions are reported from internal TMS. Mass spectra were determined with an A. E. I. MS902 spectrometer at 70 eV and 150~200°C using the direct probe insert. Silica gel for chromatographies was that of Merck (Darmstadt), 70~230 mesh. Ratios for chromatography solvents are expressed by volume. Workups by chloroform extraction were carried out by shaking the reaction solutions or mixtures with mixtures of chloroform and 5% aqueous sodium bicarbonate. The chloroform extracts were separated and dried ($MgSO_4$), and the solvent was evaporated under diminished pressure using a rotary evaporator.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*O*-methanesulfonylfortimicin B (12)

To a stirred solution of 2.98 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-tri-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**10**)[3] in 100 ml of tetrahydrofuran was added 50 ml of 0.4 N hydrochloric acid. The solution was kept at room temperature for 46 hours and then shaken with a mixture of 200 ml of chloroform and 500 ml of 5% aqueous sodium bicarbonate. The chloroform solution was separated and shaken for 5 minutes with 500 ml of 5% aqueous sodium bisulfite. The chloroform solution was separated and washed with 500 ml of water. The aqueous solutions were washed in series with five 100 ml portions of chloroform. The chloroform solutions were combined and dried ($MgSO_4$). Evaporation of the solvent under diminished pressure left 2.62 g of **12**: $\delta(CDCl_3)$ 1.04 d ($J_{6',7'}$=5.0 Hz, 6′-CH_3); 2.52 (NCH_3); 2.93, 3.18 (OSO_2CH_3's).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (20)

1. A stirred solution of 0.4856 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*O*-methanesulfonylfortimicin B (**12**), 0.1 ml of acetic anhydride and 25 ml of chloroform was kept at room temperature for 22 hours. The resulting solution was shaken with a mixture of 80 ml of chloroform and 200 ml of 5% aqueous sodium bicarbonate. The chloroform solution was separated and the aqueous solution was extracted twice with 50 ml portions of chloroform. The chloroform solutions were combined and dried ($MgSO_4$). The chloroform was evaporated under reduced pressure and residual acetic anhydride was removed by codistillation with toluene under reduced pressure to leave 0.7464 g of a mixture of 5-*O*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-2-*O*-methanesulfonylfortimicin

B (**15**), 4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-2-*O*-methanesulfonylfortimicin B (**16**), and 3-*O*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-2-*O*-methanesulfonylfortimicin B (**17**): $\tilde{\nu}_{max}$ ($CDCl_3$) 3442, 3337, 1715, 1622, 1177 cm^{-1}. δ($CDCl_3$) 1.00~1.08 m (6′-CH_3); 1.94, 2.11 (sharp singlets, $COCH_3$'s); 2.05 (broadened singlet, $NCOCH_3$); 2.53, 2.57 ($NHCH_3$'s); peaks between 2.7~3.15 [OSO_2CH_3's, $N(CH_3)COCH_3$].

a. A stirred solution of 0.3482 g of a mixture of **15**, **16** and **17**, prepared as described above, 5 ml of 5% aqueous sodium bicarbonate, and 30 ml of methanol was kept at room temperature for 18 hours. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to give from the chloroform phase 0.3195 g of a mixture (~1: 1 by TLC and ^1H NMR assay) of 4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**19**) and 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**): $\tilde{\nu}_{max}$ ($CDCl_3$) 3438, 3338, 1713, 1626 cm^{-1}. δ($CDCl_3$) 1.0~1.2 (6′-CH_3, complex multiplet); 2.05 ($COCH_3$); 2.52 ($NHCH_3$); 3.09 (NCH_3COCH_3).

A solution of 0.227 g of a mixture of **19** and **20**, prepared as described above, 20 ml of methanol and 6.5 ml of 5% aqueous sodium bicarbonate was stirred at room temperature for 135 hours. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to give from the chloroform phase 0.2167 g of **20** identical with that prepared as described below.

b. A solution of a mixture (2.26 g) of **15**, **16**, and **17** prepared as described above, 35 ml of 5% aqueous sodium bicarbonate and 200 ml of methanol was stirred at room temperature for ten days. The major portion of the methanol was evaporated under reduced pressure and the product was isolated as a white foam (1.72 g) which was chromatographed on a column of 180 g of silica gel packed and eluted with a solvent system composed of 10 : 1 : 0.1 of chloroform - methanol - concentrated ammonium hydroxide to yield 0.937 g of pure **20**: $[\alpha]_D^{30}$ +49° (*c* 1.0, CH_3OH), $\tilde{\nu}_{max}$ ($CDCl_3$) 3567, 3437, 3327, 1712 cm^{-1}. δ($CDCl_3$) 1.03 d ($J_{6',7'}$=6.0 Hz, 6′-CH_3); 2.50 (NCH_3).

Anal. Calcd. for $C_{38}H_{48}N_4O_{11}$: C 61.94, H 6.57, N 7.61.
Found: C 61.62, H 6.62, N 7.46.

2. To an ice bath-cooled, stirred solution of 1.72 g of the tetrahydrochloride of 3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**21**) (prepared by base-catalyzed hydrolysis of 4-*N*-acetyl-2′,6′-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**27**) which was prepared as described below), 2 ml of triethylamine, 17 ml of water, and 68 ml of acetonitrile was added 2.94 g of *N*-benzyloxycarbonyloxysuccinimide. Stirring was continued with cooling for 3 hours and then at room temperature overnight. The product (2.71 g) of a glass was isolated by chloroform - 5% aqueous sodium bicarbonate extraction and chromatographed on a column of 250 g of silica gel packed and eluted with a solvent system composed of 18: 2: 0.1 of chloroform - methanol - concentrated ammonium hydroxide. Early fractions contained 0.884 g of product which was not characterized. Later fractions contained 1.30 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**) identical with that prepared as described above.

3-*O*-Demethyl-2,3-di-*epi*-fortimicin B (**21**)

1. A sample (0.603 g) of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**), prepared from 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-tri-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**10**) as described above was hydrogenated for 4 hours in a solution of 66 ml of 0.2 N hydrochloric acid in methanol and 30 ml of methanol under 3 atmospheres of hydrogen in the presence of 0.6 g of 5% Pd/C to give 0.384 g of the tetrahydrochloride of **21**: δ(D_2O) 1.37 d ($J_{6',7'}$=6Hz, 6′-CH_3); 1.87 (NCH_3); 5.50 d ($J_{1',2'}$=3.0 Hz). *m/z*: (M+H), Meas. 335.2285, Calcd. for $C_{14}H_{31}N_4O_5$: 335.2294. Diaminosugar, Meas. 143.1176, Calcd. for $C_7H_{15}N_2O$: 143.1184. Cyclitol, Meas. 193.1184, Calcd. for $C_7H_{17}N_2O_4$: 193.1188.

An aqueous solution of 0.340 g of the tetrahydrochloride of **21** was passed through a column of excess AG1-X2 (SO_4^{2-}) resin. Lyophilization of the fractions containing the product gave 0.221 g of the disulfate salt of **11**: $[\alpha]_D^{30}$ +55° (*c* 1.0, H_2O).

2. A solution of 9.00 g of 4-*N*-acetyl-2′,6′-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**27**) in 100 ml of 6 N aqueous potassium hydroxide and 200 ml of ethanol was heated at 90°C under nitrogen overnight. The resulting solution was cooled, diluted with 100 ml of water and brought to pH 2 by addition of 2 N hydrochloric acid. The solvent was evaporated under

reduced pressure and residual hydrochloric acid was removed by co-distillation under reduced pressure first with ethanol and then with methanol. The residue was triturated several times with methanol and the suspensions were filtered. The filtrates were combined and the methanol was evaporated under reduced pressure leaving 6.42 g of a brown solid which was chromatographed on a column of 400 g of silica gel packed and eluted with a solvent system composed of the lower phase of a mixture of 1 : 1 : 1 of chloroform - methanol - concentrated ammonium hydroxide to give 2.14 g of **21**. The latter was converted in 0.2 N hydrochloric acid in methanol to the tetrahydrochloride identical with that described above prepared from **20**.

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**22**)

To a stirred solution of 2.06 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**20**) in 40 ml of tetrahydrofuran was added 0.942 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Stirring was continued overnight at room temperature. To the resulting solution was added an additional 0.942 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide and stirring was continued overnight. The product was isolated by chloroform - 5% sodium bicarbonate extraction which gave 2.61 g of a white glass. The latter was allowed to stand for 2 days in a solution prepared from 22 ml of triethylamine, 44 ml of water, and 180 ml of tetrahydrofuran. Chloroform - 5% sodium bicarbonate extraction gave 2.25 g of pink glass which was chromatographed on a column of 120 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - ethanol, 18 : 1 to yield 1.71 g of **22**: $[\alpha]_D^{30}+36°$ (*c* 1.0, CH_3OH), $\tilde{\nu}_{max}$ ($CDCl_3$) 3548, 3433, 1713, 1643 cm^{-1}. δ($CDCl_3$) 1.14 d ($J_{6',7'}$=6 Hz, 6′-CH_3); 3.13 (NCH_3).

Anal. Calcd. for $C_{48}H_{57}N_5O_{14}$: C 62.12, H 6.19, N 7.55.
Found: C 62.03, H 5.93, N 7.47.

3-*O*-Demethyl-2,3-di-*epi*-fortimicin A (**6**)

A sample of 0.219 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin A (**22**) in 9.5 ml of 0.2 N hydrochloric acid in methanol and 10 ml of methanol was hydrogenated for 2 hours under 3 atmospheres of hydrogen in the presence of 0.219 g of 5% Pd/C to yield 0.128 g of **6** as the tetrahydrochloride. A sample (0.900 g) of **6** prepared as described above, in water, was passed through a column of AG1-X2 (SO_4^{2-}) resin. Fractions containing product were combined and lyophilized to give 0.856 g of the disulfate salt of **6**: $[\alpha]_D^{28}+60°$ (*c* 1.0, H_2O). $\tilde{\nu}_{max}$ (KBr) 1642 cm^{-1}, δ (D_2O) 1.35 d ($J_{6',7'}$=6.6 Hz, 6′-CH_3); 3.39 (NCH_3); 5.39 d ($J_{1',2'}$=3.2 Hz). *m/z*: M^+, Meas. 391.2452, Calcd. for $C_{16}H_{33}N_5O_6$: 391.2431; Diaminosugar, Meas. 114.1163, Calcd. for $C_7H_{15}N_2O$: 143.1184; Cyclitol, Meas. 250.1392, Calcd. for $C_9H_{20}N_3O_5$: 250.1403.

Anal. Calcd. for $C_{16}H_{37}N_5O_{14}S_2\cdot 2H_2O$: C 30.81, H 6.63, N 11.20.
Found: C 30.73, H 6.65, N 11.60.

2′,6′-Di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2-*epi*-di-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**23**) and 2,3-Anhydro-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-demethoxy-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**11**)

1. A stirred suspension of 5.0 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-tri-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidinefortimicin B (**10**), 27 g of zinc dust, 53.6 g of sodium iodide and 210 ml of dry dimethylformamide was heated overnight at 100°C in an oil bath. The warm reaction mixture was poured into 1 liter of chloroform and the resulting suspension was filtered through a sintered glass funnel. The filter cake was washed thoroughly with chloroform. The filtrate and washings were combined and washed twice with 500-ml portions of sodium thiosulfate and then with 500 ml of 5% sodium bicarbonate and dried ($MgSO_4$). The chloroform was evaporated under reduced pressure and residual dimethylformamide was removed by co-distillation with toluene leaving 3.99 g of a yellow glass. The latter was chromatographed on a column of 250 g of silica gel packed and eluted with a solvent system composed of 20 : 1 : 0.2 of ethyl acetate - ethanol - triethylamine to yield 2.67 g of **11** and 0.518 g of **23** which were identical with the products prepared as described below.

2. A stirred suspension of 18.0 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-tri-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**10**), 10.9 g of zinc dust, 19.7 g of sodium iodide, and 360 ml of dry dimethylformamide was heated overnight at 100°C in an oil bath. The product mixture was isolated as described in part 1 to yield 14.0 g of yellow glass which was chromatographed on a column

of 750 g of silica gel to yield 6.35 g of **11** identical with that described previously[3], and 6.34 g of **23**: $[\alpha]_D^{28}+46°$ (*c* 1.0, CH_3OH), $\tilde{\nu}_{max}$ ($CDCl_3$) 3441, 1781, 1715, 1178 cm^{-1}. δ($CDCl_3$) 1.17 d ($J_{6',7'}$=6.8 Hz, 6'-CH_3); 2.42 (NCH_3); 3.04, 3.21 (OSO_2CH_3's).

Anal. Calcd. for $C_{40}H_{48}N_4O_{15}S_2$: C 54.04, H 5.44, N 6.30, S 7.21.
Found: C 54.40, H 5.46, N 6.30, S 6.79.

4-*N*-Acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2,3-di-*epi*-fortimicin B (**27**)

A solution of 2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-2-*epi*-di-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**23**) in 160 ml of 0.4 N hydrochloric acid in methanol was kept overnight at room temperature. The resulting solution was cooled in an ice bath and 40 ml of acetic anhydride and 18 g of solid sodium bicarbonate were added. The solution was stirred for one hour with cooling and then at room temperature overnight. The resulting solution was shaken with a mixture of 500 ml of 5% sodium bicarbonate and 250 ml of chloroform. The chloroform solution was separated and the aqueous solution was extracted with 250 ml of chloroform. The chloroform solutions were combined and dried ($MgSO_4$). Evaporation of the chloroform under reduced pressure left 9.0 g of **27**. For analysis a sample of 0.870 g of product was chromatographed on a column of 80 g of silica gel packed and eluted with a solvent system composed of 17 : 2 of ethyl acetate - ethanol to yield 0.612 g of pure **27**: $[\alpha]_D^{30}+77°$ (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3569, 3439, 3349, 1763, 1709, 1629 cm^{-1}. δ ($CDCl_3$) 1.19 d ($J_{6',7'}$=6.4 Hz, 6'-CH_3); 2.06 ($COCH_3$); 3.26 (NCH_3).

Anal. Calcd. for $C_{33}H_{42}N_4O_{11}$: C 59.09, H 6.31, N 8.36.
Found: C 59.01, H 6.13, N 8.21.

2',6'-Di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-di-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**29**)

To a stirred solution of 4.0 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-4-*N*,5-*O*-salicylidenefortimicin B (**9**)[3] in 134 ml of dimethylformamide under nitrogen and cooled in an ice bath, was added 1.67 g of 57% oily sodium hydride. Stirring was continued with cooling for one hour and then at room temperature for 20 hours. The resulting solution was cooled in an ice bath and 18 ml of acetic acid - water, 1 : 2 solution was added cautiously. The resulting solution was shaken. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction. Residual dimethylformamide was removed by co-distillation with toluene under reduced pressure leaving the crude product (**28**) (3.61 g) as a yellow glass: $\tilde{\nu}_{max}$ ($CDCl_3$) 3437, 1771, 1712 cm^{-1}. δ($CDCl_3$) 1.12 d ($J_{6',7'}$=6.5 Hz, 6'-CH_3), 2.34 (NCH_3).

To a stirred solution of the above product (3.61 g) in 60 ml of pyridine, cooled in an ice bath, was added 1.52 ml of methanesulfonyl chloride. Stirring was continued with cooling for one hour and then overnight at room temperature. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction. Residual pyridine was removed by co-distillation with toluene under reduced pressure leaving 4.69 g of a sticky, brown-orange glass. Chromatography of the latter on a column of 300 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - toluene - triethylamine, 10 : 10 : 0.1 gave 2.12 g of **29**: $[\alpha]_D^{30}+30°$ (*c* 1.0, CH_3OH), $\tilde{\nu}_{max}$ ($CDCl_3$) 3441, 1775, 1712, 1177 cm^{-1}. δ($CDCl_3$) 1.10 d ($J_{6',7'}$=6.0 Hz, 6'-CH_3); 2.29 (NCH_3); 3.04, 3.11 (OSO_2CH_3's).

Anal. Calcd. for $C_{40}H_{48}N_4O_{15}S_2$: C 54.04, H 5.44, N 6.30, S 7.21.
Found: C 54.22, H 5.72, N 6.27, S 7.34.

2',6'-Di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**30**)

A solution of 1.41 g of 2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-di-*O*-methanesulfonyl-4-*N*,5-*O*-salicylidenefortimicin B (**29**), 23 ml of 0.4 N hydrochloric acid and 45 ml of tetrahydrofuran was kept at room temperature for 3 days. The resulting solution was shaken with a mixture of 200 ml of chloroform and 500 ml of 5% aqueous sodium bicarbonate. The chloroform solution was separated and shaken for 5 minutes with 500 ml of 5% aqueous sodium bisulfite, and then with water. The aqueous solutions were washed in series with four 100-ml portions of chloroform. The chloroform solutions were combined and dried ($MgSO_4$). Evaporation of the chloroform under reduced pressure

left 1.19 g of **30** as a white glass: $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 1775, 1711, 1176 cm^{-1}. δ($CDCl_3$) 1.14 d ($J_{6',7'}$= 6.8 Hz, 6'-CH_3); 2.43 (NCH_3); 3.04 (OSO_2CH_3).

4-*N*-Acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**31**)

To a stirred solution of 7.70 g of 2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**30**) in 380 ml of chloroform, cooled in an ice bath, was added 32 ml of acetic anhydride. Stirring was continued with cooling for one hour and then at room temperature overnight. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction. Residual acetic anhydride was removed by co-distillation with toluene under reduced pressure to give 7.0 g of **31** as a glass: $\tilde{\nu}_{max}$ ($CDCl_3$) 3447, 1775, 1712, 1625, 1177 cm^{-1}.

4-*N*-Acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,5-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**32**)

To a stirred solution of 7.0 g of 4-*N*-acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**31**) in 600 ml of methanol was added 200 ml of 5% aqueous sodium bicarbonate. Stirring was continued at room temperature for 22 hours. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to give 6.93 g pale yellow glass. Chromatography of the latter on a column of 400 g of silica gel packed and eluted with a solvent system composed of chloroform - methanol, 15 :1 gave 4.36 g of **32**: $[\alpha]_D^{30}$ +59° (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3528, 3438, 1718, 1636, 1178 cm^{-1}. δ ($CDCl_3$) 1.14 d ($J_{6',7'}$=6.5 Hz, 6'-CH_3); 1.95 ($COCH_3$); 3.03 (NCH_3, OSO_2CH_3).

Anal. Calcd. for $C_{33}H_{44}N_4O_{13}S$: C 54.53, H 5.92, N 7.48, S 4.28.
Found: C 54.04, H 5.99, N 7.35, S 4.15.

2',6'-Di-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-1-*N*-methoxycarbonylfortimicin B (**33**) and 2',6'-di-*N*-benzyloxycarbonyl-1,4-*N*-carbonyl-3-*O*-demethyl-3-*epi*-fortimicin B (**34**)

A stirred suspension of 1.03 g of 4-*N*-acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,5-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**32**), 1.77 g of sodium bicarbonate and 100 ml of methanol was heated under reflux for 23 hours. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 0.814 g of orange glass. A sample (1.01 g) of product prepared as described above was chromatographed on a column of 100 g of silica gel packed and eluted with a solvent system prepared from chloroform - methanol, 5: 1. Earlier fractions gave 0.182 g of **34**: $[\alpha]_D^{30}$ +60° (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3434, 1704, 1629 cm^{-1}. δ($CDCl_3$) 1.05 d (broadened doublet, 6'-CH_3); 2.91 (NCH_3).

Anal. Calcd. for $C_{31}H_{40}N_4O_{10}$: C 59.22, H 6.41, N 8.91.
Found: C 59.23, H 6.50, N 8.71.

Intermediate fractions gave 0.156 g of a mixture of **33** and **34**. Later fractions gave 0.218 g of **33**: $[\alpha]_D^{30}$+30° (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3438, 3338, 1708 cm^{-1}. δ ($CDCl_3$) 1.11 d ($J_{6',7'}$=6.0 Hz, 6'-CH_3); 2.55 (NCH_3); 3.58 (CO_2CH_3).

Anal. Calcd. for $C_{32}H_{44}N_4O_{11}\cdot\frac{1}{2}H_2O$: C 57.38, H 6.77, N 8.37.
Found: C 57.00, H 6.77, N 8.12.

3-*O*-Demethyl-3-*epi*-fortimicin B (**35**)

A sample of 2.46 g of a mixture of 2',6'-di-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-1-*N*-methoxycarbonylfortimicin B (**33**) and 2',6'-di-*N*-benzyloxycarbonyl-1,4-*N*-carbonyl-3-*O*-demethyl-3-*epi*-fortimicin B (**34**), prepared by methanolysis of 3.04 g of 4-*N*-acetyl-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,5-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonylfortimicin B (**32**) as described above, was dissolved in a solution of 32 ml of 6 N potassium hydroxide and 64 ml of ethanol. The resulting solution was heated at 90°C under nitrogen overnight. The solution was cooled, diluted with 75 ml of water and then brought to pH 2 by addition of 1 N hydrochloric acid. The solvent was evaporated under reduced pressure. The residue was triturated with several portions of methanol. The supernatants were separated by filtration. The methanol was evaporated under reduced pressure from the combined filtrates leaving 2.76 g of brown solid. The latter was chromatographed on a column of 200 g of silica gel packed and eluted with a solvent system composed of the lower phase of a 1: 1: 1 mixture of chloroform - methanol -

concentrated ammonium hydroxide to yield 0.847 g of **35**. A sample (2.14 g) of **35** thus prepared was dissolved in 40 ml of 0.4 N hydrochloric acid in methanol. The methanol was evaporated under reduced pressure and residual hydrochloric acid was removed by co-distillation with methanol to give **35** as the tetrahydrochloride (2.67 g): $[\alpha]_D^{30}+46°$ (*c* 1.0, CH_3OH). δ (D_2O) 1.34 ($J_{6',7'}=7.0$ Hz, 6'-CH_3); 2.90 (NCH_3); 5.64 d ($J_{1',2'}=4$ Hz, $H_{1'}$). *m/z*: (M+H), Meas. 335.2323, Calcd. for $C_{14}H_{31}N_4O_5$: 335.2294; Diaminosugar, Meas. 143.1187, Calcd. for $C_7H_{15}N_2O$: 143.1184; Cyclitol, Meas. 193.1174, Calcd. for $C_7H_{17}N_2O_4$: 193.1188.

1,2',6'-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-fortimicin B (**36**)

To a stirred solution of the tetrahydrochloride of 3-*O*-demethyl-3-*epi*-fortimicin B (**35**), 2.8 ml of triethylamine, 24 ml of water, and 96 ml of methanol, cooled in an ice-bath, was added 4.07 g of *N*-benzyloxycarbonyloxysuccinimide. Stirring was continued with cooling for 3 hours and then at room temperature overnight. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to give 3.56 g of tan glass. The latter was chromatographed on a column of 250 g of silica gel packed and eluted with a solvent system composed of chloroform - methanol - concentrated ammonium hydroxide, 9 : 1 : 0.1 to yield 1.92 g of **36**: $[\alpha]_D^{30}+34°$ (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3579, 3439, 1705 cm^{-1}. δ ($CDCl_3$) 0.994 d ($J_{6',7'}=7$ Hz, 6'-CH_3); 2.55 (NCH_3).

Anal. Calcd. for $C_{38}H_{48}N_4O_{11}$: C 61.94, H 6.59, N 7.63.
Found: C 62.05, H 6.59, N 7.63.

1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-fortimicin A (**37**)

To a stirred solution of 1.10 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-fortimicin B (**36**) in 40 ml of tetrahydrofuran was added 1.19 g of *N*-benzyloxycarbonylglycine anhydride. Stirring was continued overnight at room temperature. Solvent was evaporated under reduced pressure leaving 2.39 g of white solid. A solution of the latter, 8 ml of triethylamine, 16 ml of water, and 66 ml of tetrahydrofuran was kept at room temperature for 5 days. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 1.36 g of white glass which was chromatographed on a column of 100 g of silica gel packed and eluted with ethyl acetate to yield 0.696 g of **37**: $[\alpha]_D^{23}+36°$ (*c* 1.0, CH_3OH). $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 3350, 1712, 1648 cm^{-1}. δ ($CDCl_3$) 1.07 d (broadened doublet, 6'-CH_3); 2.93 (NCH_3).

Anal. Calcd. for $C_{48}H_{57}N_5O_{14}$: C 62.12, H 6.19, N 7.55.
Found: C 62.06, H 6.22, N 7.51.

3-*O*-Demethyl-3-*epi*-fortimicin A (**7**)

A sample of 0.323 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-3-*epi*-fortimicin A (**37**) in 14 ml of 0.2 N hydrochloric acid in methanol and 11 ml of methanol was hydrogenated for 2 hours in the presence of 0.33 g of 5% Pd/C under 3 atmospheres of hydrogen to yield 0.194 g of **7** as the tetrahydrochloride. The latter was converted to the disulfate by passage through a column of excess AG1-X2 (SO_4^{2-}) in water. Lyophilization of the resulting aqueous solution gave 0.154 g of **7** as the disulfate: $[\alpha]_D^{28}+66°$ (*c* 1.0, H_2O). $\delta(D_2O)$ 1.36 d ($J_{6',7'}=7$ Hz, 6'-CH_3); 3.17 (NCH_3); 5.80 d ($J_{1',2'}=4$ Hz, $H_{1'}$). *m/z*: M^+, Meas. 391.2408, Calcd. for $C_{16}H_{33}N_5O_6$ 391.2431: Diaminosugar, Meas. 143.1186, Calcd. for $C_7H_{15}N_2O$: 143.1184; Cyclitol, Meas. 250.1410, Calcd. for $C_9H_{20}N_3O_5$, 250.1403.

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