SYNTHESIS OF 2-DEOXYFORTIMICINS AND 1-DEAMINO-2-DEOXY-2-*EPI*-AMINOFORTIMICINS *VIA* 2-*O*-METHANESULFONYLFORTIMICIN B

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The synthesis of 2-deoxyfortimicins A (**15**) and B (**11**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicins A (**18**) and B (**12**) is described. Two routes have been developed for synthesis of the key intermediate 2-*O*-methanesulfonylfortimicin B (**7**). One route involves selective blocking of fortimicin B with *N*-benzyloxycarbonyl groups followed by formation of a 4,5-salicylaldehyde oxazolidine derivative. Subsequent mesylation followed by deblocking gave **7**. A more efficient route to **7** involves concomitant salicylaldehyde SCHIFF base and 4,5-oxazolidine formation followed by mesylation and hydrolysis. The formation of 1,2(*R*)-epiminofortimicin B (**8**) from **7** followed by RANEY nickel reduction gave 2-deoxyfortimicin B and 1-deamino-2-deoxy-2-*epi*-aminofortimicin B, which were converted to the corresponding fortimicin A derivatives by selective *N*-blocking, *N*-acylation and subsequent deblocking. The antibacterial activities of the new fortimicin A derivatives are presented.

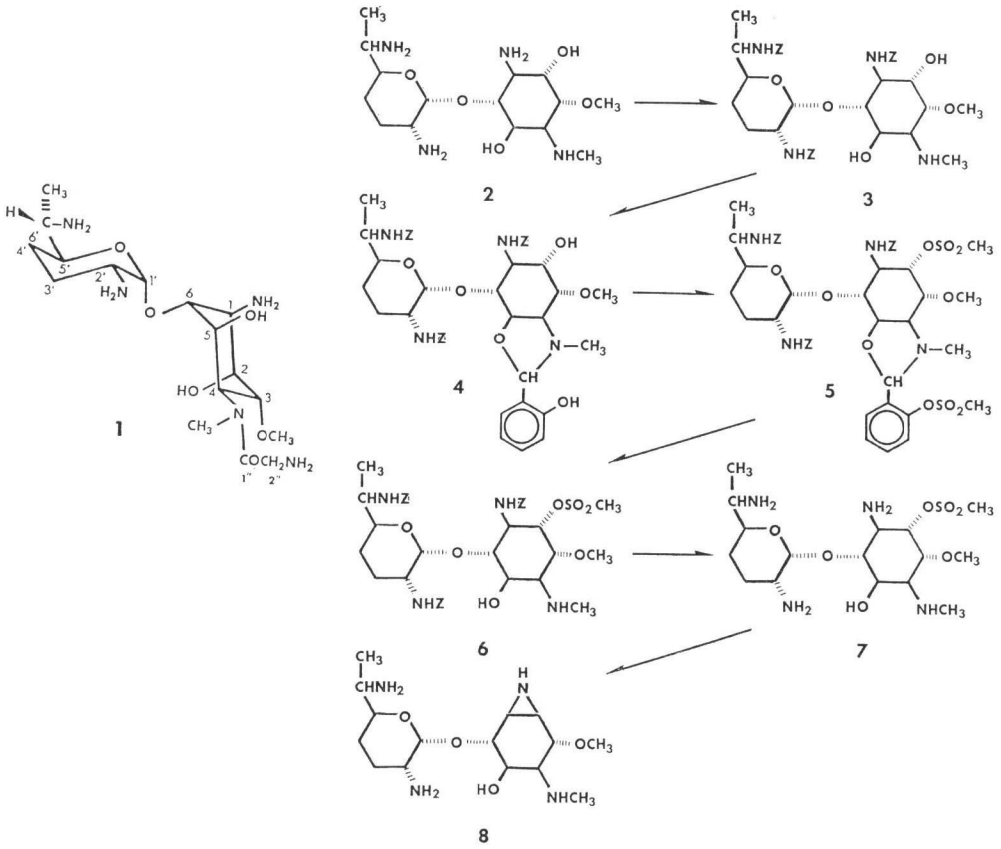
Recently an unusual family of pseudo-disaccharide aminoglycoside antibiotics, the fortimicins, were isolated from fermentation broths of *Micromonospora olivoasterospora*.[1] Members of the fortimicin family have unusual 1,4-diaminocyclitol moieties.[2,3] The first member isolated, fortimicin A (**1**), is of high interest due to its broad spectrum and high antibacterial activity. Of particular note, fortimicin A has excellent activity against a wide range of microorganisms resistant to the current clinically important aminoglycoside antibiotics.[4]

We have initiated efforts to systematically modify the fortimicins in an attempt to develop structure-activity and structure-toxicity relationships. Previous work in this laboratory resulted in the preparation of 4-*N*-acyl derivatives[5] and purpurosamine modified derivatives.[6,7,8] We now describe the synthesis of some cyclitol modified fortimicins from 2-*O*-methanesulfonylfortimicin B (**8**), a key intermediate for the synthesis of C_1-C_2 modified fortimicins.

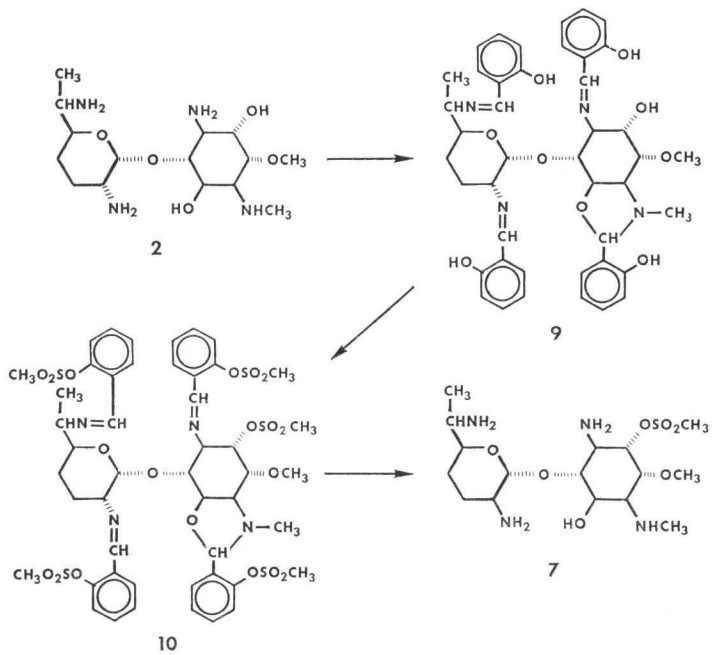
Schemes 1 ~ 3 illustrate the synthetic transformations carried out in this study. The strategy for the preparation of C_1-C_2 modified fortimicins was to effect selective mesylation at C_2 *via* a suitably blocked intermediate. We anticipated that on *N*-deblocking, the C_1 amino group would displace the neighboring *trans* C_2 methanesulfonyl group to form a stable 2-deoxy-1,2(*R*)-epiminofortimicin. Literature precedents[9] led us to expect that reductive opening of the epimino function would give deoxyfortimicins.

As outlined in Scheme 1, we initially envisioned a synthetic route which would use 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3**) readily prepared from fortimicin B (**2**).[5] Accordingly, treatment of **3** with salicylaldehyde gave a quantitative yield of the expected 4,5-oxazolidine **4**, an intermediate with the requisite free C_2 hydroxyl group. Sulfonylation of **4** with methanesulfonyl chloride in pyridine gave, in high yield, the desired mesylate **5**. Hydrolysis of the 4,5-oxazolidine ring was smoothly effected with hydrochloric acid in tetrahydrofuran leading to 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonyl-

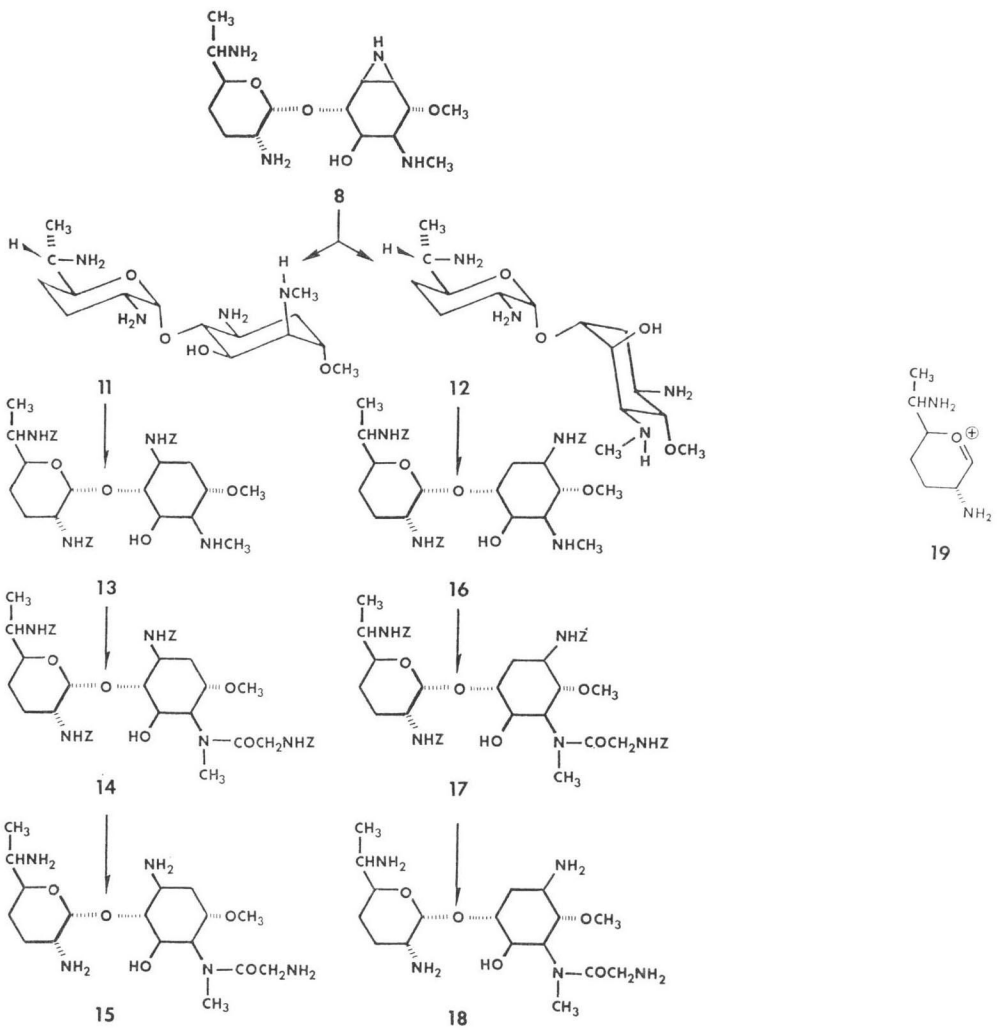
Scheme 1.



Scheme 2.



Scheme 3.



fortimicin B (**6**). Facile hydrogenolysis of the *N*-blocking benzyloxycarbonyl groups with palladium on carbon in acidic methanol afforded, in quantitative yield, 2-*O*-methanesulfonylfortimicin B (**7**) as the tetrahydrochloride.

The *trans* arrangement of the C_1 amino group and the C_2 mesyl substituent provides for neighboring group participation by nitrogen to displace the mesyl group to form a C_1-C_2 epimino function. The hydrogenolysis of the *N*-blocking groups was performed in an acidic medium to prevent formation of the epimino function. To effect epimino formation, the free base of **7** was generated by passing an aqueous solution of the perhydrochloride salt through a column of an anion-exchange resin. Basic elutes were collected and allowed to stand at room temperature. Under these conditions, epimine formation was complete in 16 hours. To prepare pure 2-deoxy-1,2(*R*)-epiminofortimicin B (**8**), a second treatment with an anion-exchange resin was necessary to remove the displaced methanesulfonic acid. The epimine **8** was characterized by the high-field absorption attributed to the C_1 and C_2 carbon atoms in the c.m.r. spectrum[10] (32.5 and 33.5 ppm respectively), and the H_1 and H_2 protons in the p.m.r. spectrum.[11]

An alternate, more direct synthesis of the 1,2(*R*)-epimino derivative **8** was accomplished *via* 1,2′, 6′-tri-*N*-salicylidene-fortimicin B-4,5-salicylaldehyde oxazolidine (**9**).[12] The latter **9** was prepared on treatment of fortimicin B with four equivalents of salicylaldehyde in methanol. Mesylation of **9** without purification gave 1,2′,6′-tri-*N*-(2-*O*-methanesulfonylsalicylidene)-2-*O*-methanesulfonylfortimicin B-4, 5-(2-*O*-methanesulfonylsalicylaldehyde) oxazolidine (**10**). Acid catalyzed hydrolysis of the crude re-

Table 1. 100 MHz c.m.r. parameters for fortimicin B (**2**), 2-deoxyfortimicin B (**11**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**) free bases in D_2O solution.

Carbon No.	**2**		**11**		**12**	
	pD 10.3	β-Shift	pD 10.9	β-Shift	pD 11.4	β-Shift
1	53.8		50.9		33.2	3.3
2	71.2	5.7	34.7	3.4	50.5	
3	79.9	5.8	74.1	3.0	85.9	7.5
4	60.8		63.3		61.0	
5	71.2	4.6	71.0	5.4	66.8	2.4
6	84.1	9.9	83.6	10.4	74.9	
NCH_3	35.4	3.1	31.2	3.4	33.6	2.4
OCH_3	59.2		56.9		60.4	
1′	102.5	6.5	101.4	5.4	99.8	4.5
2′	50.6		50.4		50.3	
3′	27.0	5.5	26.8	5.3	27.3	
4′	27.3		27.3		27.3	
5′	75.1	4.1	76.1	4.4	74.9	4.2
6′	50.4		49.3		49.9	
7′	18.5	3.4	18.0	2.8	18.5	3.5

Table 2. p.m.r. parameters of 2-deoxyfortimicin B (**11**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**).

11				**12**			
Chemical shift ppm		Coupling constant Hz		Chemical shift ppm		Coupling constant Hz	
H-1	3.45	$J_{1,2a}$	—				
H-2a	2.23	$J_{1,2e}$	4.5	H-1a	2.0~2.5	$J_{1a,2}$	9.2
H-2e	2.45	$J_{2a,3}$	—	H-1e	2.0~2.5	$J_{1e,2}$	4.0
H-3	4.14	$J_{2e,3}$	—	H-2	3.50	$J_{2,3}$	9.2
H-4	3.33	$J_{2a,2e}$	14	H-3	3.58	$J_{3,4}$	9.2
H-5	4.45	$J_{3,4}$	~4.0	H-4	3.14	$J_{4,5}$	2.5
H-6	3.98	$J_{4,5}$	4.3	H-5	4.63	$J_{5,6}$	2.5
H-1′	5.48	$J_{5,6}$	8.2	H-6	4.38	$J_{1a,6}$	2.5
H-2′	—	$J_{1,6}$	8.2	H-1′	5.31	$J_{1e,6}$	2.5
H-3′	1.8~2.6	$J_{1',2'}$	3.0	H-2′	—	$J_{1',2'}$	4.0
H-4′		$J_{6',7'}$	6.5	H-3′	1.4~2.6	$J_{6',7'}$	6.2
H-5′	—			H-4′			
H-6′	—			H-5′	—		
C-6′-CH_3	1.50			H-6′	—		
NCH_3	2.82			C-6′-CH_3	1.51		
OCH_3	3.86			NCH_3	2.83		
				OCH_3	4.02		

action gave 2-*O*-methanesulfonyl fortimicin B (**7**) tetrahydrochloride in excellent purity identical with that prepared as described above. The scope of the aldehydes useful in the route shown in Scheme 2 is unknown as the same reaction sequence using benzaldehyde gave a complex mixture of products. The utility of salicylaldehyde may be a function of the rate of formation of 4,5-salicylaldehyde oxazolidine. We have previously noted that in the case of the fortimicins, the formation of the 4,5-benzaldehyde oxazolidine is slower than formation of the 4,5-salicylaldehyde oxazolidine.[7]

Reductive opening of the C_1-C_2 epimino function would be expected to generate 2-deoxyfortimicin B (**11**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**). Accordingly, catalytic hydrogenation of **8** in the presence of RANEY nickel under a hydrogen atmosphere gave a mixture of **11** and **12** which were cleanly separated by ion-exchange chromatography.

The high resolution mass spectra of both **11** and **12** revealed 6-*epi*-purpurosamine derived fragments at *m/e* 143 (**19**). Also observed in the mass spectra of both **11** and **12** was a prominent cyclitol derived peak of $C_7H_{15}O_3N_2$ or 16 mass units less than the similar cyclitol derived fragment seen in the spectrum of fortimicin B (**2**). These results confirmed the empirical formulas of the aminocyclitol moiety of both **11** and **12**.

The structures of **11** and **12** were established by c.m.r. and p.m.r. spectroscopy (Tables 1, 2). The c.m.r. spectrum of **11** exhibited 15 resonances, seven of which could be closely correlated with the resonances of 6-*epi*-purpurosamine B. Further, the resonances at 31.2 ppm and 56.9 ppm were attributed to the *N*- and *O*-methyl groups by their quartet multiplicities displayed on the SFORD spectrum. The six remaining resonances were associated with the aminocyclitol ring carbons. Of these latter resonances, three in the range 71.1 ~ 86.3 ppm are deshielded by oxygen and two at 50.9 ppm and 63.3 ppm are deshielded by nitrogen. Of particular note was the large upfield shift in the resonances assigned to C_2 which was shown to be a triplet by SFORD. This resonance, at 34.7 ppm, was attributed to a methylene carbon derived from the deoxygenation. Protonation of the amine groups of **11** produced β shifts of four cyclitol ring carbon resonances as expected of a 1,4-diaminocyclitol. Finally, the p.m.r. spectrum of **11** (Table 2) was consistent with the proposed structure although resonance overlap, even at 270 MHz, prevented complete analysis of the cyclitol ring protons.

As expected, the c.m.r. spectrum of **12** (Table 1) showed the presence of 15 carbon atoms in the molecule. The presence of the 6-*epi*-purpurosamine moiety was readily confirmed when the chemical shifts of carbon atoms 1′-7′ were compared with the corresponding resonances of fortimicin B (**2**). Resonances attributed to the *N*- and *O*-methyl groups were assigned from quartet multiplicities shown on SFORD experiments. The chemical shifts of the six resonances attributed to the aminocyclitol carbons are consistent with the assigned structure. Noteworthy was the resonance at 33.2 ppm confirming the presence of a C_1 methylene carbon. The occurrence of an amino group at C_2 was confirmed by the three β shifts observed in the spectrum upon protonation which was consistent with a 1,3-diaminocyclitol.

Final confirmation of the structure of **12** was obtained from consideration of the p.m.r. spectrum (Table 2). A total analysis of the cyclitol ring proton resonances was possible from consideration of the 100 MHz and 270 MHz spectra. Spin decoupling experiments performed at 100 MHz determined the coupling pattern and coupling constants shown in Table 2. The large coupling constants found for $J_{2,3}$ and $J_{3,4}$ requires that protons at C_2, C_3 and C_4 be axial, while protons at C_5 and C_6 must be equatorial. This pattern is only consistent if the cyclitol moiety has adopted the cyclitol conformation found in fortimicin A (**1**) as shown in **12**.

The fortimicin B derivatives **11** and **12** were converted to the corresponding fortimicin A derivatives

15 and **18** by a procedure previously used.[5] Treatment of **11** and **12** with a slight excess of three equivalents of *N*-(benzyloxycarbonyloxy) succinimide gave, respectively, 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**13**) and 2,2′,6′-tri-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**16**). Acylation with *N*-(*N*-benzyloxycarbonylglycyloxy) succinimide gave, respectively, the tetra-*N*-benzyloxycarbonyl-4-*N*-glycyl derivatives **14** and **17**. Catalytic hydrogenolysis of the benzyloxycarbonyl groups with palladium on carbon in 0.2 N hydrochloric acid in methanol led, respectively, to 2-deoxyfortimicin A (**15**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**18**), both isolated as the tetrahydrochloride salts.

Antibacterial activities of 2-deoxyfortimicin A (**15**) tetrahydrochloride and 1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**18**) tetrahydrochloride, compared with fortimicin A (**1**) tetrahydrochloride, were determined by an agar dilution method in MUELLER-HINTON medium. When compared against 208 varied bacterial strains, 2-deoxyfortimicin A (**15**) was 35% more active than fortimicin A (**1**) without a significant change in breadth of the spectrum. 1-Deamino-2-deoxy-2-*epi*-aminofortimicin A (**18**) tetrahydrochloride was inactive against a wide variety of bacteria.

Experimental

General

Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter. I.r. spectra were recorded using a Perkin-Elmer Model 521 grating spectrometer. P.m.r. spectra were determined at either 100 MHz with a Varian Associates HA-100 spectrometer or at 270 MHz with a Bruker WH-270 spectrometer at ambient temperatures. Chemical shifts in $CDCl_3$ are reported relative to internal TMS. Chemical shifts for p.m.r. spectra determined in D_2O are reported relative to external TMS. C.m.r. spectra were measured on a Varian Associated/Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 70 eV and 100~150°C using the direct probe insert. Silica gel for chromatography refers to that of Merck (Darmstadt) 70~230 mesh. All evaporations were carried out under diminished pressure. Microanalytical results are reported for those compounds which could be freed of solvent.

1,2′,6′-Tri-*N*-benzyloxycarbonylfortimicin B-4,5-salicylaldehyde oxazolidine (**4**)

A solution of 22 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3**)[5] in 396 ml of methanol was treated with 3.96 ml of salicylaldehyde and refluxed for 1 hour. Evaporation of the methanol gave 26 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B-4,5-salicylaldehyde oxazolidine (**4**) as a brownish-yellow solid which was not purified further: i.r. ($CDCl_3$) 1661, 1629 and 1579 cm^{-1}; p.m.r. ($CDCl_3$) δ 0.94 (d, 3H, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.34 (s, 3H, C_4-NCH_3), 3.49 (s, 3H, C_3-OCH_3), 7.31 (m, 15H, Cbz-aromatic).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B-4,5-(2-*O*-methanesulfonylsalicylaldehyde) oxazolidine (**5**)

A stirring solution of 26 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B-4,5-salicylaldehyde oxazolidine (**4**) in 154 ml of dry pyridine was treated with 12.2 ml of freshly distilled methanesulfonyl chloride. After stirring for 20 hours, the reaction mixture was poured into 2,000 ml of 5% aqueous sodium bicarbonate solution. Chloroform extraction followed by evaporation and repeated co-distillation with benzene to remove the residual pyridine gave 31.2 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B-4,5-(2-*O*-methanesulfonylsalicylaldehyde) oxazolidine (**5**): i.r. ($CDCl_3$) 1150, 1352 and 1639 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.0 (d, 3H, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.19 (s, 3H, C_4-NCH_3), 2.94 (s, 3H, C_2-OSO_2CH_3), 3.15 (s, 3H, aromatic-OSO_2CH_3), 3.60 (s, 3H, C_3-OCH_3), 7.33 (m, 15H, Cbz-aromatic).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**6**)

A stirring solution of 31.2 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B-4,5-(2-*O*-methanesulfonylsalicylaldehyde) oxazolidine (**5**) in 1,000 ml of tetrahydrofuran was treated with 262 ml of 0.4 N hydrochloric acid. After stirring for 4 hours, the reaction mixture was poured into 5,700 ml of 6 N ammonium hydroxide and extracted with chloroform. The chloroform extract was washed with 7% aqueous sodium hydrogen sulfite solution followed by a water wash. Evaporation gave 27.3 g of residue which was chromatographed on a column of Sephadex LH-20 prepared and eluted with 95% ethanol. Fractions containing the major component were concentrated to dryness to give 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**6**): $[\alpha]_D^{23}+19°$ (*c* 1.0, methanol); i.r. ($CDCl_3$) 3436, 3350, 1703 and 1502 cm^{-1}; p.m.r. ($CDCl_3$) 1.07 (d, 3H, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 2.34 (s, 3H, C_4-NCH_3), 2.87 (s, 3H, C_2-OSO_2CH_3), 3.48 (s, 3H, C_3-OCH_3).

Anal. Calcd for $C_{40}H_{52}N_4O_{13}S$: C, 57.96; H, 6.32; N, 6.76.
Found: C, 57.65; H, 6.52; N, 6.62.

2-*O*-Methanesulfonylfortimicin B tetrahydrochloride (**7**)

A solution of 4.42 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**6**) in 310 ml of 0.2 N hydrochloric acid in methanol was treated for 4 hours with 4.5 g of 5% palladium on carbon under 3 atmospheres of hydrogen. The catalyst was filtered off and the filtrate was concentrated to dryness. Excess hydrochloric acid was removed by repeated co-distillation with methanol to leave 2.79 g of 2-*O*-methanesulfonylfortimicin B tetrahydrochloride (**7**): $[\alpha]_D^{25}+92°$ (*c* 1.01, methanol); i.r. (KBr) 3400, 2920 and 1590 cm^{-1}; p.m.r. (D_2O) δ 1.82 (d, 3H, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 3.31 (s, 3H, C_4-NCH_3), 3.88 (s, 3H, C_2-OSO_2CH_3), 4.07 (s, 3H, C_3-OCH_3), 5.88 (d, 1H, $H_{1'}$, $J_{1',2'}=4.0$ Hz).

2-Deoxy-1,2 (*R*)-epiminofortimicin B (**8**)

A solution prepared from 2.8 g of 2-*O*-methanesulfonylfortimicin B (**7**) tetrahydrochloride in 20 ml of water was passed through a column (2.2×20 cm) of an anion-exchange resin (AG® 2-X8, hydroxyl form). Basic eluates were allowed to stand at room temperature for 72 hours. Evaporation of the water gave a residue containing 2-deoxy-1,2(*R*)-epiminofortimicin B (**8**) and methanesulfonic acid. A second treatment with an anion-exchange resin, sufficient to remove the methanesulfonic acid, gave pure 2-deoxy-1,2(*R*)-epiminofortimicin B (**8**): $[\alpha]_D^{24}+102°$ (*c* 1.01, methanol); i.r. (KBr) 3360, 3270 and 1585 cm^{-1}; p.m.r. (D_2O) 1.55 (d, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 2.83 (s, C_4-NCH_3), 4.02 (s, C_3-OCH_3), 5.42 (d, $H_{1'}$, $J_{1',2'}=3.1$ Hz); mass spec. (M+·) meas. 330.2272, calcd. for $C_{15}H_{30}N_4O_4$ 330.2267.

2-Deoxyfortimicin B (**11**) and 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**)

A solution prepared from 3.22 g of 2-deoxy-1,2(*R*)-epiminofortimicin B (**8**) in 250 ml of wet ethanol was treated for 24 hours with 12 g of RANEY nickel under 3 atmospheres of hydrogen. The catalyst was removed by filtration and the filtrate was concentrated to dryness to give 2.90 g of colorless froth. The froth was chromatographed on a column (2.9×50 cm) of a cation-exchange resin (Bio Rex 70), ammonium form, and eluted with a gradient of water to 1 N ammonium hydroxide. The first eluates were lyophilized to give 1.347 g of 2-deoxyfortimicin B (**11**): $[\alpha]_D^{21}+86°$ (*c* 1.3, methanol); i.r. (KBr) 1590 and 1450 cm^{-1}; c.m.r. (see Table 1); p.m.r. (see Table 2); mass spec. (M+·) meas. 332.2412, calcd. for $C_{15}H_{32}N_4O_4$ 332.2424.

Later eluates were collected and lyophilized to give 1.172 g of 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**): $[\alpha]_D^{23}+134°$ (*c* 1.0, methanol); i.r. (KBr) 1582 and 1445 cm^{-1}; c.m.r. (see Table 1); p.m.r. (see Table 2); mass spec. (M+·) meas. 332.2412, calcd. for $C_{15}H_{32}N_4O_4$ 332.2424.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**13**)

A stirring, ice-bath cooled solution of 0.834 g of 2-deoxyfortimicin B (**11**) in 13 ml of water and 25 ml of methanol was treated with 2.09 g of *N*-(benzyloxycarbonyloxy) succinimide. After stirring in the cold for 3 hours and then at room temperature for 20 hours, the major portion of the methanol was evaporated. After the addition of water, the product was isolated by chloroform extraction. Evaporation gave a foam which was chromatographed on a column (2.3×70 cm) of silica gel prepared and eluted with a mixture of chloroform - methanol - concentrated ammonium hydroxide (23.4: 1.4: 0.1, v/v). Fractions containing the major component were taken to dryness to give 0.936 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**13**): $[\alpha]_D^{23}+63°$ (*c* 1.0, methanol); i.r. ($CDCl_3$) 1710 and 1502 cm^{-1};

p.m.r. ($CDCl_3$) δ 1.12 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.0 Hz), 2.26 (s, C_4-NCH_3), 3.29 (s, C_3-OCH_3), 4.78 (d, $H_{1'}$, $J_{1',2'}$=4.0 Hz), 7.31 (m, Cbz-aromatic).

Anal. Calcd. for $C_{39}H_{50}O_{10}N_4$: C, 63.74; H, 6.86; N, 7.62.
Found: C, 63.59; H, 7.02; N, 7.61.

1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**14**)

A stirring solution of 0.807 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**13**) in 14 ml of dry tetrahydrofuran was treated for 18 hours with 0.439 g of *N*-(benzyloxycarbonylglycyloxy) succinimide. The tetrahydrofuran was evaporated to give 1.231 g of colorless solid. The solid was chromatographed on a column (2.0×44 cm) of silica gel prepared and eluted with benzene - methanol - 95% ethanol - concentrated ammonium hydroxide (23.5: 1.5: 1.9: 0.2, v/v). Fractions containing the major component were taken to dryness and rechromatographed on a column of Sephadex LH-20 prepared and eluted with 95% ethanol. Eluates containing the major product were evaporated to give 0.623 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**14**): $[\alpha]_D^{23}$+114° (*c* 1.0, methanol); i.r. ($CDCl_3$) 1705, 1638 and 1500 cm^{-1}; p.m.r. ($CDCl_3$) δ 2.87 (s, C_4-NCH_3), 3.24 (s, C_3-OCH_3), 7.25 (Cbz-aromatic).

Anal. Calcd. for $C_{49}H_{59}O_{13}N_5$: C, 63.56; H, 6.42; N, 7.56.
Found: C, 63.60; H, 6.59; N, 7.58.

2-Deoxyfortimicin A (**15**)

A solution of 0.463 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**14**) in 60 ml of 0.2 N hydrochloric acid in methanol was hydrogenolyzed in the presence of 0.463 g of 5% palladium on carbon for 4 hours under 3 atmospheres of hydrogen. The catalyst was collected on a filter and the filtrate was concentrated to dryness to give a white solid. Repeated co-distillation with methanol removed excess hydrochloric acid to give 0.305 g of 2-deoxyfortimicin A (**15**) isolated as the tetrahydrochloride: i.r. (KBr) 1640 and 1489 cm^{-1}; p.m.r. (D_2O) δ 1.79 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 3.58 (s, -NCH_3), 3.90 (s, -OCH_3), 5.82 (d, $H_{1'}$, $J_{1',2'}$=3.5 Hz); mass spec., (M+•) meas. 389.2630. $C_{17}H_{35}N_5O_5$ requires 389.2638.

2,2',6'-Tri-*N*-benzyloxcarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**16**)

To a stirring ice bath cooled solution of 0.868 g of 1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**12**) in 12.5 ml of water and 25 ml of methanol was added 2.153 g of *N*-(benzyloxycarbonyloxy) succinimide. After stirring in the cold for 3 hours and then at room temperature for 20 hours, the major portion of the methanol was evaporated. Chloroform extraction followed by evaporation gave 1.537 g of residue which was chromatographed on a column (2.2×70 cm) of silica gel prepared and eluted with chloroform - methanol - concentrated ammonium hydroxide (23.4: 1.4: 0.1, v/v). Fractions containing the major polar component were taken to dryness to give 0.769 g of 2,2',6'-tri-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin B (**16**): $[\alpha]_D^{23}$+86° (*c* 1.25, methanol); i.r. ($CDCl_3$) 1707 and 1502 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.13 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 2.32 (s, C_4-NCH_3), 3.34 (s, C_3-OCH_3), 7.32 (Cbz-aromatic).

Anal. Calcd. for $C_{39}H_{50}O_{10}N_4$: C, 63.74; H, 6.86; N, 7.62.
Found: C, 63.65; H, 7.00; N, 7.76.

2,2',6',2''-Tetra-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**17**)

A stirring solution of 0.50 g of 2,2',6'-tri-*N*-benzyloxycarbonyl-1-deamino-2-*epi*-aminofortimicin B (**16**) in 8.5 ml of dry tetrahydrofuran was treated with 250 mg of *N*-(benzyloxycarbonylglycyloxy) succinimide. The tetrahydrofuran was evaporated to give a colorless froth which was chromatographed on a column (1.6×58 cm) of silica gel with ethyl acetate - hexane (80: 20, v/v) to give 0.386 g of 2,2',6',2''-tetra-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**17**): $[\alpha]_D^{23}$+37° (*c* 1.0, methanol); i.r. ($CDCl_3$) 1705, 1638 and 1502 cm^{-1}; p.m.r. ($CDCl_3$) δ 1.14 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.0 Hz), 2.89 (s, C_4-NCH_3), 3.23 (s, C_3-OCH_3), 7.30 (Cbz-aromatic).

Anal. Calcd. for $C_{49}H_{59}O_{13}N_5$: C, 63.56; H, 6.42; N, 7.56.
Found: C, 63.53; H, 6.73; N, 7.43.

1-Deamino-2-deoxy-2-*epi*-aminofortimicin A (**18**)

2,2',6',2''-Tetra-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**17**) (0.09 g)

was hydrogenolyzed and worked up as described for the preparation of **15**, to give 0.070 g of 1-deamino-2-deoxy-2-*epi*-aminofortimicin A (**18**) isolated as the tetrahydrochloride: i.r. (KBr) 1640 and 1487 cm^{-1}; p.m.r. (D_2O) δ 1.77 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 3.63 (s, C_4-NCH_3), 3.89 (s, C_3-OCH_3), 5.72 (d, $H_{1'}$, $J_{1',2'}$=3.5 Hz); mass spec., (M+•) meas. 389.2626. $C_{17}H_{35}N_5O_5$ requires 389.2638.

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