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PREPARATIONS OF 2-*epi*-FORTIMICINS A FROM 2-*epi*-FORTIMICIN B BY INTRAMOLECULAR BASE-CATALYZED 2-*O*-ACYLATION OF 1,2′,6′-TRI-*N*-BENZYLOXYCARBONYL-2-*epi*-FORTIMICIN B*

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ABSTRACT

Preparations of 2-*epi*-fortimicin A (**4**) from 2-*epi*-fortimicin B (**3**) are described. In contrast to the previously reported, selective 4-*N*-acylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**) with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B (**5**) underwent predominant 2-*O*,4-*N*-diacylation under similar conditions. Proof of the structure of the diacylated product is presented, with evidence that the diacylated product is formed by initial intramolecular, base-catalyzed 2-*O*-acylation. The *in vitro* antibacterial activities of 2-*epi*-fortimicin A (**4**), 2-*O*-glycyl-2-*epi*-fortimicin A (**11**), 1-*N*-glycyl-2-*epi*-fortimicin A (**12**), and 5-deoxy-2-*epi*-fortimicin A (**13**) are reported.

DISCUSSION

Methods for conversion of fortimicin B (**1**) into 2-*epi*-fortimicin B (**3**) were described in the previous paper[1]. It was hoped that conversion of **3** into 2-*epi*-fortimicin A (**4**) could be accomplished by the process previously reported for conversion[2] of fortimicin B (**1**) into fortimicin A (**2**). Although the synthesis of **4** from **3** has been accomplished, the chemistry of the 2-*epi*-fortimicins B, as a consequence of the *cis*-relationship of the 2-hydroxyl and the 4-methylamino groups, presented an interesting contrast with the chemistry of the fortimicins B in which the 2- and 4-substituents are *trans*.

Treatment of 2-*epi*-fortimicin B (**3**) with *N*-(benzyloxycarbonyloxy)succinimide gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B (**5**). Attempted conversion of **5** into 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**6**) with slightly more than one equivalent of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave recovered starting material and 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(*N*-benzyloxycarbonyl)glycyl-2-*epi*-fortimicin A (**7**), proof of the structure of which is described

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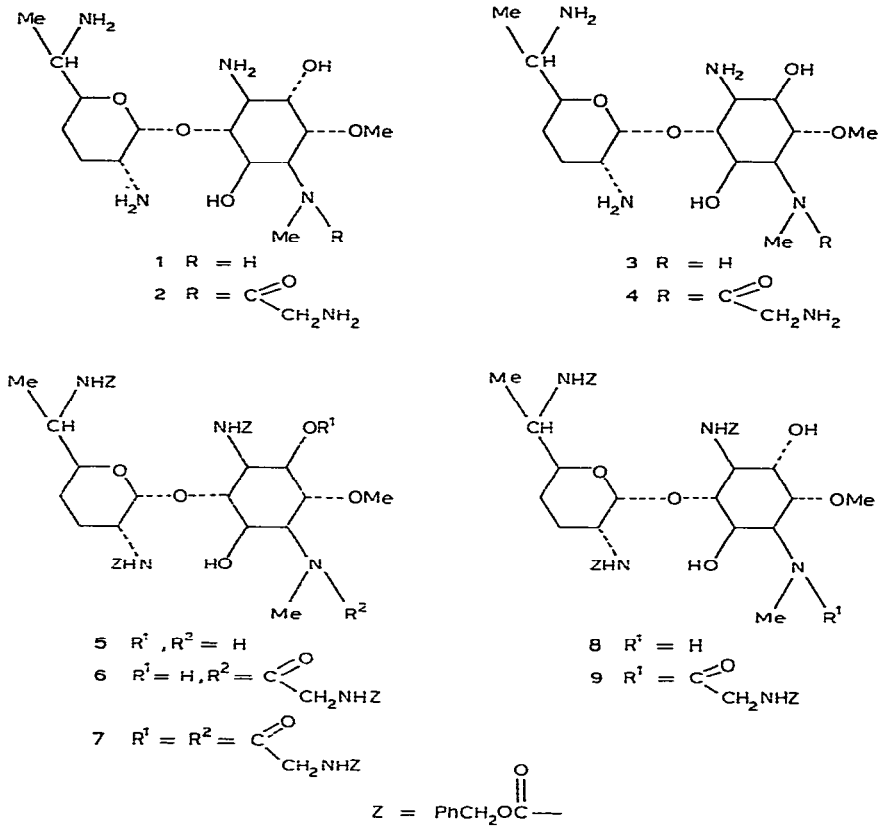
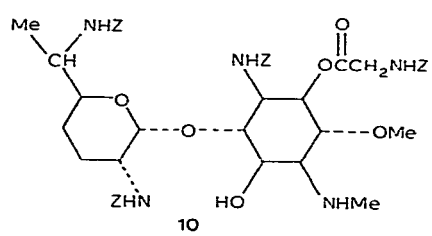


here. Under similar conditions, the 2-epimeric 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**) was converted into 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A (**9**) in ~70% yield[2]. Treatment of **5** with slightly more than 2 mol of *N*-(benzyloxycarbonylglycyloxy)succinimide gave the diacylated product **7** in 65% yield, together with 15% of the desired 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**6**). Mild, base-catalyzed hydrolysis, converted the diacylated product **7** into **6** in good yield.

Attempted acylation of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**6**) with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, under conditions that converted **5** primarily into the diacylated product **7**, gave only recovered starting material. This result established that **7** was formed from **5** by initial 2-*O*-acylation to form 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(*N*-benzyloxycarbonyl)glycyl-2-*epi*-fortimicin B (**10**), followed by 4-*N*-acylation of the latter. Formation of both the diacylated product **7** and 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**6**) on treatment of **5** with *N*-(benzyloxycarbonylglycyloxy)succinimide must be the result of competitive 4-*N*- *vs.* 2-*O*-acylation. Formation of the *O*-acylated intermediate **10** is



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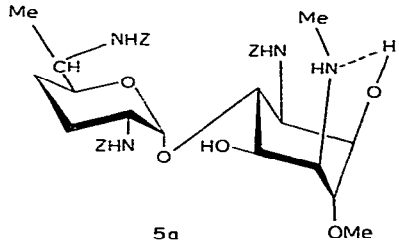

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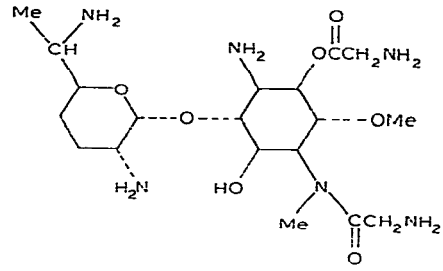

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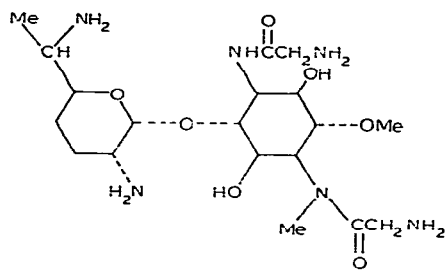

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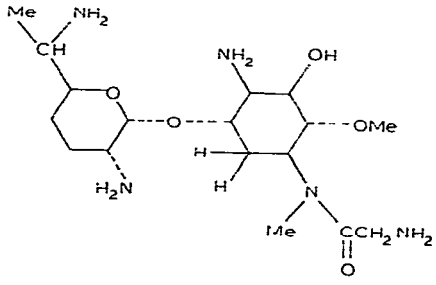

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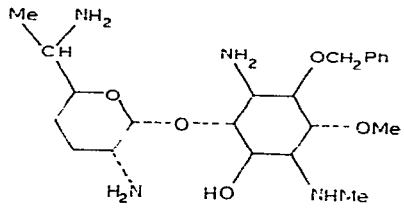

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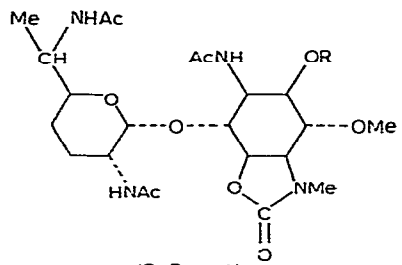
most probably the result of intramolecular, base-catalyzed *O*-acylation resulting from hydrogen bonding between the 2-hydroxyl and the 4-methylamino groups in that cyclitol conformation (**5a**) in which these groups have a 1,3-diaxial relationship.

Catalytic hydrogenolysis of **6** and **7** gave 2-*epi*-fortimicin A (**4**) and 2-*O*-glycyl-2-*epi*-fortimicin A (**11**), respectively, isolated as their perhydrochloride salts. The latter (**11**) was characterized by an ester-carbonyl band in its i.r. spectrum. Treatment of the pentahydrochloride salt of **11** with AG2-X8 (OH^-) resin in aqueous solution gave, by 2-*O*- to 1-*N*-migration, 1-*N*-glycyl-2-*epi*-fortimicin A (**12**), isolated as the tetrahydrochloride salt.

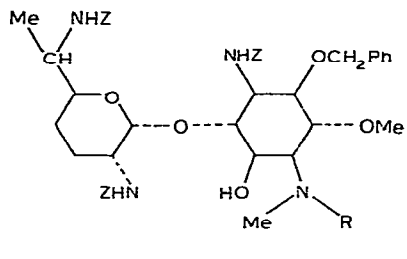
Comparison of the ^{1}H-n.m.r. spectra of the perhydrochloride salts of 2-*epi*-fortimicin A (**4**) and 2-*O*-glycyl-2-*epi*-fortimicin A (**11**) showed quartets for both, at δ 4.37 and δ 5.73, respectively, which may be attributed to the protons attached to C-2. [Because of peak overlap at 100 MHz, the H-2 resonance of 2-*epi*-fortimicin A (**4**) was determined at 270 MHz.] The difference between the chemical shifts of the quartets ($\Delta\delta$ 1.36) was that expected for the downfield shift on acylation of a

proton attached to the hydroxyl-bearing carbon of a secondary alcohol[3]. The magnitude of the coupling constants for **4** ($J_{1,2}$ 5.0 and $J_{2,3}$ 9.5 Hz) and **11** ($J_{1,2}$ 4.8 and $J_{2,3}$ 9.2 Hz) established that H-2 of both were axial, and thus that the solution conformations of the cyclitol rings of the salts of **4** and **11** were the same as the solution conformations of fortimicin A and its salts[4].

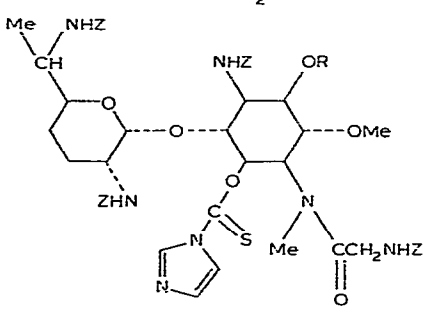
Chemical proof that the *O*-acyl groups of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(*N*-benzyloxycarbonyl)glycyl-2-*epi*-fortimicin A (**7**), and the pentahydrochloride salt of 2-*O*-glycyl-2-*epi*-fortimicin A (**11**) were attached to O-2 and not O-5 was



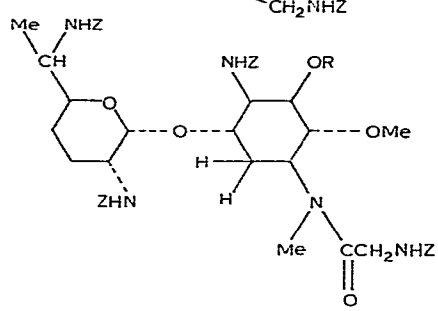
15 R = H
16 R = CH_2Ph



17 R = H
18 R = $C(=O)CH_2NHZ$



19 R = $C(=O)CH_2NHZ$
20 R = CH_2Ph



21 R = $C(=O)CH_2NHZ$
22 R = CH_2Ph
23 R = H

obtained by conversion of **7** into 5-deoxy-2-*epi*-fortimicin A (**13**) in a process whose key step is the Barton deoxygenation procedure[5]. An unambiguous synthesis of 5-deoxy-2-*epi*-fortimicin A (**13**) *via* the Barton procedure was performed, starting with 2-*O*-benzyl-2-*epi*-fortimicin B (**14**). The latter was prepared by conversion of 1,2′,6′-tri-*N*-acetyl-2-*epi*-fortimicin B 4,5-carbamate[1] (**15**) into the 2-benzyl ether (**16**), which gave **14** on base-catalyzed hydrolysis.

2-*O*-Benzyl-2-*epi*-fortimicin B (**14**) was converted into 2-*O*-benzyl-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B (**17**) with *N*-(benzyloxycarbonyloxy)succinimide. Treatment of **17** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave

2-*O*-benzyl-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**18**). Catalytic hydrogenation of **18** removed both the *N*-benzyloxycarbonyl groups and the *O*-benzyl group to give 2-*epi*-fortimicin A (**4**), identical with that prepared from 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**6**) as already described.

Both 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(*N*-benzyloxycarbonyl)glycyl-2-*epi*-fortimicin A (**7**) and 2-*O*-benzyl-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**18**) were converted into the 5-*O*-thiocarbonylimidazole esters **19** and **20**, respectively. Deoxygenation of **19** and **20** with tributylstannane gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(*N*-benzyloxycarbonyl)glycyl-5-deoxy-2-*epi*-fortimicin A (**21**) and 2-*O*-benzyl-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-5-deoxy-2-*epi*-fortimicin A (**22**), respectively. Mild base-catalyzed hydrolysis of the 2-*O*-(*N*-benzyloxycarbonyl)-glycyl group of **21** gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-5-deoxy-2-*epi*-fortimicin A (**23**). Catalytic hydrogenolysis of **23** gave 5-deoxy-2-*epi*-fortimicin A (**13**) identical with material of unambiguous structure prepared by hydrogenation of 2-*O*-benzyl-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*epi*-5-deoxyfortimicin A (**22**), thus providing conclusive chemical evidence that the *O*-acyl groups of the diacylated products **7** and **11** were attached to O-2.

Relevant ^{13}C-n.m.r. data are recorded in Table I. It may be noted that the ^{13}C-n.m.r. titration data for 1-*N*-glycyl-2-*epi*-fortimicin A (**12**) showed on protonation shifts only for the resonances of carbon atoms attached to the amino groups of the diamino sugar moiety. This confirmed that the acyl groups of **12** were attached to the nitrogen atoms of the cyclitol ring.

TABLE I

^{13}C-N.M.R. DATA[a]

C	**4**		**12**		**13**		**14**	
	pD 1.95	*β-Shift*	*pD 1.39*	*β-Shift*	*pD 3.9*	*β-Shift*	*pD 1.4*	*β-Shift*
1′	95.4	5.2	95.5	5.7	93.9	4.6	94.9	4.3
2′	51.9		51.9		51.3		51.6	
3′	21.5	5.6	21.8	6.0	21.6	5.7	21.4	5.6
4′	26.3		26.3		26.4		26.3	
5′	71.1	4.4	71.6	3.9	70.9	2.3	70.8	2.4
6′	49.5		49.4		49.3		49.0	
7′	15.2	3.6	15.3	3.5	15.1	2.2	15.5	3.3
1	54.5		54.6		51.7		53.9	
2	69.8	3.9	71.4		69.2	2.6	64.6	3.2
3	75.0		75.6		78.7		75.2	4.4
4	55.6		55.5		55.3		62.2	
5	70.9		71.0		26.4		71.2	3.4
6	73.3	3.7	75.6		70.9	4.3	76.5	3.1

[a] ^{13}C-N.m.r. spectra were determined as described in the accompanying paper. Solutions in D_2O ~ 10% (w/v), were used throughout.

TABLE II

in vitro ANTIBACTERIAL ACTIVITIES[a]

Organism	*Fort. A*[b] **2**	2-epi-*Fort. A*[b] **4**	*5-Deoxy-2-epi-Fortimicin A*[b] **13**	*2-O-Glycyl-2-epi-Fortimicin A*[c] **11**	*1-N-Glycyl-2-epi-Fortimicin A*[c] **12**
Staphylococcus aureus Smith	0.78	0.78	6.2	50	12.5
Streptococcus faecalis 10541	50	50	>100	>100	>100
Enterobacter aerogenes 13048	3.1	3.1	50	100	100
Escherichia coli Juhl	6.2	6.2	100	>100	>100
E. coli BL 3676 (Res)	25	25	>100	>100	>100
E. coli 76-2	3.1	3.1	50	100	100
Klebsiella pneumoniae 10031	1.56	3.1	50	100	50
K. pneumoniae KY 4262	6.2	12.5	>100	>100	>100
Providencia 1577	1.56	1.56	25	100	50
Pseudomonas aeruginosa BMH No. 10	0.78	0.78	25	50	25
P. aeruginosa KY 8512	12.5	12.5	>100	>100	>100
P. aeruginosa KY 8516	50	50	50	>100	>100
P. aeruginosa 209	>100	>100	>100	>100	>100
P. aeruginosa 27853	25	12.5	>100	>100	>100
Salmonella typhimurium Ed. No. 9	3.1	3.1	25	100	50
Serratia marcescens 4003	1.56	1.56	25	100	50
Shigella sonnei 9290	6.2	6.2	100	>100	>100
Proteus rettgeri U6333	6.2	6.2	>100	>100	>100
Proteus vulgaris JJ	6.2	6.2	50	>100	100
Proteus mirabilis Fin. No. 9	6.2	12.5	25	>100	100

[a]*in vitro* Antibacterial activities were determined by the serial, two-fold dilution method using Mueller–Hinton agar. [b]Assayed as disulfate salts. Activities expressed as μg of free base per mL. [c]Assayed as tetrahydrochloric salts. Activities expressed as μg of tetrahydrochloride salt per mL.

The *in vitro* antibacterial activities of the 2-*epi*-fortimicins are recorded in Table II. 2-*epi*-Fortimicin A (**4**) had activity about equal to that of fortimicin A. In contrast, 5-deoxy-2-*epi*-fortimicin A (**13**) and both the 2-*O*-glycyl- and 1-*N*-glycyl-2-*epi*-fortimicins A, (**11**) and (**12**), respectively, were essentially devoid of activity.

EXPERIMENTAL

General methods. — General procedures are reported in the accompanying paper[1].

1,2',6'-Tri-N-*benzyloxycarbonyl-2*-epi-*fortimicin B* (**5**). — To a magnetically stirred solution of 2-*epi*-fortimicin B (**3**, 2.9 g) in water (42 mL) and methanol (84 mL), cooled in an ice bath, was added 6.4 g of *N*-(benzyloxycarbonyloxy)succinimide. Stirring was continued for 3 h with cooling, and then overnight at room temperature. The resulting solution was added to 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The extract was dried (magnesium sulfate) and evaporated to give a glass (6.91 g). The latter was chromatographed on a column of 450 g of silica gel with 9:1 1,2-dichloroethane–ethanol to yield 3.1 g (50%) of **5**; $[\alpha]_D^{23}$ +59° (*c* 1.0, methanol), $\tilde{\nu}_{max}^{CDCl_3}$ 3440, 3330, and 1708 cm^{-1}; $\delta(CDCl_3)$: 1.04 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.40 (NCH_3), and 3.39 (OCH_3).

Anal. Calc. for $C_{39}H_{50}N_4O_{11}$: C, 62.38; H, 6.71; N, 7.46. Found: C, 62.11; H, 6.79; N, 7.36.

1,2',6',2''-Tetra-N-*benzyloxycarbonyl-2*-O-(N-*benzyloxycarbonyl*)*glycyl-2*-epi-*fortimicin A* (**7**) *and 1,2',6',2''-tetra*-N-*benzyloxycarbonyl-2*-epi-*fortimicin A* (**6**). — A solution of compound **5** (1.2 g), *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide (1.24 g), and oxolane (90 mL) was kept for 3 days at room temperature. The resulting solution was poured into 5% aqueous sodium hydrogencarbonate and extracted with chloroform. Evaporation of the extract left a glass (1.82 g). The product was chromatographed on a column of 200 g of silica gel with 19:1:0.1 1,2-dichloroethane–ethanol–water. Earlier fractions gave 1.17 g (65%) of **7**; $[\alpha]_D^{23}$ +22° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3432, 1752 (s), 1712, and 1638 cm^{-1}, $\delta(CDCl_3)$: 1.16 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3); 2.85, 3.0 (NCH_3, rotamers), and 3.33 (OCH_3).

Anal. Calc. for $C_{59}H_{68}N_6O_{17}$: C, 62.53; H, 6.05; N, 7.41. Found: C, 62.40; H, 6.05; N, 7.34.

Further elution of the column gave 0.222 g (15%) of **6**; $[\alpha]_D^{22}$ +43° (*c* 1.0, methanol), $\tilde{\nu}_{max}^{CDCl_3}$ 3436, 1710, and 1635 cm^{-1}; $\delta(CDCl_3)$: 1.15 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.87, 3.04 (NCH_3, rotamers), and 3.48 (OCH_3).

Anal. Calc. for $C_{49}H_{59}N_5O_{14}$: C, 62.47; H, 6.31; N, 7.47. Found: C, 62.67; H, 6.55; N, 7.26.

1,2',6',2''-Tetra-N-*benzyloxycarbonyl-2*-epi-*fortimicin A* (**6**). — A solution of compound **7** (1.0 g), 5 mL of 5% aqueous sodium hydrogencarbonate, and methanol (50 mL) was stirred overnight at room temperature. The mixture was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform. Evaporation

of the dried extract left a glass (0.972 g). Chromatography of the latter on a column of 90 g of silica gel with 9 : 1 ethyl acetate–hexane gave 0.628 g (76%) of **6**, identical with that already described.

1,2',6'-Tri-N-*acetyl-2*-O-*benzyl-2*-epi-*fortimicin* B *4,5-carbamate* (**16**). — To a stirred suspension of 1,2',6'-tri-*N*-acetyl-2-*epi*-fortimicin B 4,5-carbamate[1] (**15**, 2.67 g), barium oxide (2.22 g), and barium hydroxide octahydrate (2.86 g) in 134 mL of *N,N*-dimethylformamide, cooled in a 2-propanol–ice bath, was added 2.3 mL of α-bromotoluene. The mixture was stirred for 15 min in the cold bath, in a water–ice bath for 3.5 h, and then at room temperature overnight. The mixture was filtered through a Celite mat, and the mat was washed thoroughly with chloroform. The filtrates were combined and the solvent was evaporated. The residue was dissolved in chloroform and the solution again filtered through a Celite mat. The solvent was evaporated and residual *N,N*-dimethylformamide was removed by evaporation of toluene from the residue, leaving an oil (3.00 g). The latter product was chromatographed on a column of 250 g of silica gel with 9 : 1 1,2-dichloroethane–methanol to yield 1.83 g (58%) of **16**; $[\alpha]_D^{23}$ +52° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3439, 3312, 1742, and 1644 cm^{-1}; $\delta(CDCl_3)$: 1.20 d ($J_{6',7'}$ 6.6 Hz, 6'-CH_3), 1.90, 1.94; 1.96 (NCOCH_3), 2.82 (NCH_3), 3.42 (OCH_3), 4.62, 4.64 q (OCH_2Ph, central peaks of AB quartet, outer peaks too small to be observable), and 5.14 ($J_{1',2'}$ 3 Hz, 1'-H); *m/z* (M + 1) calc. for $C_{29}H_{43}N_4O_9$: 591.3030, measured 591.3053; (cyclitol fragment) calc. for $C_{18}H_{25}N_2O_6$: 365.1713, measured 365.1706; (diamino sugar fragment) calc. for $C_{11}H_{19}N_2O_3$: 227.1396, measured 227.1401.

2-O-*Benzyl-2*-epi-*fortimicin B* (**14**). — A solution of compound **16** (6.39 g) in 800 mL of 2M aqueous sodium hydroxide was heated for 3 days at 85° under nitrogen. The resulting solution was cooled to room temperature and brought to pH 7 by addition of M hydrochloric acid. The water was evaporated and residual water removed by evaporation of ethanol from the residue. The residue was treated with several portions of boiling ethanol, and the supernatants were filtered. The ethanol was evaporated, and the residue treated with several portions of boiling chloroform, and the supernatants were filtered and combined. Evaporation of the chloroform left a glass (5.53 g), which was chromatographed on a column of 450 g of silica gel packed and eluted with 10 : 1 : 1 dichloromethane–methanol–concentrated ammonium hydroxide to yield 3.24 g (68%) of **14**; $[\alpha]_D^{23}$ +96° (*c* 1.0, methanol), $\tilde{\nu}_{max}^{CDCl_3}$ 3372 and 3292 cm^{-1}; $\delta(CDCl_3)$: 1.05 d ($J_{6',7'}$ 6.3 Hz, 6'-CH_3), 2.44 (NCH_3), 3.51 (OCH_3), 4.69 (OCH_2Ph), and 4.90 d ($J_{1',2'}$ 3.4 Hz, 1'-H); (M^+·) calc. for $C_{22}H_{38}N_4O_5$: 438.2842, measured 438.2853; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1191; (cyclitol fragment) calc. for $C_{15}H_{25}N_2O_4$: 297.1814, measured 297.1811.

2-O-*benzyl-1,2',6'-tri*-N-*benzyloxycarbonyl-2*-epi-*fortimicin B* (**17**). — To a stirred solution of compound **14** (2.51 g), water (28 mL), and methanol (110 mL), cooled in an ice bath, was added *N*-(benzyloxycarbonyloxy)succinimide (4.4 g). Stirring was continued with cooling for 3 h and then overnight at room temperature. The resulting solution was poured into 5% aqueous sodium hydrogencarbonate and

extracted with several portions of chloroform. The combined extracts were dried and evaporated to give a glass (4.64 g). A sample (0.998 g) of this glass was chromatographed on a column of 100 g of silica gel with 99:1 ethyl acetate–triethylamine to yield 0.584 g (58%) of **17**; $[\alpha]_D^{23}$ +37° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3444, 3347, and 1704 cm^{-1}; $\delta(CDCl_3)$: 1.06 d ($J_{6',7'}$ 5.9 Hz, 6'-CH_3), 2.31 (NCH_3), and 3.44 (OCH_3).

Anal. Calc. for $C_{46}H_{56}N_4O_{11}$: C, 65.69; H, 6.71; N, 6.66. Found: C, 65.13; H, 7.01; N, 6.45.

*2-O-Benzyl-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-*epi-*fortimicin A* (**18**). — To a stirred solution of compound **17** (0.500 g) in oxolane (30 mL), cooled in an ice bath, was added *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide (0.182 g). Stirring was continued with cooling for 3 h and then overnight at room temperature. The resulting solution was poured into 5% aqueous sodium hydrogencarbonate and the suspension extracted with several portions of chloroform. Evaporation of the dried extract gave a glass (0.607 g), of which 0.600 g was chromatographed on a column of 60 g of silica gel with 1:1 1,2-dichloroethane–ethyl acetate to yield 0.413 g (67%) of **18**; $[\alpha]_D^{23}$ +25° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3433, 3335, 1710 and 1640 cm^{-1}; $\delta(CDCl_3)$: 1.18 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 2.36 (NCH_3), and 3.50 (OCH_3).

Anal. Calc. for $C_{56}H_{65}N_5O_{14} \cdot H_2O$: C, 64.04; H, 6.43; N, 6.67. Found: C, 64.46; H, 6.49; N, 6.74.

2-epi-Fortimicin A (**4**). — (*a*) A sample (1.25 g) of 2-*O*-benzyl-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-*epi*-fortimicin A (**18**) was hydrogenated for 4 h in 0.2M methanolic hydrochloric acid (100 mL) under 3 atm of hydrogen in the presence of 2.5 g of 5% palladium-on-carbon. The catalyst was removed by filtration and the solvent evaporated. Residual hydrochloric acid was removed by evaporation of methanol, leaving 0.668 g (100%) of **4** as the tetrahydrochloride salt: $[\alpha]_D^{22}$ +55° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{KBr}$ 1640 cm^{-1}; $\delta(D_2O)$: 1.81 d ($J_{6',7'}$ 6.7 Hz, 6'-CH_3), 3.63 (NCH_3), 4.06 (OCH_3), and 5.79 d ($J_{1',2'}$ 3.7 Hz, H-1'): ($M^{+\cdot}$) calc. for $C_{17}H_{35}N_5O_6$: 405.2587, measured 405.2580; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1178; (cyclitol fragment) calc. for $C_{10}H_{20}N_3O_4$: 246.1454, measured 246.1429.

A solution of the tetrahydrochloride salt of **4** (6.48 g) in water (25 mL) was applied to a column of AG1-X2 (SO_4^{2-}) resin, and the product was eluted with water. Lyophilization of the combined fractions containing the product gave 6.66 g (84%) of the disulfate salt of **4**; $[\alpha]_D^{23}$ +58° (*c* 1.0, water); $\delta(D_2O)$: 1.82 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 3.66 (NCH_3), 4.09 (OCH_3), 5.82 d ($J_{1',2'}$ 3.4 Hz, 1'-H).

Anal. Calc. for $C_{17}H_{39}N_5O_{14}S \cdot 4\ H_2O$: C, 30.30; H, 7.03; N, 10.39. Found: C, 30.61; H, 6.29; N, 10.49.

(*b*) Compound **6** (0.110 g) was hydrogenated for 4 h in 0.1M methanolic hydrogen-chloride (19 mL) under 3 atm of hydrogen in the presence of 0.110 g of 5% palladium-on carbon. Isolation as before gave 0.063 g (98%) of **4** as the tetrahydrochloride salt, identical with that prepared as already described.

*2-O-Glycyl-2-*epi*-fortimicin A* (**11**). — Compound **7** (0.1819 g) was hydrogenated for 4 h in a solution containing 16 mL of 0.2M hydrochloric acid in methanol and

14 mL of methanol under 3 atm of hydrogen in the presence of 0.2 g of 5% palladium-on-carbon. Conventional isolation gave 0.077 g (74%) of **11** as the pentahydrochloride salt; $[\alpha]_D^{23}$ +50° (*c* 1.0, methanol) $\tilde{\nu}_{max}^{KBr}$ 1750 cm^{-1}; $\delta(D_2O)$: 1.82 d ($J_{6',7'}$ 6.7 Hz, 6'-CH_3), 3.67 (NCH_3), 4.02 (OCH_3), 5.84 d ($J_{1',2'}$ 3 Hz, 1'-H), and 6.16 q ($J_{1,2}$ 4, $J_{2,3}$ 9 Hz, H-2); ($M^{+\cdot}$) calc. for $C_{19}H_{38}N_6O_7$: 462.2802, measured 462.2777; (cyclitol fragment); calc. for $C_{12}H_{23}N_4O_5$: 303.1668, measured 303.1672; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1178.

1-N-*Glycyl-2*-epi-*fortimicin A* (**12**). — An aqueous solution of the pentahydrochloride salt of 2-*O*-glycyl-2-*epi*-fortimicin A (**11**, 0.431 g) was applied to a column of 25 mL of AG2-X8 (OH) resin (50–100 mesh). The basic eluate was collected, and the resulting aqueous solution was kept for 1 h at room temperature. The resulting solution was then brought to pH 1 by addition of 0.2M hydrochloric acid. The water was evaporated and residual water was removed by evaporation of ethanol and then methanol, leaving 0.345 g (85%) of **12** as the tetrahydrochloride salt: $[\alpha]_D^{24}$ +69° (*c* 1.0, methanol), $\tilde{\nu}_{max}^{KBr}$ 1642 cm^{-1}; $\delta(D_2O)$: 1.82 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 3.64 (NCH_3), 4.06 (OCH_3), and 5.72 ($J_{1',2'}$ 3.6 Hz, 1'-H); ($M^{+\cdot}$) calc. for $C_{19}H_{38}N_6O_7$: 462.2802, measured 462.2777; (cyclitol fragment) calc. for $C_{12}H_{23}N_4O_5$: 303.1668, measured 303.1683; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1173.

1,2',6',2''-Tetra-N-*benzyloxycarbonyl-2*-O-(N-*benzyloxycarbonyl*)*glycyl-5*-O-*thiocarbonylimidazoyl-2*-epi-*fortimicin A* (**19**). — A solution of compound **7** (0.600 g), 1,1'-thiocarbonyldiimidazole (0.436 g), triethylamine (0.6 mL), and 1,2-dichloroethane (22 mL) was boiled under reflux for 1.5 h. The solution was cooled and the solvent evaporated leaving a dark-brown oil (1.25 g), which was chromatographed on a column of 60 g of silica gel with ethyl acetate to yield 0.565 g (88%) of pure **19**: $\tilde{\nu}_{max}^{CDCl_3}$ 3440, 3360(s), 1755(s), 1712, and 1652 cm^{-1}; $\delta(CDCl_3)$: 1.13 d ($J_{6',7'}$ 6.2 Hz, 6'-CH_3), 2.80 (NCH_3), and 3.42 (OCH_3).

1,2',6',2''-Tetra-N-*benzyloxycarbonyl-2*-O-(N-*benzyloxycarbonyl*)*glycyl-5-deoxy-2*-epi-*fortimicin A* (**21**). — To a stirred refluxing solution of tributylstannane (2 mL) in 1,4-dioxane (45 mL) under an atmosphere of nitrogen was added, dropwise, a solution of compound **19** (0.532 g) in 1,4-dioxane (15 mL). After the addition was complete, refluxing was continued for 2 h. The resulting solution was cooled to room temperature, and the solvent evaporated. Hexane (~20 mL) was added and the resulting mixture was kept overnight at room temperature. The hexane supernatant was removed by decantation and the residue chromatographed on a column of 50 g of silica gel with 4:1 ethyl acetate–hexane to yield 0.307 g (63%) of **21**; $[\alpha]_D^{24}$ +18° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3435, 1752, 1714, and 1652 cm^{-1}; $\delta(CDCl_3)$: 1.15 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.80 (NCH_3), and 3.31 (OCH_3).

Anal. Calc. for $C_{59}H_{68}N_6O_{16} \cdot H_2O$: C, 62.42; H, 6.22; N, 7.41. Found: C, 62.20; H, 6.41; N, 8.53.

2-O-*Benzyl-1,2',6',2''-tetra*-N-*benzyloxycarbonyl-5*-O-*thiocarbonylimidazoyl-2*-epi-*fortimicin A* (**20**). — A stirred solution of compound **18** (1.64 g) 1,1'-thiocarbonyldiimidazole (1.31 g) of triethylamine (1.3 mL), and 1,2-dichloroethane (65 mL) was

boiled under reflux for 8 h. The solvent was evaporated and the residue chromatographed on a column of 200 g of silica gel with 9:1 (v/v) ethyl acetate–hexane to yield 1.95 g (110%, not further purified) of **20**; $\tilde{\nu}_{max}^{CDCl_3}$ 3425, 3370(s), 1710, and 1647 cm^{-1}; $\delta(CDCl_3)$: 1.16 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.80 (NCH_3), and 3.09 (OCH_3).

*2-O-Benzyl-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-5-deoxy-2-*epi*-fortimicin A* (**22**). — To a stirred, refluxing solution of tributylstannane (8 mL) in 150 mL of 1,4-dioxane, under an atmosphere of nitrogen, was added, dropwise, a solution of compound **20** (1.95 g) in 50 mL of 1,4-dioxane. After the addition had been completed, heating was continued for 2 h. The resulting solution was cooled to room temperature and the 1,4-dioxane was evaporated. Hexane (60 mL) was added, and the resulting mixture was kept overnight at room temperature. The hexane was removed by decantation, and the residue (2.9 g) was chromatographed on a column of silica gel with 7:3 ethyl acetate–hexane to yield 0.694 g (40%) of **22**; $[\alpha]_D^{24}$ +33° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3432, 3350(s), 1712, and 1647 cm^{-1}; δ(pyridine, room temperature, rotamers): 1.42 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 2.74, and 2.87 (NCH_3), 3.40, 3.44 (OCH_3); δ(pyridine, 110°): 1.32 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 2.80 (NCH_3), and 3.42 (OCH_3).

Anal. Calc. for $C_{56}H_{65}N_5O_{13}$: C, 66.19; H, 6.45; N, 6.89. Found: C, 65.43; H, 6.54; N, 6.82.

*1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-5-deoxy-2-*epi*-fortimicin A* (**23**). — A solution of **21** (0.270 g), 5% aqueous sodium hydrogencarbonate (1.6 mL), and methanol (16 mL) was stirred for 2 days at room temperature. The mixture was poured into 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The dried extract was evaporated to give a glass (0.253 g), chromatography of which, (0.218 g) on a column of 25 g of silica gel with ethyl acetate, gave 0.139 g (62%) of **23**; $[\alpha]_D^{23}$ +43° (*c* 1.0, methanol); $\tilde{\nu}_{max}^{CDCl_3}$ 3437, 3337, 1710, and 1647 cm^{-1}; δ(pyridine, room temperature, rotamers): 1.36 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.76, 2.89 (NCH_3), 3.48, 3.51 (OCH_3); δ(pyridine, 110°): 1.29 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.84 (NCH_3), and 3.49 (OCH_3).

Anal. Calc. for $C_{49}H_{59}N_5O_{13}$: C, 63.55; H, 6.42; N, 7.56. Found: C, 64.02; H, 6.79; N, 7.07.

*5-Deoxy-2-*epi*-fortimicin A* (**13**). — (*a*) Compound **23** (0.579 g) in 0.2M hydrochloric acid in methanol (50 mL) was hydrogenated under 3 atm of hydrogen for 4 h in the presence of 0.58 g of 5% palladium-on-carbon. The catalyst was removed by filtration and the solvent evaporated, leaving 0.307 g (92%) of **13** as the tetrahydrochloride salt. An aqueous solution of the latter was passed through a column of 16 mL of AG1-X2 (SO_4^{2-}) resin (50–100 mesh). Lyophilization of the eluate containing the product gave 0.319 g (82%) of **13** as the disulfate salt; $[\alpha]_D^{23}$ +50° (*c* 1.0, water); $\delta(D_2O)$: 1.35 d ($J_{6',7'}$ 6.8 Hz, 6'-CH_3), 3.05 (NCH_3), 3.54 (OCH_3), 4.09 ($COCH_2NH_2$), and 5.37 ($J_{1',2'}$ 3.0 Hz, 1'-H); ($M^{+\cdot}$) calc. for $C_{17}H_{35}N_5O_5$: 389.2638, measured 389.2641; (cyclitol fragment) calc. for $C_{10}H_{22}N_3O_4$: 248.1610, measured 248.1608; (diamino sugar fragment) calc. for $C_7H_{15}N_2O$: 143.1184, measured 143.1184.

Anal. Calc. for $C_{17}H_{39}N_5O_{13}S_2 \cdot 5\ H_2O$: C, 32.37; H, 7.03; N, 11.11. Found:

C, 32.30; N, 6.92; N, 11.11.

(*b*) Compound **22** (0.514 g) in 0.2M hydrochloric acid in methanol (50 mL) was hydrogenated for 4 h under 3 atm of hydrogen in the presence of 1.0 g of 5% palladium-on-carbon. The catalyst was removed by filtration and the solvent evaporated leaving 0.262 g (97%) of **13** as the tetrahydrochloride salt. An aqueous solution of the latter was passed through 17 mL of AG1-X2 (SO_4^{2-}) resin. Lyophilization of the eluate containing the product gave 0.270 g (82%) of **13** as the disulfate salt, identical with that prepared as already described from **21**.

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