3-AMINO-3-DEMETHOXYFORTIMICIN A AND THE C-2 EPIMERIC-2-AMINO-3-*O*-DEMETHYL-2-DEOXYFORTIMICINS A

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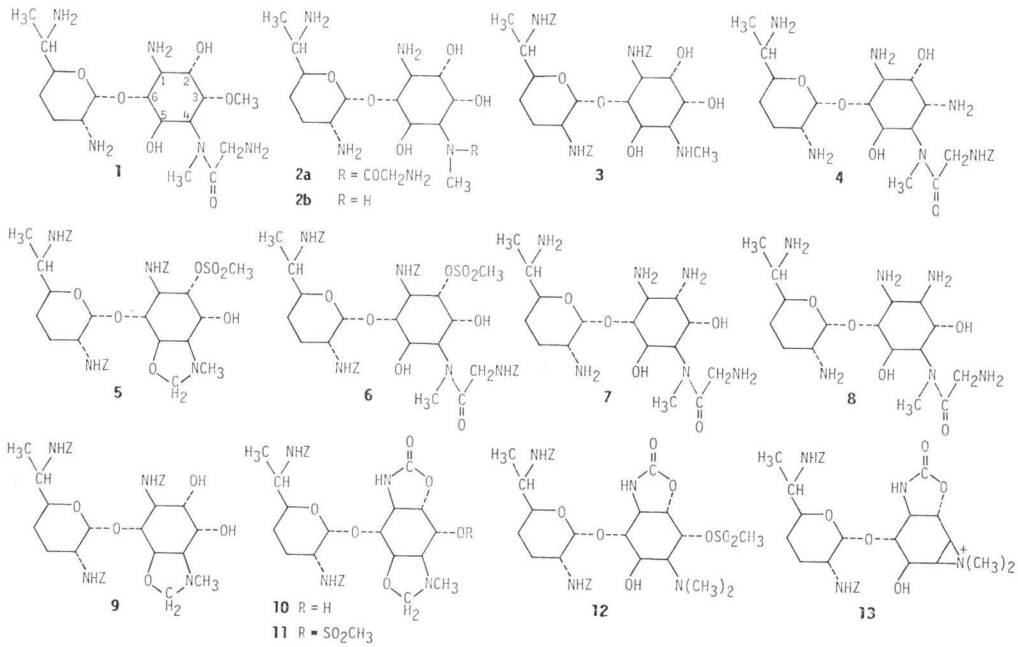
Selective reactions of 3-*O*-demethyl-3-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin derivatives have been used as the key steps in the syntheses of 3-amino-3-demethoxyfortimicin A and the C-2 epimeric 2-amino-3-*O*-demethyl-2-deoxyfortimicins A. *In vitro* antibacterial activities of the new fortimicin derivatives are reported.

Among the more active derivatives of fortimicin A (**1**)[1,2], are those modified at the C-2 and C-3 positions of the cyclitol ring. These include 2-deoxyfortimicin A[3], 2-*epi*-fortimicin A[4], 3-*O*-demethylfortimicin A[5], and 3-demethoxy-2-deoxyfortimicin A[6]. The 3-*O*-demethylfortimicins (**2a** and **2b**) are of particular interest, not only because of the high antibacterial activity of 3-*O*-demethylfortimicin A (**2a**)[5], but also because the presence of the C-3 hydroxyl groups in the 3-*O*-demethylfortimicins offers unique possibilities for preparation of both C-2 and C-3-modified fortimicins. We have reported elsewhere the preparation from 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**3**)[5] of 3-demethoxy-2-deoxyfortimicin A[6] and the C-2 epimeric 3-*O*-demethylfortimicins A[7]. We here report the preparations from **3** of 3-amino-3-demethoxyfortimicin A (**4**) as well as a novel process for converting **3** to



the 3-*O*-demethyl-2-*O*-methanesulfonylfortimicin derivatives **5** and **6** which were used to prepare the C-2 epimeric 2-amino-3-*O*-demethylfortimicins A (**7** and **8**) employing methodology developed in the fortimicin series[8].

3-Amino-3-demethoxyfortimicin A (4)

Treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**3**) with formaldehyde in aqueous methanol gave the oxazolidine **9** which was converted to the 1,2-carbamate **10** with sodium hydride in dimethylformamide. The carbamate was converted to the methanesulfonate **11** with methanesulfonyl chloride in pyridine. Treatment of **11** with sodium cyanoborohydride and sodium azide in methanol in the presence of formaldehyde and acetic acid gave the azide **14**. Based on the conversion of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B to the 4-*N*-methyl derivative with sodium cyanoborohydride and formaldehyde in methanol[9], the conversion of **11** to **14** may be formulated as proceeding by initial reduction of **11** to the dimethylamine **12**, cyclization of **12** to the dimethylaziridinium ion **13**, and *trans* opening of the latter by attack of azide ion at C-3 to give the 3-azide **14**. Rearrangement of the 1,2-carbamate **14** to the isomeric 1,5-carbamate **15** was carried out in aqueous methanol in the presence of sodium bicarbamate. A similar rearrangement was observed in the parent fortimicin series[9].

Hydrolysis of **15** with potassium hydroxide in aqueous ethanol gave 3-azido-3-demethoxy-4-*N*-methylfortimicin B (**16**) which was converted to the tri-*N*-benzyloxycarbonyl derivative **17**. Treatment of the latter with iodine and sodium acetate in methanol with irradiation by a 150 W flood lamp, followed by addition of formaldehyde, gave the 4,5-formaldehyde oxazolidine **18***. The latter, **18**, was converted to the hydroxy amine **19** by mild, acid-catalyzed hydrolysis in the presence of hydroxylamine hydrochloride as an aldehyde scavenger. The azide **19** proved resistant to the normal conditions of 4-*N*-acylation using *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide which were successful with other fortimicin derivatives[3~10]. The desired conversion of **19** to **20** was carried out using *N*-benzyloxycarbonylglycyl anhydride**. Catalytic hydrogenation of **20** reduced the azide group to the amino group and removed the *N*-benzyloxycarbonyl protecting groups to give 3-amino-3-demethoxyfortimicin A (**4**).

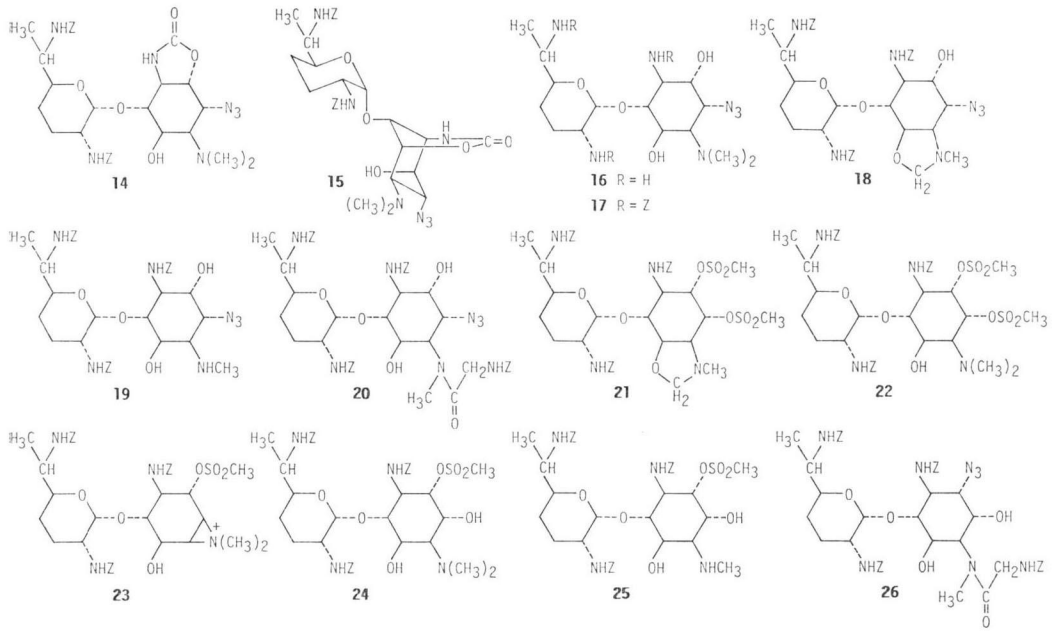
2-Amino-3-*O*-demethyl-2-deoxyfortimicin A (7) and 2-Amino-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (8)

The oxazolidine **9** was converted to the dimethanesulfonate **21** with methanesulfonyl chloride in pyridine. Treatment of **21** with sodium cyanoborohydride and formaldehyde in methanol in the presence of acetic acid gave the dimethylamino monomethanesulfate **24** in a process which is believed to proceed *via* the dimethylamino dimethanesulfonate **22** and the aziridinium ion **23** in which formation of **24** would occur by *trans* opening of the aziridinium ring of **23** by attack of water at C-3. Treatment of **24** with iodine and sodium acetate in methanol followed by addition of formaldehyde gave **5**. Mild acid-catalyzed hydrolysis of **5** in the presence of hydroxylamine hydrochloride as an aldehyde scavenger gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin B (**25**). Acylation of **25** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin A (**6**).

Treatment of **6** with sodium azide in dimethylformamide gave 2-azido-1,2′,6′,2″-tetra-*N*-benzyl-

* Preliminary studies indicated that the oxazolidine **18** was formed directly in the de-*N*-methylation reaction. In the reaction cited, formaldehyde was added to facilitate isolation of the oxazolidine **18** uncontaminated by the free hydroxy amine **19**.

** The authors are grateful to Dr. A. M. THOMAS for suggesting this reagent.



oxycarbonyl-3-*O*-demethyl-2-deoxyfortimicin A (**26**). Catalytic hydrogenation of **26** gave 2-amino-3-*O*-demethyl-2-deoxyfortimicin A (**7**), the ^{1}H NMR spectrum of which was identical to that of a sample prepared by boron tribromide-catalyzed 3-*O*-demethylation[11] of 2-amino-2-deoxyfortimicin A[8].

Treatment of the 2-*O*-methanesulfonyl oxazolidine **5** with sodium azide in dimethylformamide gave the 2-*epi* azide **27**. The contrast of the stereochemistries at C-2 of the displacements **6**→**26** (retention) and **5**→**27** (inversion) is analogous to observations in the parent fortimicin series, and is believed to be conformationally derived[8].

Mild acid-catalyzed hydrolysis of **27** in the presence of hydroxylamine hydrochloride gave 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin B (**28**). The latter was converted to the fortimicin A derivative **29** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Treatment of **29** with zinc in acetic acid gave the 2-*epi* amine **30** which on catalytic hydrogenation gave 2-amino-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**8**).

The *in vitro* antibacterial activities are recorded in Table 1. Whereas the antibacterial activities of both 3-amino-3-demethoxyfortimicin A (**4**) and 2-amino-3-*O*-demethyl-2-deoxyfortimicin A (**7**) are equal to or somewhat better than fortimicin A, 2-amino-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**8**) is almost devoid of activity.

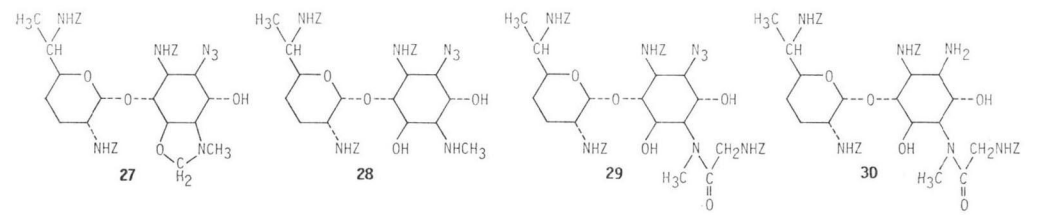


Table 1. *In vitro* antibacterial activities* (minimum inhibitory concentrations, μg/ml).

Organism	Fortimicin A	3-Amino-3-demethoxy-fortimicin A (4)	2-Amino-3-*O*-demethyl-2-deoxy-fortimicin A (7)	2-Amino-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (8)
Staphylococcus aureus Smith	1.56	0.78	0.78	25
Streptococcus faecalis 10541	25	100	50	>100
Enterobacter aerogenes 13048	3.1	3.1	6.2	50
Escherichia coli JUHL	3.1	3.1	6.2	50
E. coli BL 3676 (Res)	25	25	25	>100
E. coli 76-2	3.1	3.1	3.1	50
Klebsiella pneumoniae 10031	1.56	1.56	1.56	50
K. pneumoniae KY 4262	3.1	6.2	6.2	100
Proteus inconstans 1577	1.56	3.1	1.56	50
Pseudomonas aeruginosa BMH #10	0.78	0.78	1.56	25
P. aeruginosa KY 8512	6.2	3.1	6.2	50
P. aeruginosa KY 8516	6.2	3.1	3.1	—
P. aeruginosa 209	>100	>100	100	>100
P. aeruginosa 27853	6.2	3.1	3.1	100
Salmonella typhimurium Ed. #9	3.1	3.1	3.1	50
Serratia marcescens 4003	1.56	3.1	3.1	100
Shigella sonnei 9290	6.2	6.2	6.2	50
Proteus rettgeri U6333	6.2	6.2	12.5	50
P. vulgaris JJ	3.1	3.1	3.1	100
P. mirabilis Fin #9	6.2	3.1	3.1	100

* 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.

Experimental

General

Optical rotations were determined with a Perkin Elmer Model 421 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 on an A.E.I. MS-902 spectrometer at 70 eV and 150~200°C using the direct probe insert. Silica gel for column chromatography 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 reaction mixtures with mixtures of chloroform and 5% aqueous $NaHCO_3$. 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-4-*N*,5-*O*-methylenefortimicin B (9)

A solution of 4.02 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**3**), 2 ml of 37% formaldehyde, and 400 ml of methanol was stirred at room temperature for 18 hours. Solvent was evaporated under reduced pressure leaving 4.08 g of **9** as a white glass: δ ($CDCl_3$) 1.02 d ($J_{6',7'}$=6.4 Hz, 6′-CH_3), 2.15 (NCH_3).

2′,6′-Di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-4-*N*,5-*O*-methylenefortimicin B (10)

To a stirred solution of 6.01 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-4-*N*,5-*O*-methylenefortimicin B (**9**) in 120 ml of dimethylformamide, under a nitrogen atmosphere, was added 1.84 g of a 57% oily dispersion of sodium hydride. Stirring was continued with cooling for 1 hour and then at room temperature for 18 hours. The resulting solution was cooled in an ice bath, and 20 ml of a solu-

tion of 1 : 2 acetic acid - water was added cautiously. The product (6.13 g of brown foam) was isolated by chloroform extraction. Chromatography of the product on a column of 450 g of silica gel packed and eluted with ethyl acetate - triethylamine (20: 0.1) gave 3.21 g of **10** as a white glass: δ ($CDCl_3$) 1.14 d ($J_{6',7'}$=6.8 Hz, 6'-CH_3), 2.25 (NCH_3), 3.78 d, 4.59 d (J=2 Hz, OCH_2NCH_3). ν_{max} ($CDCl_3$) 3602, 3440, 3320 sh, 1770, 1718 cm^{-1}.

2',6'-Di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**11**)

To a stirred solution of 1.01 g of 2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-4-*N*,5-*O*-methylenefortimicin B (**10**) in 18 ml of pyridine, cooled in an ice bath, was added 0.36 ml of methanesulfonyl chloride. Stirring was continued with cooling for 1 hour and then at room temperature overnight. The product (1.20 g of **11** as a glass) was isolated by chloroform extraction: δ ($CDCl_3$) 1.26 d ($J_{6',7'}$=6.7 Hz, 6'-CH_3), 2.39 (NCH_3), 3.10 (OSO_2CH_3), 3.89 d, 4.62 d (J=2.6 Hz, OCH_2-NCH_3). ν_{max} ($CDCl_3$) 3443, 2298, 1778, 1715, 1178 cm^{-1}.

3-Azido-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-demethoxy-4-*N*-methylfortimicin B (**14**) and 3-Azido-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,5-*O*-carbonyl-3-demethoxy-4-*N*-methylfortimicin B (**15**)

A suspension prepared from 1.03 g of 2',6'-di-*N*-benzyloxycarbonyl-1-*N*,2-*O*-carbonyl-3-*O*-demethyl-3-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**11**), 0.236 g of sodium cyanoborohydride, 0.8 g of sodium azide, 0.8 ml of 37% formaldehyde, 0.4 ml of acetic acid, and 10 ml of methanol was stirred at room temperature for 40 hours. Isolation of the product by chloroform extraction gave 0.895 g of **14** as a glass: δ ($CDCl_3$) 1.15 d ($J_{6',7'}$=6 Hz, 6'-CH_3), 2.38 [$N(CH_3)_2$]. ν_{max} ($CHCl_3$) 3442, 3318, 2106, 1774, 1712 cm^{-1}.

A milky suspension of 0.832 g of **14**, 80 ml of methanol and 25 ml of 5% aqueous sodium bicarbonate was stirred at room temperature for 24 hours. Chloroform extraction gave 0.824 g of a glass. Chromatography of a sample of 9.80 g of product prepared as described above, on a column of 400 g of silica gel packed and eluted with ethyl acetate gave 5.61 g of **15**: $[\alpha]_D^{23}$ +93° (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.15 d ($J_{6',7'}$=6.5 Hz, 6'-CH_3), 2.39 [$N(CH_3)_2$]. ν_{max} ($CHCl_3$) 3520, 3442, 2112, 1718 cm^{-1}.

Anal. Calcd. for $C_{32}H_{41}N_7O_9$: C 57.56, H 6.19, N 14.68.
Found: C 56.94, H 6.03, N 14.57.

3-Azido-3-demethoxy-4-*N*-methylfortimicin B (**16**)

A solution prepared from 5.61 g of 3-azido-2',6'-di-*N*-benzyloxycarbonyl-1-*N*,5-*O*-carbonyl-3-demethoxy-4-*N*-methylfortimicin B (**15**), 70 ml of 6 N potassium hydroxide, and 140 ml of ethanol was heated at 85~90°C overnight. Water (100 ml) was added, and the resulting solution was brought to pH 1 by addition of 1 N hydrochloric acid. Solvent was evaporated under diminished pressure. The residue was triturated with methanol, and insoluble salts were removed by filtration. Evaporation of the methanol from the filtrate under diminished pressure left 5.67 g of product which was chromatographed on a column of 350 g of silica gel packed and eluted with a solvent system composed of chloroform - methanol - concentrated ammonium hydroxide (1: 1: 0.1) to yield 2.73 g of **16**. The latter was dissolved in 200 ml of methanol and the resulting solution was brought to pH 1 by addition of 0.4 N hydrochloric acid in methanol. Solvent was evaporated under diminished pressure and residual hydrochloric acid was removed by co-distillation under reduced pressure first with ethanol and then with methanol to give 3.10 g of the tetrahydrochloride of **16**: δ (D_2O, pH 4.86) 1.36 d ($J_{6',7'}$=6.8 Hz, 6'-CH_3), 3.09 [$N(CH_3)_2$], 5.37 d ($J_{1',2'}$=3.2 Hz, 1'-H). *m/z*: M^+; Meas. 374.2528, Calcd. for $C_{15}H_{32}N_7O_4$ 374.2516, cyclitol; meas. 232.1413, calcd. for $C_8H_{18}N_5O_3$ 232.1410, diaminosugar; meas. 143.1184, calcd. for $C_7H_{15}N_2O$ 143.1184.

3-Azido-1,2',6'-tri-*N*-benzyloxycarbonyl-3-demethoxy-4-*N*-methylfortimicin B (**17**)

A suspension of 3.07 g of the tetrahydrochloride of 3-azido-3-demethoxy-4-*N*-methylfortimicin B (**16**), 4.85 g of *N*-benzyloxycarbonyloxysuccinimide, 3.63 ml of triethylamine, 24 ml of water, and 96 ml of methanol was stirred in an ice bath for 3 hours and then at room temperature overnight. Isolation of the product by chloroform extraction gave 4.38 g of product which was chromatographed on a column of 400 g of silica gel packed and eluted with ethyl acetate - hexane - triethylamine (4 : 1 : 0.1) to yield 4.05 g

of **17**: $[\alpha]_D^{25}+71°$ (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.02 d ($J_{6',7'}=6.0$ Hz, 6'-CH_3), 2.41 [$N(CH_3)_2$]. ν_{max} ($CHCl_3$) 3441, 3341, 2106, 1711 cm^{-1}.

Anal. Calcd. for $C_{39}H_{49}N_7O_{10}$: C 60.37, H 6.36, N 12.64.
Found: C 60.57, H 6.18, N 12.61.

3-Azido-1,2',6'-tri-*N*-benzyloxycarbonyl-3-demethoxy-4-*N*,5-*O*-methylenefortimicin B (**18**) and 3-Azido-1,2',6'-tri-*N*-benzyloxycarbonyl-3-demethoxyfortimicin B (**19**)

A stirred solution of 3-azido-1,2',6'-tri-*N*-benzyloxycarbonyl-3-demethoxy-4-*N*-methylfortimicin B (**17**), 1.86 g of iodine, 6.53 g of sodium acetate trihydrate and 160 ml of methanol was irradiated for 4 hours with a 150 W flood lamp. Sodium thiosulfate (2.95 g) was added and stirring was continued until the solution became colorless. Formaldhyde (0.7 ml, 37%) was added and the product (3.64 g) was isolated as a white glass, **18**: δ ($CDCl_3$) 0.99 d ($J_{6',7'}=6.5$ Hz, 6'-CH_3), 2.29 (NCH_3), 3.84 d, 4.58 d ($J=2.5$ Hz, OCH_2NCH_3). ν_{max} ($CHCl_3$) 3570, 3440, 3340, 2108, 1712 cm^{-1}.

A solution of 3.60 g of **18**, 1.84 g of hydroxylamine hydrochloride, 3.8 ml of acetic acid and 130 ml of methanol was heated under reflux for one hour. The product (3.46 g of **19**) was isolated by chloroform extraction: δ ($CDCl_3$) 0.99 d ($J_{6',7'}=6.3$ Hz, 6'-CH_3), 2.38 (NCH_3). ν_{max} ($CHCl_3$) 3440, 3350, 2110, 1712 cm^{-1}.

3-Azido-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-3-demethoxyfortimicin A (**20**)

A magnetically stirred solution of 3.42 g of 3-azido-1,2',6'-tri-*N*-benzyloxycarbonyl-3-demethoxyfortimicin B (**19**), 2.68 g of *N*-benzyloxycarbonylglycyl anhydride, and 100 ml of tetrahydrofuran was kept overnight at room temperature. Isolation of the product by chloroform extraction gave 4.92 g of a glass. Chromatography of the latter on a column of 250 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane gave 1.8 g of **20**: $[\alpha]_D^{25}+43°$ (*c* 1.0, CH_3OH), δ ($CDCl_3$) 1.18 d ($J_{6',7'}=6.2$ Hz, 6'-CH_3), 2.91, 3.06 (NCH_3, rotamers). ν_{max} ($CHCl_3$) 3438, 3338, 2108, 1714, 1644 cm^{-1}.

Anal. Calcd. for $C_{48}H_{57}N_8O_{13}$: C 60.49, H 5.62, N 11.76.
Found: C 60.87, H 5.85, N 11.66.

3-Amino-3-demethoxyfortimicin A (**4**)

3-Azido-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-3-demethoxyfortimicin A (**20**, 0.876 g) was catalytically hydrogenated under three atmospheres of hydrogen for 3 hours in 92 ml of 0.2 N hydrochloric acid in the presence of 0.9 g of 5% palladium on carbon to give 0.551 g of the pentahydrochloride of **4**. The latter was converted to the salt ($C_{16}H_{34}N_6O_5\cdot 5/2H_2SO_4\cdot 5H_2O$) with AG1-X2 ($SO_4^{2-}$) resin: $[\alpha]_D^{25}+79°$ (*c* 1.0, H_2O). δ (D_2O) 1.06 d ($J_{6',7'}=7.0$ Hz, 6'-CH_3), 3.19 (NCH_3), 5.33 d ($J_{1',2'}=3.5$ Hz, H-1'). *m/z* M^+; meas. 390.2609, calcd. for $C_{16}H_{34}N_6O_5$ 390.2591. Cyclitol; Meas. 249.1560, calcd. for $C_9H_{21}N_4O_4$ 249.1563. Diaminosugar; Meas. 143.1176, Calcd. for $C_7H_{15}N_2O$ 143.1184.

Anal. Calcd. for $C_{16}H_{34}N_6O_5\cdot 5/2H_2SO_4\cdot 5H_2O$: C 28.23, H 6.52, N 12.35, S 11.77.
Found: C 28.16, H 6.54, N 12.59, S 11.96.

1,2',6'-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**21**)

A solution of 2.03 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-4-*N*,5-*O*-methylenefortimicin B (**9**), 1.5 ml of methanesulfonyl chloride, and 50 ml of pyridine was kept at 0°C for 0.5 hour and then at room temperature overnight. Workup by chloroform - 5% aqueous sodium bicarbonate extraction gave 2.68 g of **21** as an orange foam: δ ($CDCl_3$) 1.08 d ($J_{6',7'}=6.5$ Hz, 6'-CH_3), 2.32 (NCH_3), 2.93, 3.17 (OSO_2CH_3's), 3.89 d, 4.65 d ($J=2.2$ Hz, OCH_2NCH_3). ν_{max} ($CDCl_3$) 3447, 3317, 1711, 1179 cm^{-1}.

1,2',6'-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonyl-4-*N*-methylfortimicin B (**24**)

A stirred solution of 0.5062 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2,3-di-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**21**), 0.1105 g of sodium cyanoborohydride, 0.4 ml of 37% formaldehyde, 0.2 ml of acetic acid, and 5.0 ml of methanol was kept at room temperature for 22 hours. Isolation of the product by chloroform - 5% sodium bicarbonate extraction gave 0.4453 g of **24** as a white glass: δ ($CDCl_3$) 1.09 (unresolved doublet, 6'-CH_3), 2.35 [$N(CH_3)_2$], 2.94 (OSO_2CH_3). ν_{max} ($CDCl_3$) 3438, 3338 sh, 1713, 1177 cm^{-1}.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**5**) and 1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin B (**25**)

A magnetically stirred solution of 2.92 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonyl-4-*N*-methylfortimicin B (**24**), 1.37 g of iodine, 4.80 g of sodium acetate trihydrate, and 120 ml of methanol was irradiated for 5 hours with a 150 W flood lamp. Sodium thiosulfate (2.17 g) was added and stirring was continued for 45 minutes. To the resulting colorless suspension was added 0.5 ml of 37% formaldehyde. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to yield 4.26 g of **5** as a glass: δ ($CDCl_3$) 1.07 d ($J_{6',7'}$=6.6 Hz, 6′-CH_3), 2.24 (NCH_3), 2.92 (OSO_2CH_3), 3.81 d, 4.63 d (J=2.3 Hz, OCH_2NCH_3). ν_{max} ($CDCl_3$) 3597, 3442, 3327, 1709, 1177 cm^{-1}.

A solution of 0.547 g of **5**, 0.207 g of hydroxylamine hydrochloride, 0.5 ml of acetic acid, and 28 ml of methanol was heated under reflux for 0.5 hour. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 0.4935 g of **25** as a white glass: δ ($CDCl_3$) 1.06 d ($J_{6',7'}$=6.5 Hz, 6′-CH_3), 2.32 (NCH_3), 2.88 (OSO_2CH_3). ν_{max} ($CDCl_3$) 3598, 3442, 3358, 1713, 1176 cm^{-1}.

2-Azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-4-*N*,5-*O*-methylenefortimicin B (**27**) and 2-Azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin B (**28**)

A magnetically stirred suspension of 4.24 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonyl-4-*N*,5-*O*-methylenefortimicin B (**5**), 4.23 g of sodium azide, and 100 ml of dimethylformamide was heated at 92°C for 22 hours. The product was isolated by chloroform - 5% aqueous bicarbonate extraction to yield 3.61 g of **27** as a glass: δ ($CDCl_3$) 1.13 d ($J_{6',7'}$=6.0 Hz, 6′-CH_3), 2.40 (NCH_3), 4.62 d (1H, J=4.5 Hz, OCH_2NCH_3)*. ν_{max} ($CHCl_3$) 3582, 3447, 3347, 2109, 1713 cm^{-1}.

A solution of 4.55 g of **27**, 1.24 g of hydroxylamine hydrochloride, 3.4 ml of acetic acid, and 200 ml of methanol was heated under reflux for 0.5 hour. The product was isolated by chloroform - sodium bicarbonate extraction to yield 3.38 g of **28** as a foam: δ ($CDCl_3$) 1.17 d ($J_{6',7'}$=6 Hz, 6′-CH_3), 2.27 (NCH_3). ν_{max} ($CHCl_3$) 3587, 3447, 3357, 2112, 1737 cm^{-1}.

2-Azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**29**)

A magnetically stirred solution of 3.29 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin B (**28**), 1.6 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide and 35 ml of tetrahydrofuran was kept at room temperature for 22 hours. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 4.04 g of brown foam. To convert any 5-*O*-acylated product to **29** (see reference 9 for an example where this was necessary) the product was kept overnight in a solution of 16 ml of triethylamine, 32 ml of water, and 130 ml of tetrahydrofuran. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 3.82 g of tan glass. Chromatography of 3.80 g of the latter on a column of 250 g of silica gel packed and eluted with ethyl acetate gave 2.73 g of **29**: $[\alpha]_D^{23}$ +26.3° (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.15 (unresolved doublet, 6′-CH_3), 2.88 (major), 2.99 (minor) (NCH_3 rotamers). ν_{max} ($CHCl_3$) 3443, 3353, 2118, 1717, 1647 cm^{-1}.

Anal. Calcd. for $C_{48}H_{56}N_8O_{13}$: C 60.49, H 5.92, N 11.76.
Found: C 60.79, H 6.11, N 11.72.

2-Amino-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**30**)

To a stirred solution of 1.43 g of 2-azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**29**) in 30 ml of glacial acetic acid was added 4.5 g of zinc dust. The resulting suspension was stirred at room temperature for two days. The suspension was filtered and the acetic acid was evaporated from the filtrate under reduced pressure leaving 1.57 g of a light yellow glass. Chromatography of the latter (1.50 g) on a column of silica gel packed and eluted with ethyl acetate - ethanol - triethylamine (10: 1: 0.1) gave 0.933 g of **30** as a white glass: $[\alpha]_D^{23}$ +29° (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.10 d ($J_{6',7'}$=6.5 Hz, 6′-CH_3), 2.87 (major), 3.01 (minor) (NCH_3, rotamers). ν_{max} ($CDCl_3$) 3436, 3336, 1716, 1638 cm^{-1}.

Anal. Calcd. for $C_{48}H_{58}N_6O_{13}$: C 62.19, H 6.31, N 9.07.
Found: C 62.05, H 6.38, N 9.11.

* High-field doublet obscured.

2-Amino-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**8**)

A sample of 0.598 g of 2-amino-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxy-2-*epi*-fortimicin A (**30**) in a solution of 65 ml of 0.2 N hydrochloric acid in methanol and 35 ml of methanol was hydrogenated for 4 hours under 3 atm. of hydrogen in the presence of 0.6 g 5% Pd/C. The catalyst was removed by filtration. Solvent was evaporated under diminished pressure, and residual hydrochloric acid was removed by co-distillation with methanol under reduced pressure leaving 0.336 g of the pentahydrochloride of **8** as a white glass. The latter was converted to the salt ($C_{16}H_{34}N_6O_5 \cdot 5/2H_2SO_4 \cdot 3H_2O$) with AG1-X2 ($SO_4^{2-}$) resin: $[\alpha]_D^{25}+69°$ (*c* 1.0, H_2O). δ (D_2O, pD 5.0) 1.34 d ($J_{6',7'}=7.6$ Hz, 6′-CH_3), 3.18 (NCH_3), 4.09 ($COCH_2N$), 5.36 d ($J_{1',2'}=3.6$ Hz, $H_{1'}$).

Anal. Calcd. for $C_{16}H_{34}N_6O_5 \cdot 5/2H_2SO_4 \cdot 3H_2O$: C 29.00, H 6.39, N 12.68, S 12.09.
Found: C 29.19, H 6.29, N 12.67, S 11.64.

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin A (**6**)

A solution of 5.56 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin B (**5**), 2.58 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, and 60 ml of tetrahydrofuran was stirred at room temperature for 26 hours. Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 6.82 g of a glass. To convert any 5-*O*-acylated product to **6** (see reference 9 for an example) the product was treated for 24 hours with 140 ml of a solution of tetrahydrofuran - triethylamine - water (8 : 2 : 1). Isolation of the product by chloroform - 5% aqueous sodium bicarbonate extraction gave 6.26 g of **6**: $[\alpha]_D^{27}+38.4°$ (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.1 (unresolved doublet, 6′-CH_3), 2.88 (NCH_3), 3.04 (OSO_2CH_3). ν_{max} ($CDCl_3$) 3441, 1712, 1642, 1177 cm^{-1}.

Anal. Calcd. for $C_{49}H_{59}N_5O_{16}S$: C 58.50, H 5.91, N 6.96, S 3.19.
Found: C 57.92, H 5.93, N 6.82, S 3.78.

2-Azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-deoxyfortimicin A (**26**)

A stirred suspension of 1.00 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethyl-2-*O*-methanesulfonylfortimicin A (**6**), 1.00 g of sodium azide, and 30 ml of dimethylformamide was heated at 93°C for 25 hours. The product was isolated by chloroform - 5% aqueous sodium bicarbonate extraction to yield 0.945 g of brown glass. Chromatography of the latter (0.877 g) on a column of 40 g of silica gel packed and eluted with ethyl acetate gave 0.268 g of **26**: $[\alpha]_D^{24}+28°$ (*c* 1.0, CH_3OH). δ ($CDCl_3$) 1.13 d ($J_{6',7'}=5$ Hz, 6′-CH_3), 2.92 (NCH_3). ν_{max} ($CHCl_3$) 3438, 3358, 2118, 1713 cm^{-1}.

Anal. Calcd. for $C_{48}H_{56}N_8O_{13}$: C 60.50, H 5.92, N 11.76.
Found: C 60.13, H 5.92, N 11.43.

2-Amino-3-*O*-demethyl-2-deoxyfortimicin A (**7**)

A sample of 0.512 g of **26** in 27 ml of 0.2 N hydrochloric acid in methanol was hydrogenated for 4 hours under 3 atm. of hydrogen in the presence of 0.5 g of 5% Pd/C. The catalyst was removed by filtration and the methanol was evaporated under diminished pressure to yield 0.319 g of the pentahydrochloride of **7**. The latter was converted to the salt $C_{16}H_{35}N_6O_5 \cdot 5/2H_2SO_4 \cdot 6H_2O$ with AG1-X2 (SO_4^{2-}) resin: $[\alpha]_D^{25}+67°$ (*c* 1.0, H_2O). δ (D_2O, pD 5.04) 1.36 d ($J_{6',7'}=6.4$ Hz, 6′-CH_3), 3.18 (NCH_3), 4.10 ($COCH_2N$), 5.33 d ($J_{1',2'}=3.8$ Hz, $H_{1'}$). *m/z* (M+H); meas. 391.2658, calcd. for $C_{16}H_{35}N_6O_5$ 391.2668, cyclitol; meas. 249.1542, calcd. for $C_9H_{21}N_4O_4$ 249.1562, diaminosugar; meas, 143.1183, calcd. for $C_7H_{15}N_2O$ 143.1184.

Anal. Calcd. for $C_{16}H_{35}N_6O_5 \cdot 5/2H_2SO_4 \cdot 6H_2O$: C 27.86, H 6.57, N 12.19, S 11.62.
Found: C 28.01, H 6.35, N 11.76, S 11.10.

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