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2′-*N*-ACYLFORTIMICINS AND 2′-*N*-ALKYLFORTIMICINS *via* THE ISOFORTIMICIN REARRANGEMENT[1]

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ABSTRACT

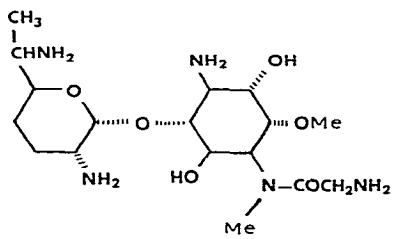
Fortimicin A and a number of 4-*N*-acylfortimicins B, although stable as either the fully protonated hydrochloride or sulfate salts, undergo degradation as the free bases in aqueous solution. Detailed studies with fortimicin A and 4-*N*-acetylfortimicin B have shown that degradation occurs, in part, by simple cleavage of the 4-*N*-acyl groups with formation of fortimicin B, and, in part, by rearrangement to the 2′-*N*-acylfortimicins B (the isofortimicin rearrangement). The conversions of the rearrangement products into 2′-*N*-glycylfortimicin A, 2′-*N*-acetylfortimicin A, and the 2′-*N*-(2-aminoethyl)fortimicins A and B are described. The antibacterial activities of the new fortimicin A derivatives are presented.

INTRODUCTION

Fortimicin A (**1**) and fortimicin B (**2**) are aminocyclitol (aminoglycoside) antibiotics formed in fermentations by *Micromonospora olivoasterospora*[2]. Fortimicin A differs from fortimicin B only by the attachment of a glycyl group to the 4-methylamino group of fortimicin B. Structural studies[3] have shown that the conformation in D_2O solution of the aminocyclitol ring of fortimicin A, which has by far the greater antibacterial activity[4], is that chair form (**1a**) having the 4-glycylamide group equatorially attached. In contrast, the conformation of fortimicin B in D_2O solution is that chair form (**2a**) having the 4-methylamino group axial. Fortimicin A is stable as either the disulfate or the tetrahydrochloride salt, as the dry salts or in aqueous solution. As the free base, however, fortimicin A undergoes degradation in aqueous solution ($t_{1/2}$ ~3 days) to form, in part, fortimicin B by simple cleavage of the 4-*N*-glycyl group, and in part, an isomeric glycyl amide, isofortimicin, by transfer of the glycyl group to one of the three primary amino groups (the isofortimicin rearrangement). A similar rearrangement was found to occur[5] in the case of 4-*N*-acetylfortimicin B (**6a**). The present report describes the determination of the structures of the rearranged products, and their conversions into new fortimicin A derivatives for which antibacterial activities are presented.

DISCUSSION

The presence of the glycyl group in isofortimicin (**3a**) was established by the presence of a carbonyl absorption (1660 cm^{-1}) in the i.r. spectrum, and by the presence in the ^{13}C-n.m.r. spectrum (Table I) of a peak attributed to the glycyl



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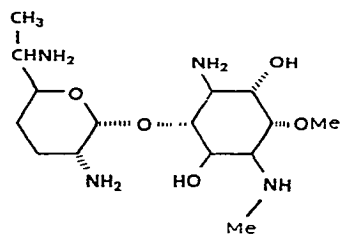

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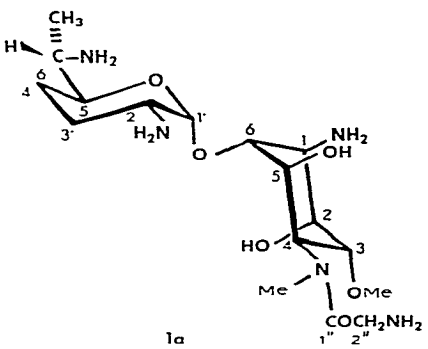
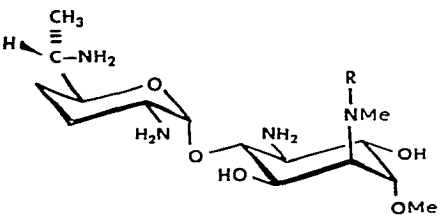
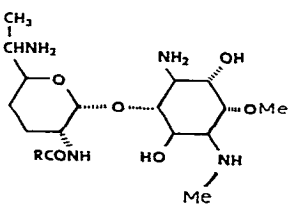


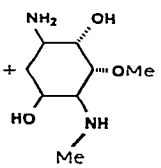
1a



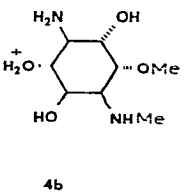
2a R = H
1b R = $COCH_2NH_2$



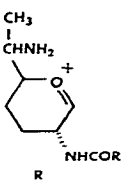
3a R = CH_2NH_2
3b R = CH_3



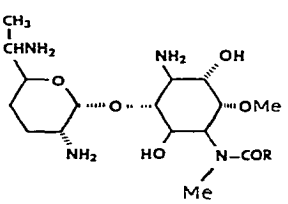
4a



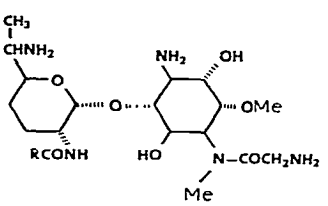
4b



5a R = CH_2NH_2
5b R = CH_3



6a R = CH_3
6b R = $CHCH_2CH_2NH_2$ (OH)



7a R = CH_2NH_2
7b R = CH_3

TABLE I

25-MHz ^{13}C-N.M.R. PARAMETERS IN D_2O SOLUTION*

Fortimicin B (2)			*Isofortimicin* (3a)			*2'*-N-*Acetylfortimicin B* (3b)		
Carbon	*pD* 10.3	1.8	*Carbon*	*pD* 10.4	1.0	*Carbon*	*pD* 10.3	1.1
1	53.8	53.5	1	54.3	53.4	1	54.3	53.7
2	71.2	65.5	2	71.1	66.4	2	71.0	65.6
3	79.9	74.1	3	79.8	74.0	3	79.8	73.9
4	60.8	58.1	4	61.2	57.9	4	61.1	57.8
5	71.2	66.6	5	71.0	65.5	5	71.0	65.4
6	84.1	74.2	6	82.3	74.2	6	83.0	72.9
1'	102.5	96.0	1'	98.9	97.7	1'	99.2	96.9
2'	50.6	51.9	2'	50.8	52.0	2'	50.6	52.1
3'	27.0	21.5	3'	23.7	22.6	3'	23.7	22.7
4'	27.3	26.3	4'	26.9	27.1	4'	26.8	27.2
5'	75.1	71.0	5'	74.4	70.7	5'	75.0	70.7
6'	50.4	49.4	6'	49.9	49.1	6'	50.2	48.9
7'	18.5	15.1	7'	18.2	15.2	7'	18.5	15.1
OCH_3	59.2	56.1	OCH_3	59.2	57.7	OCH_3	59.2	57.8
NCH_3	35.4	32.3	NCH_3	35.4	32.1	NCH_3	35.3	31.8
			Gly	44.8	41.3	$COCH_3$	22.8	22.5

*^{13}C-N.m.r. spectra were measured with a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal 1,4-dioxane (67.4 p.p.m.) and are reported in p.p.m. downfield from Me_4Si. Reported pD values are uncorrected, pH-meter readings of solutions in D_2O.

methylene carbon atom. That the 4-*N*-methyl group of isofortimicin was not acylated was established by the chemical shift (D_2O) of the protons of the methylamino group (δ 2.85 p.p.m.), which is in good agreement with that of the free base of fortimicin B (δ 2.85 p.p.m.)[3], and at much higher field than that (δ 3.50 p.p.m.)[3] of the free base of fortimicin A. The mass spectrum of isofortimicin first established that the glycyl group was attached to one of the nitrogen atoms of the diamino sugar portion. In addition to the molecular ion, there were also present the unsubstituted aminocyclitol fragment **4a**, also characteristic[3] of fortimicin B, and the *N*-glycylated diamino sugar fragment **5a**.

That the glycyl group of isofortimicin (**3a**) was attached to N-2' was established by ^{13}C-n.m.r. spectroscopy with the criterion that both acylation[6] and protonation[6,7] of aliphatic amines result in upfield shifts of the resonances of the carbon atoms β to the amino groups that undergo change. In the case of acylation, the magnitude of the β-shifts may depend on the conformational tendency of the acyl group[8].

The chemical shifts of the carbon atoms of fortimicin B (**2**) and isofortimicin (**3a**) are listed in Table I. Comparison of the data for the free bases (pH 10.3–10.4) shows that, while acylation does not appreciably affect either the chemical shifts of the cyclitol carbon atoms or C-2',4',5',6', and 7' of the diamino sugar moiety, the

resonances of C-1′ and C-3′ of the glycyl derivative are at higher field than are those of fortimicin B as a consequence of the upfield β-shifts due to 2′-*N*-acylation. In addition, comparison of the chemical shifts of the free bases with those of the fully protonated species (pH 1.0–1.8) established that the resonances of the carbon atoms β to the amino groups at C-1, C-4, and C-6′ showed upfield shifts, on protonation of the amino groups, essentially equal to those of fortimicin B. In contrast, the resonances of C-1′ and C-2′ of the 2′-*N*-glycylated product were much less sensitive to pH than were those of fortimicin B (which exhibited the expected upfield shifts on protonation of the 2′-amino group).

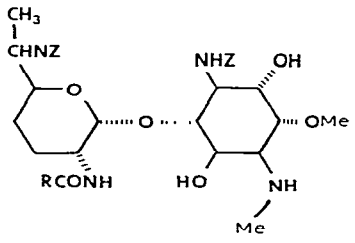
Thus far it has not been established whether the isofortimicin rearrangement is intra- or inter-molecular. Inspection of molecular models showed that formation of isofortimicin (**3a**) by direct intramolecular transfer of the 4-*N*-glycyl group to the 2′-amino group is sterically feasible only from that conformation of the aminocyclitol ring which has the 4-glycylamide group axial (**1b**), but not from that which has this group equatorial (**1a**).

4-*N*-Acetylfortimicin B[5] (**6a**), like fortimicin A[1], was found to react as the free base in aqueous solution ($t_{1/2} \sim 1$ day) to give a mixture of fortimicin B and 2′-*N*-acetylfortimicin B (**3b**). The structure of the latter was established by n.m.r. (Table I and Experimental) and mass spectrometry by the same criteria used for isofortimicin. The mass spectrum of **3b** showed the unsubstituted aminocyclitol fragment **4b** and the *N*-acylated diamino sugar fragment **5b**.

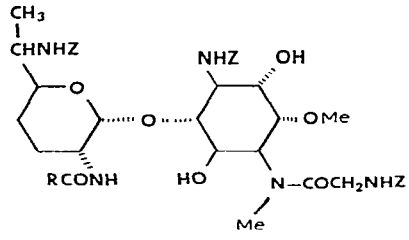
Preliminary investigation by means of ^{1}H-n.m.r. spectroscopy showed that most 4-*N*-acylfortimicins[5], as the free bases in aqueous solution, undergo cleavage of the 4-*N*-acyl groups at room temperature, with half lives varying between several h and several days, as shown by ^{1}H-n.m.r., but the nature of the products has not been determined in all instances. Noteworthy is 4-*N*-DL-(4-amino-2-hydroxybutanoyl)-fortimicin B[5] (**6b**), which undergoes cleavage to fortimicin B in aqueous solution within 15 min after conversion into the free base with an excess of AG2-X8 (OH^-) resin. We attribute the exceptional lability of the aminohydroxybutanoyl group of **6b** to intramolecular participation of the 4-amino group reminiscent of that which accounts for the facile cleavage of 4-hydroxycarboxylic acid esters[9] by participation of the 4-hydroxyl groups. A cyclic mechanism involving intramolecular participation of the 4-amino group of the 4-amino-2-hydroxybutanoyl side-chain was invoked[10] to explain the thermal elimination on mass spectrometry of the acyl group of 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]gentamicin C_1.

2′-*N*-Glycylfortimicin A (**7a**) was prepared by a sequence starting with the treatment of **3a** with *N*-(benzyloxycarbonyloxy)succinimide to give 1,6′-di-*N*-benzyloxycarbonyl-2′-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin B (**8a**). Coupling of the latter with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave 1,6′,2″-tri-*N*-benzyloxycarbonyl-2′-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin A (**9a**). Catalytic hydrogenolysis of **9a** in 0.2M methanolic hydrochloric acid gave 2′-*N*-glycylfortimicin A (**7a**), isolated as the perhydrochloride.

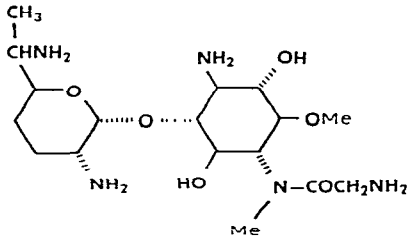
Similarly, 2′-*N*-acetylfortimicin B (**3b**) was converted into 2′-*N*-acetyl-1,6′-



8a R = CH_2NHZ
8b R = CH_3



9a R = CH_2NHZ
9b R = CH_3



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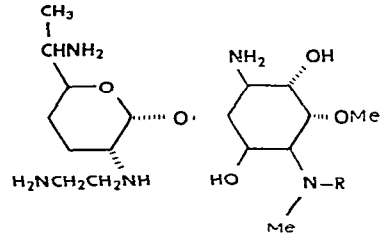


11 R = H
12 R = $COCH_2NH_2$

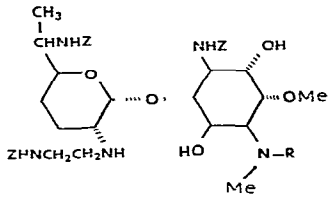
di-*N*-benzyloxycarbonylfortimicin B (**8b**) with *N*-(benzyloxycarbonyloxy)succinimide. Coupling of **8b** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave 2′-*N*-acetyl-1,6′,2″-tri-*N*-benzyloxycarbonylfortimicin A (**9b**). Catalytic hydrogenolysis of **9b** in 0.2M methanolic hydrochloric acid gave 2′-*N*-acetylfortimicin A (**7b**), isolated as the perhydrochloride.

That the free 2-amino group is not necessary for labilization of the 4-*N*-glycyl group of fortimicin A derivatives was shown by cleavage of the 4-*N*-glycyl group of 2′-*N*-acetylfortimicin A (**7b**) as the free base in aqueous solution ($t_{1/2}$ ~3 days) to give 2′-*N*-acetylfortimicin B (**3b**).

In contrast to the labile 4-*N*-acylfortimicins just described, 4-*N*-glycylfortimicin E[11] (**10**), which is that diastereomer of fortimicin A having both C-3 and C-4 substituents of opposite configuration to the corresponding carbon positions of fortimicin A, was found to be indefinitely stable as the free base*.

Reduction of isofortimicin (**3a**) with lithium aluminum hydride gave 2′-*N*-(2-aminoethyl)fortimicin B (**11**). Treatment of the latter with *N*-(benzyloxycarbonyloxy)succinimide gave a mixture from which 1,6′-di-*N*-benzyloxycarbonyl-2′-*N*-(2-*N*-benzyloxycarbonylaminoethyl)fortimicin B (**13**) was isolated by chromatography.

*A referee has suggested that the isofortimicin rearrangement may be the result of a double acyl migration, first from N-4 to the *cis*-related O-5, and then to N-2′. Accordingly, it was suggested that the stability of fortimicin E may be a consequence of the *trans* relationship of its 4- and 5-substituents. Although a double rearrangment has been suggested[1], further work will be necessary to completely elucidate the rearrangement mechanism.



13 R = H
14 R = $COCH_2NHZ$

TABLE II

ANTIBACTERIAL ACTIVITY OF 2′-*N*-SUBSTITUTED FORTIMICINS A AND B PERHYDROCHLORIC ACID SALTS COMPARED WITH FORTIMICIN A TETRAHYDROCHLORIDE

Organisms	*Minimum inhibitory concentration (μg/mL)*					
	1	**7a**	**12**	**7b**	**3a**	**3b**
Staphylococcus aureus (Smith)	0.78	1.56	1.56	100	> 100	> 100
Streptococcus faecalis 10541	100	100	100	> 100	> 100	> 100
Enterobacter aerogenes 13048	3.1	6.2	6.2	100	> 100	> 100
Escherichia coli (Juhl)	6.2	12.5	12.5	100	> 100	> 100
Klebsiella pneumoniae 10031	3.1	25	3.1	50	> 100	> 100
Providencia 1577	3.1	6.2	6.2	> 100	> 100	> 100
Pseudomonas aeruginosa KY-8512	25	50	50	> 100	> 100	> 100
Serratia marcescens 4003	3.1	3.1	3.1	> 100	> 100	> 100
Shigella sonnei 9290	6.2	12.5	6.2	100	> 100	> 100
Proteus rettgeri U-6333	12.5	25	25	—	> 100	> 100

The absence of an acyl group at N-4 of **13** was established by the chemical shift (δ 2.35) of the 4-*N*-methyl protons of **13**, which was almost identical with that (δ 2.32) of the 4-*N*-methyl protons of the free methylamino group of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B[5]. Tentative assignment of the structure of **13** was based on the expected relative ease of acylation of the primary nitrogen atom of the 2′-*N*-(2-aminoethyl) group of **11** rather than the (secondary) N-2′ atom. In addition, the selective 4-*N*-acylation of **13** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide to give **14** was compatible with the attachment of benzyloxycarbonyl groups to N-1 and N-6′ of **13** and **14**. That the acylation of **13** to give **14** occurred at N-4 was established by the chemical shift (δ 2.92) of the 4-*N*-methyl protons of **14**, which was in good agreement with that (δ 2.82) of the 4-*N*-methyl protons of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A[5]. The difference between the chemical shifts of the 4-*N*-acylated and the 4-methylamino derivatives ($\Delta\delta$ ~0.5) is compatible with the downfield shift of the 4-*N*-methyl protons expected on 4-*N*-acylation[12].

The minimum inhibitory concentration of the perhydrochlorides of the 2′-*N*-substituted fortimicins A were determined by a two-fold dilution method in Mueller–Hinton agar, with fortimicin A tetrahydrochloride as the reference antibiotic (Table

II). All of the 2′-*N*-substituted fortimicins A were less active than fortimicin A (**1**). 2′-*N*-Glycylfortimicin A (**7a**) and 2′-*N*-(2-aminoethyl)fortimicin A (**12**) were approximately one-half as active as fortimicin A (**1**), whereas 2′-*N*-acetylfortimicin A (**7b**) showed only weak activity against the microorganisms tested.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Hilger and Watts polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 521 grating spectrometer. ^{1}H-N.m.r. spectra were recorded at 100 MHz with a Varian Associates HA-100 spectrometer. Chemical shifts for ^{1}H-n.m.r. spectra determined in $CDCl_3$ are reported relative to internal tetramethylsilane. Chemical shifts for ^{1}H-n.m.r. spectra determined in D_2O are reported relative to external tetramethylsilane. Mass spectra were obtained with an AEI MS-902 spectrometer at 70 eV. All evaporations were performed under diminished pressure with rotary evaporators and organic solutions dried with magnesium sulfate. Microanalytical results are reported for *N*-benzyloxycarbonyl derivatives. Other compounds, which could not be freed of solvent, were characterized by ^{1}H-n.m.r., ^{13}C-n.m.r., and mass spectra, and as single spots in t.l.c. with several solvent systems and detection with ceric sulfate reagent (1.0 g of ceric sulfate, 2.5 g of ammonium molybdate, and 10 mL of concentrated sulfuric acid diluted to 100 mL with water).

Cleavage of the 4-*N*-acyl groups in fortimicin A and the 4-*N*-acylfortimicins B was monitored by ^{1}H-n.m.r. spectroscopy (D_2O), observing the disappearance of the acylated 4-*N*-methyl signals (δ ~3.5) and the appearance of the 4-*N*-methyl signals of the free methylamino groups (δ ~2.8)[4]. The free bases were generated by treatment of aqueous solutions of sulfate or hydrochloride salts with an excess of AG2-X8 (OH^-) resin.

Isofortimicin (**3a**). — Fortimicin A tetrahydrochloride (**1**, 10.0 g) was converted into the free base by passing an aqueous solution through a column of anion-exchange resin (BioRad AG1-X2, OH^-). The column eluate was diluted to 1%, calculated from starting fortimicin, and kept for 14 days at room temperature. Evaporation of the water left a syrup, a portion of which (2.08 g) was chromatographed on a column (2.2 × 52 cm) of cation-exchange resin (Bio Rex 70, 100–200 mesh, NH_4^+ form) eluted with a gradient of water to M ammonium hydroxide. The first fractions eluted were evaporated to give 1.349 g of isofortimicin (**3a**); $[\alpha]_D^{25}$ +41.6° (*c* 1.09, methanol); ν_{max}^{KBr} 3370, 1660, and 1540 cm^{-1}; ^{1}H-n.m.r. (D_2O); δ 1.52 (d, 3 H, $J_{6',7'}$ 7.0 Hz, 6′-CH_3), 2.85 (s, 3 H, 4-*N*CH_3), 3.75 (s, 2 H, COCH_2N), 3.93 (s, 3 H, C_3-*O*CH_3), 5.60 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′); ^{13}C-n.m.r.: see Table I); *m/e* 405.2566 (M^+), calc. for $C_{17}H_{35}N_4O_6$ 405.2588; sugar fragment **5a** *m/e* 200.1394, calc. for $C_9H_{18}N_3O_2$ 200.1399; aminocyclitol **4a** fragment *m/e* 189.1249, calc. for $C_8H_{17}N_2O_3$ 189.1239.

Further elution gave 0.441 g of fortimicin B (**2**) identical (t.l.c., i.r., ^{1}H-n.m.r.) with an authentic sample.

1,6′-Di-N-*benzyloxycarbonyl-2′*-N-(N-*benzyloxycarbonylglycyl)fortimicin B*

(**8a**). — A stirred, ice bath-cooled solution prepared from isofortimicin (**3a** 1.0 g), water (15 mL), and methanol (30 mL) was treated with 2.22 g of *N*-(benzyloxycarbonyloxy)succinimide. Stirring was continued for 3 h in the cold and then for 20 h at room temperature. The methanol was evaporated off and the syrup shaken with a mixture of chloroform and water. The chloroform layer was separated, washed with water, and dried. Evaporation of the chloroform gave 2.075 g of residue, which was chromatographed on a column (2.0 × 53 cm) of silica gel prepared and eluted with 23.4:1.4:0.1 (v/v) chloroform–methanol–concentrated ammonium hydroxide. Fractions containing the major component were evaporated to give 0.904 g of 1,6′-di-*N*-benzyloxycarbonyl-2′-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin B (**8a**); $[\alpha]_D^{23}$ +15.8° (*c* 1.04, methanol); $\nu_{max}^{CDCl_3}$ 3410, 3330, 1705, and 1515 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 0.95 (d, 3 H, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.38 (s, 3 H, 3-OCH_3), 3.40 (s, 3 H, 4-NCH_3), and 7.32 (m, Z aromatic).

Anal. Calc. for $C_{41}H_{53}N_5O_{12}$: C, 60.96; H, 6.61; N, 8.67. Found: C, 60.85; H, 6.86; N, 8.49.

1,6′,2″-Tri-N-benzyloxycarbonyl-2′-N-(benzyloxycarbonylglycyl)fortimicin A (**9a**). — To a stirred solution of **8a** (0.904 g) in 5.4 mL of oxolane (tetrahydrofuran) was added 0.405 g of *N*-(*N*-benzyloxycarboxylglycyloxy)succinimide. After stirring for 20 h, the solvent was evaporated and the product purified by chromatography on a column (2.2 × 76 cm) of silica gel prepared and eluted with 85:15:1 (v/v) benzene–methanol–concentrated ammonium hydroxide to give 0.928 g of **9a**: $[\alpha]_D^{24}$ +48.9° (*c* 1.0, methanol); $\nu_{max}^{CDCl_3}$ 3410, 3350, 1703, and 1636 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.14 (unresolved d, 6′-CH_3), 2.93 (s, 3 H, 4-NCH_3), 3.29 (s, 3 H, 3-OCH_3), and 7.28 (m, Z aromatic).

Anal. Calc. for $C_{51}H_{62}N_6O_{15}$: C, 61.31; H, 6.26; N, 8.41. Found: C, 61.28; H, 6.42; N, 8.30.

2′-N-Glycylfortimicin A (**7a**). — A solution of **9a** (0.235 g) in 0.2M hydrochloric acid in methanol (40 mL) was hydrogenolyzed over 0.235 g of 5% palladium-on-carbon for 4 h under 3 atm of hydrogen. The catalyst was removed by filtration and the excess of hydrochloric acid removed by repeated evaporation of methanol from the residue to give **7a** (0.153 g), isolated as the tetrahydrochloride; $[\alpha]_D^{25}$ +84.6° (*c* 1.0, methanol); ν_{max}^{KBr} 1640, 1620, and 1563 cm^{-1}; ^{1}H-n.m.r. (D_2O); δ 1.80 (d, 3 H, $J_{6',7'}$ 7.0 Hz, 6′-CH_3), 3.58 (s, 3 H, 4-NCH_3), 3.94 (s, 3 H, 3-OCH_3), and 5.55 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); *m/e* 444.2699 ($M^+ - H_2O$), calc. for $C_{19}H_{36}N_6O_6$ 444.2696.

2′-N-Acetylfortimicin B (**3b**). — The trihydrochloride salt of 4-*N*-acetylfortimicin B (**6a**, 0.840 g), prepared as previously described[5], was converted into the free base with anion-exchange resin (BioRad AG2-X8, OH^-) and treated as described for the preparation of **3a**. Similar processing and isolation by chromatography on a cation-exchange resin led to 0.391 g of **3b**; $[\alpha]_D^{25}$ +37.6° (*c* 0.95, methanol); ν_{max}^{KBr} 3350, 1642, and 1557 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.51 (d, 3 H, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.42 (s, 3 H, 2′-$NCOCH_3$), 2.84 (s, 3 H, 4-NCH_3), 3.92 (s, 3 H, 3-OCH_3), and 5.56 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); *m/e* 391.2579 $(M + H)^+$, calc. for $C_{17}H_{35}N_4O_6$ 391.2556;

sugar fragment **5b** *m/e* 185.1298, calc. for $C_9H_{17}N_2O_2$ 185.1290; aminocyclitol fragment **4b** *m/e* 207.1356, calc. for $C_8H_{19}N_2O_4$ 207.1345.

Anal. Calc. for $C_{17}H_{34}N_4O_6 \cdot 2H_2O$: C, 47.76; H, 9.19; N, 13.10. Found: C, 47.74; H, 9.28; N, 13.13.

Further elution gave 0.068 g of fortimicin B (**2**), identical (t.l.c., i.r., ^{1}H-n.m.r.) with an authentic sample.

Finally, later fractions contained 0.121 g of starting 4-*N*-acetylfortimicin B (**6a**).

2'-N-*Acetyl-1,6'-di*-N-*benzyloxycarbonylfortimicin B* (**8b**). — To a stirred, ice bath-cooled solution prepared from *N*-acetylfortimicin B (**3b**, 0.290 g), methanol (9.0 mL), and water (4.5 mL) was added *N*-(benzyloxycarbonyloxy)succinimide (0.388 g). Stirring was continued for 3 h in the cold and then for 22 h at room temperature. The product was isolated by extraction with chloroform to give 0.480 g of residue which was chromatographed on a column (2.0 × 43 cm) of silica gel eluted with 23.4:1.4:0.1 (v/v) chloroform–methanol–concentrated ammonium hydroxide. Fractions containing the major component were evaporated to yield 0.151 g of **8b**: $[\alpha]_D^{23}$ +27.1° (*c* 1.0, methanol); $\nu_{max}^{CDCl_3}$ 3550, 3440, 3338, 1708, and 1658 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 0.99 (d, 3 H, $J_{6',7'}$ 6.0 Hz, 6'-CH_3), 1.32 (s, 3 H, 2'-$NCOCH_3$), 2.43 (s, 3 H, 4-NCH_3), 3.46 (s, 3 H, 3-OCH_3), and 4.93 (unresolved d, 1 H, H-1).

Anal. Calc. for $C_{33}H_{46}N_4O_{10}$: C, 60.17; H, 7.04; N, 8.50. Found: C, 59.52; H, 6.98; N, 8.40.

2'-N-*Acetyl-1,6',2''-tri*-N-*benzyloxycarbonylfortimicin A* (**9b**). — A stirred solution of **8b** (1.0 g) in dry oxolane (6 mL) was treated with 0.443 g of *N*-(benzyloxycarbonylglycyloxy)succinimide. After stirring overnight, solvent was evaporated off, and the product (1.496 g) purified by chromatography on a column (1.9 × 60 cm) of silica gel with 20:2.0:0.04 (v/v) dichloroethane–95% ethanol–concentrated ammonium hydroxide as the developer. Fractions containing only the major product were evaporated to give 0.850 g of **9b**: $[\alpha]_D^{23}$ +56.7° (*c* 1.0, methanol); $\nu_{max}^{CDCl_3}$ 1710, 1680, and 1501 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.19 (unresolved d, 6'-CH_3), 1.94 (s, 3 H, 2'-$NCOCH_3$), 3.02 (s, 3 H, 4-NCH_3), 3.38 (s, 3 H, 3-OCH_3), and 7.30 (m, Z aromatic).

Anal. Calc. for $C_{43}H_{55}N_5O_{13}$: C, 60.77; H, 6.52; N, 8.24. Found: C, 60.45; H, 6.53; N, 8.25.

2'-N-*Acetylfortimicin A* (**7b**). — Hydrogenolysis of **9b** (0.680 g) under conditions described for **7a** gave, after processing, 0.459 g of **7b**, isolated as the trihydrochloride; $[\alpha]_D^{23}$ +105.1° (*c* 1.0, methanol); ν_{max}^{KBr} 1645 and 1535 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.83 (d, $J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.52 (s, 2'-$NCOCH_3$), 3.61 (s, 4-NCH_3), 3.97 (s, 3-OCH_3), and 5.55 (d, $J_{1',2'}$ 4.0 Hz, H-1'); ^{13}C-n.m.r.: see Table I; *m/e* 447.2674 (M^+), calc. for $C_{19}H_{37}N_5O_7$ 447.2693.

2'-N-(*2-Aminoethyl*)*fortimicin B* (**11**). — A stirred solution prepared from 4.0 g of 2'-*N*-glycylfortimicin B (**3a**) in 160 mL of oxolane was treated with 2.44 g of lithium aluminum hydride. The mixture was heated for 20 h under reflux. Water was cautiously added to consume the excess of lithium aluminum hydride, and the

mixture was then centrifuged. The supernatant solution was evaporated and the residue purified by chromatography on a column (2.0 × 46 cm) of silica gel prepared and eluted with the lower phase of a 1:1:1 (v/v) mixture of chloroform–methanol–concentrated ammonium hydroxide. Fractions containing the major component were taken to dryness to give 1.743 g of solid, which was dissolved in 170 mL of 0.2M hydrochloric acid in methanol. After 0.5 h, the methanol was evaporated and excess hydrochloric acid was removed by repeated evaporation of portions of methanol to give 2.63 g of 2′-*N*-(2-aminoethyl)fortimicin B (**11**), isolated as the pentahydrochloride: $[\alpha]_D^{25}$ +63.8° (*c* 1.0, methanol): ν_{max}^{KBr} 3420, 2950, 1595, and 1480 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.86 (d, 3 H, $J_{6',7'}$ 7.0 Hz, 6′-CH_3), 3.36 (s, 3 H, 4-*N*CH_3), 4.02 (s, 3 H, 3-*O*CH_3), and 6.07 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); *m/e* 391.2771 (M^+), calc. for $C_{17}H_{35}N_5O_5$ 391.2795.

*1,6′-Di-*N*-benzyloxycarbonyl-2′-*N*-(2-*N*-benzyloxycarbonylaminoethyl)fortimicin B* (**13**). — To an ice bath-cooled, stirred solution of the free base of **11** (0.825 g) in water (12 mL) and methanol (25 mL) was added 1.83 g of *N*-(benzyloxycarbonyloxyl)succinimide. Stirring was continued for 3 h in the cold and then for 20 h at room temperature. The methanol was evaporated off and the syrup was shaken with a mixture of chloroform and water. The chloroform layer was separated and the aqueous portion was extracted again with chloroform. The combined chloroform extract was washed with water, dried, and evaporated to a residue (1.584 g) that was chromatographed on a column (2.2 × 65 cm) of silica gel prepared and eluted with 23.5:1.4:2.0:0.2 (v/v) benzene–methanol–95% ethanol–concentrated ammonium hydroxide. The first fractions eluted (0.377 g) were thought to contain 1,4,6′-tri-*N*-benzyloxycarbonyl-2′-*N*-(2-*N*-benzyloxycarbonylaminoethyl)fortimicin B, as judged by the downfield-shifted 4-*N*CH_3 group and the 20-proton area of the resonance attributed to the z aromatic group in the ^{1}H-n.m.r. spectra.

Further elution gave fractions containing only 1,2′,6′-tri-*N*-benzyloxycarbonyl-2′-*N*-(2-*N*-benzyloxycarbonylaminoethyl)fortimicin B; $[\alpha]_D^{23}$ +30.7° (*c* 1, methanol); $\nu_{max}^{CDCl_3}$ 3435, 1703, and 1505 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 (d, 3 H, $J_{6',7}$ 6.5 Hz, 6′-CH_3), 2.26 (s, 3 H, 4-*N*CH_3), 3.38 (s, 3-*O*CH_3), and 7.31 (s, Z aromatic).

Anal. Calc. for $C_{49}H_{61}N_5O_{13}$: C, 63.42; H, 6.63; N, 7.55. Found: C, 63.24; H, 6.79; N, 7.81.

Final fractions, which contained a single component, were taken to dryness to give 0.559 g of **13**: $[\alpha]_D^{24}$ −9.5° (*c* 1, methanol): $\nu_{max}^{CDCl_3}$ 3440, 1710, and 1510 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 0.86 (d, 3 H, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.35 (s, 3 H, 4-*N*CH_3), 3.46 (s, 3 H, 3-OCH_3), and 7.35 (m, 15 H, Z aromatic).

Anal. Calc. for $C_{41}H_{55}N_5O_{11}$: C, 62.03; H, 6.98; N, 8.82. Found: C, 61.80; H, 7.03; N, 8.72.

*1,6′,2″-Tri-*N*-benzyloxycarbonyl-2′-*N*-(2-*N*-benzyloxycarbonylaminoethyl)fortimicin A* (**14**). — A stirred solution of **13** (0.503 g) in oxolane (3.4 mL) was treated with 0.223 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. After stirring for 20 h, the solvent was evaporated off. The product was purified by chromatography on a column (1.5 × 74 cm) of silica gel eluted with 23.5:1.4:2.0:0.2 (v/v) benzene–

methanol–95% ethanol–concentrated ammonium hydroxide to give 0.403 g of pure **14**; $[\alpha]_D^{24}$ +32.7° (*c* 1.03, methanol); $\nu_{max}^{CDCl_3}$ 3410, 1705, 1635, and 1500 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$); δ 1.17 (unresolved d, 3 H, 6′-CH_3), 2.92 (s, 3 H, 4-NCH_3), 3.33 (s, 3 H, 3-OCH_3), and 7.28 (m, Z aromatic).

Anal. Calc. for $C_{51}H_{46}N_6O_{14}$: C, 62.18; H, 6.55; N, 8.53. Found: C, 62.04; H, 6.56; N, 8.42.

2′-N-(2-Aminoethyl)fortimicin A (**12**). — Hydrogenolysis of **14** (0.455 g) under conditions identical to those described for **8a** gave, after processing, 0.268 g of **12** as the pentahydrochloride: $[\alpha]_D^{24}$ +67.7° (*c* 1.0, methanol); ν_{max}^{KBr} 3420, 2940, 1643, and 1600 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.78 (d, 3 H, $J_{1',2'}$ 7.0 Hz, 6′-CH_3), 3.57 (s, 3 H, 4-NCH_3), 3.93 (s, 3 H, 3-OCH_3), and 5.87 (s, 1 H, $J_{1',2'}$ 3.2 Hz, H-1′); *m/e* 448.2991 (M^+), calc. for $C_{19}H_{40}N_6O_6$ 448.3009.

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