6′-*N*-METHYLFORTIMICINS A AND B AND 6′,6′-DI-*N*-METHYLFORTIMICINS A AND B†

JACK TADANIER*, DANIEL A. DUNNIGAN, JERRY R. MARTIN,
LESLIE FREIBERG and MOMIR CIROVIC

Abbott Laboratories, Departments of Chemical Research and Analytical Research
North Chicago, Illinois 60064, U.S.A.

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Selective 6′-*N*-alkylation of 1,2′-di-*N*-benzyloxycarbonylfortimicin B was effected by both catalytic and chemical reductive alkylation in the presence of aldehydes. These facile selective 6′-*N*-alkylations were used as the basis of the preparations of the 6′,6′-di-*N*-methylfortimicins A and B, and the 6′-*N*-methylfortimicins A and B. Of these new 6′-*N*-methylated fortimicins, only 6′-*N*-methylfortimicin A has appreciable antibacterial activity, which was about half that of fortimicin A.

Fortimicin A (**1**) and fortimicin B (**2**) are novel, pseudodisaccharide, aminoglycoside antibiotics which are formed in fermentations by *Micromonospora olivoasterospora*[1]. In the context of a program of chemical modification of the fortimicin antibiotics[2], carried out with the object of obtaining fortimicin derivatives with improved therapeutic properties, we have prepared the 6′,6′-di-*N*-methylfortimicins A and B (**3** and **4**) and the 6′-*N*-methylfortimicins A and B (**5** and **6**). In the present report we describe the syntheses, structure proofs, and antibacterial activities of these semisynthetic antibiotics.

Clinical application of antibiotics frequently leads to development of resistant strains of bacteria. Studies of the mechanisms of bacterial resistance to the aminoglycoside antibiotics has shown that resistance is frequently a consequence of bacterial enzymes which effect *N*-acylation. For example, aminoglycoside antibiotics which have primary amino groups at $C_{6'}$ such as gentamicin C_{1a}, gentamicin C_2, kanamycin A, and kanamycin B are inactivated by AAC (6′) acetyltransferases[3]. In contrast gentamicin C_1, which differs from gentamicin C_2 only by the presence of the 6′-*N*-methyl group and which has antibacterial activity about equal to the activities of the gentamicins C_{1a} and C_2[4], is not subject to inactivation by AAC (6′) N-acetylating enzymes. It has been suggested[5] that one method of preventing possible deactivation of an antibiotic which occurs by acetylation of a primary amino group by a bacterial enzyme is by *N*-alkylation of the amino group involved. The present work was carried out with the object of preparing fortimicin A derivatives which would not be susceptible to inactivation by enzymes which would effect 6′-*N*-acylation of fortimicin A. Accordingly we planned the syntheses of 6′-*N*-methylfortimicin A (**5**) and 6′,6′-di-*N*-methylfortimicin A (**3**). It should be noted that the diaminosugar moieties of fortimicin A and 6′-*N*-methylfortimicin A differ from those of gentamicin C_2 and gentamicin C_1, respectively, only in the configurations of the $C_{6'}$-carbons.

Treatment of fortimicin B (**2**) with *N*-benzyloxycarbonyloxysuccinimide in aqueous methanol in the presence of acetic acid gave 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**). The positions of attachment of the two benzyloxycarbonyl groups of **7** were determined by the criterion that, for primary

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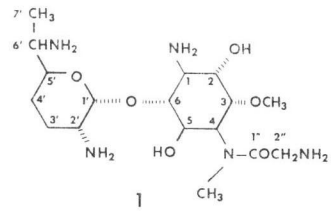
and secondary amines, the chemical shifts of the protons attached to the carbon atoms bearing amino groups undergo downfield shifts when the amino groups are acylated[6]. In the present case the reference compound was 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**)[2]. The pmr spectra of **7** and **8** were determined in pyridine-d_5 at 110°C to minimize signal broadening due to hindered rotation about the carbon-nitrogen bonds of the benzyloxycarbonyl groups. Hexamethylsiloxane was used as an internal reference. That the C_4-methylamino group of **7** was not acylated was established by the fact that the chemical shifts of the C_4-*N*-methyl groups of **7** (δ 2.25) and **8** (δ 2.21) were almost identical.

Although the $C_{2'}$-proton absorptions of **7** and **8** were not resolved from other proton absorptions, their chemical shifts were determined by double irradiation experiments. The anomeric $C_{1'}$-proton doublets of **7** (δ 5.44 d, $J_{1',2'}$=3.5 Hz) and **8** (δ 5.43 d, $J_{1',2'}$=3.2 Hz) were collapsed by irradiation at δ 3.76 and δ 3.72, respectively, which established these as the values of the chemical shifts of the $C_{2'}$-protons of **7** and **8**. The near identity of the chemical shifts of the $C_{2'}$-protons of **7** and **8** proved that the $C_{2'}$-nitrogen of **7**, like that of **8**, was acylated by one of the benzyloxycarbonyl groups present.

A pentuplet at δ 2.75 ($J_{5',6'} \cong J_{6',7'} \simeq 7.0$ Hz) in the spectrum of the di-*N*-benzyloxycarbonyl derivative **7** was shown to be the $C_{6'}$-proton by double irradiation experiments. Irradiation of **7** at δ 2.75 collapsed the $C_{6'}$-methyl doublet at δ 0.87 d ($J_{6',7'}$=6.5 Hz). Although the absorption of the $C_{6'}$-proton of the tri-*N*-benzyloxycarbonyl derivative **8** was not resolved, its chemical shift ($\sim\delta$ 3.76) was determined by collapse of the $C_{6'}$-methyl doublet (δ 1.07 d, $J_{6',7'}$=6.8 Hz) by irradiation at δ 3.76. The difference (1.01 ppm) between the chemical shifts of the $C_{6'}$-protons of **7** and **8** was attributed to the downfield shift of the $C_{6'}$-proton on $C_{6'}$-*N*-acylation of **7**. This established that the $C_{6'}$-amino group of **7** was not acylated, and thus that the second benzyloxycarbonyl group was at C_1.

Reductive methylation of 1,2-di-*N*-benzyloxycarbonylfortimicin B (**7**) with formaldehyde and 5% platinum on carbon under three atmospheres of hydrogen gave 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methyl-4,5-N,O-methylenefortimicin B (**9**), which was characterized spectroscopically by a doublet at δ 4.50 (J=3 Hz) in the pmr spectrum due to one of the methylene protons of the oxazolidine ring*, and by its conversion to 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**)



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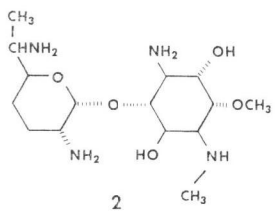

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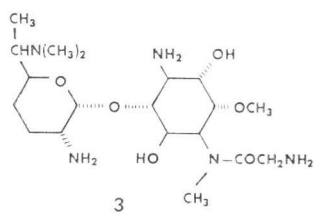

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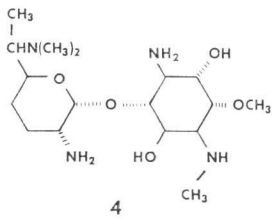

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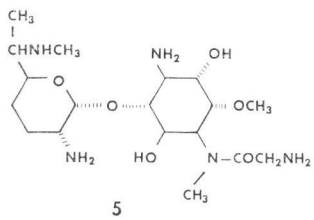

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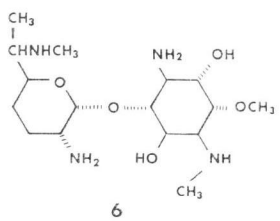

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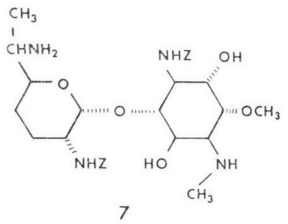
* The other doublet was obscured by overlapping peaks, but appeared to be in the region between δ 3.79 and δ 3.86.

by mild acid-catalyzed hydrolysis in the presence of hydroxylamine hydrochloride as a formaldehyde scavenger. Acylation of the C_4-methylamino group of **10** with *N*-(*N*-benzyloxycarbonylglycyloxy)-succinimide gave 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin A (**11**).

Treatment of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**) first with benzaldehyde in refluxing methanol for one hour, followed by immediate cooling of the solution and sodium borohydride reduction, gave 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) which was isolated in 67% yield by column chromatography. It must be noted that when the methanol solution prepared from **7**, benzaldehyde, and methanol, after having been heated under reflux for one hour, was kept at ambient temperature for twenty-two hours before sodium borohydride reduction, the reduced product mixture contained 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) and a new, less polar product in a ratio of about 1 : 3 as estimated by tlc. The new product is believed to be the 4,5-benzaldehyde oxazolidine **13*** since the off-resonance cmr spectrum of the mixture showed a low-field doublet at 99.7 ppm, attributable to the methine proton of the bridging phenylmethylene group. On column chromatography, the new product was eluted in the early fractions contaminated with benzyl alcohol, and 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) was isolated in only 27% yield. On mild acid-catalyzed hydrolysis of the crude reduction mixture, the new product disappeared and 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B was isolated in 70% yield by column chromatography.

Reductive methylation of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) with formaldehyde and 5% platinum on carbon under three atmospheres of hydrogen, followed by mild, acid-catalyzed hydrolysis of the resulting 4,5-formaldehyde oxazolidine **14** in the presence of hydroxylamine hydrochloride gave 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**). Acylation of the C_4-methylamino group of the latter with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin A (**16**).

Catalytic hydrogenolyses of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**) and 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin A (**11**) in the presence of palladium on



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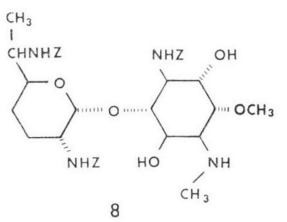

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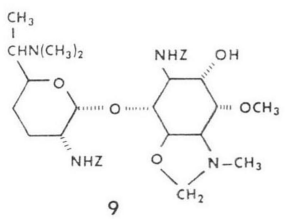

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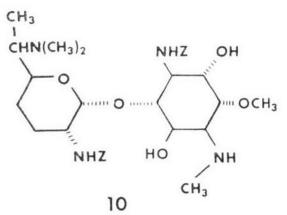

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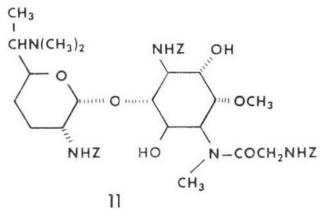

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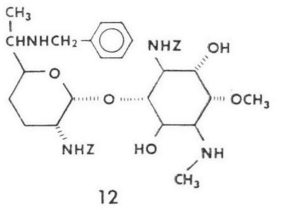

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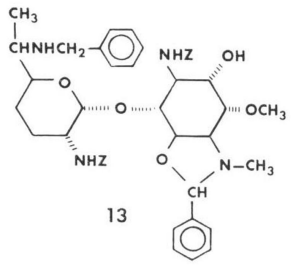
* A similar benzaldehyde oxazolidine has been reported to form with the vicinal, cis-related methylamino and hydroxyl groups of gentamicin C_2, D. J. COOPER, J. WEINSTEIN & J. A. WAITE: J. Med. Chem. 14: 1118, 1971.



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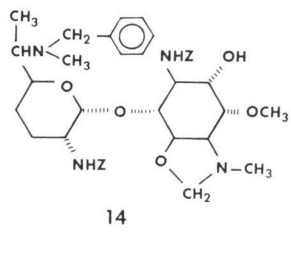

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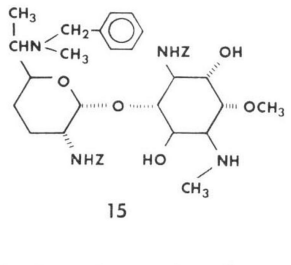

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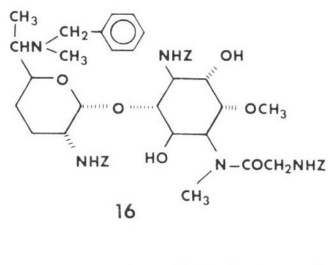

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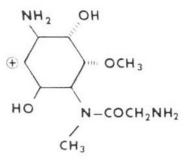
carbon removed the benzyloxycarbonyl protecting groups to give 6′,6′-di-*N*-methylfortimicin B (**4**) and 6′,6′-di-*N*-methylfortimicin A (**3**), respectively, which were isolated as their perhydrochlorides. Similar hydrogenolyses of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**) and 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin A (**16**) removed the benzyl groups as well as the benzyloxycarbonyl groups to give 6′-*N*-methylfortimicin B (**6**) and 6′-*N*-methylfortimicin A (**5**) which were isolated as their perhydrochlorides.

Confirmation that the site of alkylation of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**) was the 6′-amino group, and that the products were, thus, 6′,6′-di-*N*-methyl- and 6′-*N*-methylfortimicins, was provided by comparison of their mass spectra with those of fortimicin A and fortimicin B[7]. Both the 6′-*N*-methyl- and 6′,6′-di-*N*-methylfortimicins A and B showed fragments with masses attributable to the corresponding cyclitol fragments **17** and **18** also observed in the mass spectra of fortimicin A and fortimicin B, respectively. In addition the 6′-*N*-methyl and 6′,6′-di-*N*-methyl derivatives lacked the 6′-*epi*-purpurosamine fragment **19** observed for both fortimicin A and fortimicin B, and showed instead the characteristic mono- and dimethylated fragments, **20** and **21**, respectively.



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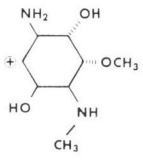

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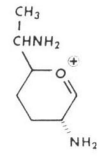

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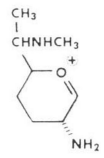

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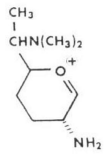

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The *in vitro* antibacterial activities of the 6′-*N*-methylfortimicins and 6′,6′-di-*N*-methylfortimicins against nineteen microorganisms are listed in Table 1. Of these, only 6′-*N*-methylfortimicin A has appreciable activity which is somewhat less than that of fortimicin A.

Experimental

General

Optical rotations were determined with a Hilger and Watts polarimeter. Ir spectra were recorded using a Perkin-Elmer Model 521 grating spectrometer. Pmr spectra were determined at 100 MHz with a Varian Associates HA-100 spec-

Table 1. *In vitro* antibacterial activities of the 6′-*N*-methyl- and 6′,6′-di-*N*-methylfortimicins.*

Organism	Minimum inhibitory concentration (mcg/ml)					
	Fortimicin B	Fortimicin A	**6**	**4**	**5**	**3**
Staph. aureus Smith	>100	1.56	>100	>100	1.56	>100
Strep. faecalis 10541	>100	50	>100	>100	>100	>100
Enterobacter aerogenes 13048	>100	3.1	>100	>100	6.2	>100
E. coli Juhl	>100	12.5	>100	>100	6.2	>100
E. coli BL 3676 (Res)	>100	25	>100	>100	50	>100
E. coli 76-2	>100	6.2	>100	>100	6.2	>100
Kleb. pneumoniae 10031	>100	6.2	>100	>100	3.1	>100
Kleb. pneumoniae KY 4262	>100	6.2	>100	>100	12.5	>100
Providencia 1577	>100	3.1	>100	>100	3.1	>100
Pseudo. aeruginosa BMH # 10	>100	1.56	>100	>100	1.56	>100
Pseudo. aeruginosa KY 8512	>100	25	>100	>100	50	>100
Pseudo. aeruginosa KY 8516	>100	50	>100	>100	>100	>100
Pseudo. aeruginosa 209	>100	>100	>100	>100	>100	>100
Pseudo. aeruginosa 27853	>100	25	>100	>100	50	>100
Sal. typhimurium Ed. # 9	>100	3.1	>100	>100	3.1	>100
Serratia marcescens 4003	>100	3.1	>100	>100	3.1	>100
Shigella sonnei 9290	>100	12.5	>100	>100	12.5	>100
Proteus rettgeri U6333	>100	12.5	>100	>100	50	>100
Proteus vulgaris JJ	>100	6.2	>100	>100	6.2	>100

* The *in vitro* activities were determined by the serial dilution method using MUELLER-HINTON agar with the tetrahydrochlorides. Activities are expressed as minimum inhibitory concentrations in micrograms of tetrahydrochloride per milliliter.

trometer. Chemical shifts are reported in ppm downfield from external TMS contained in a co-axial capillary in the sample tube. Cmr spectra were measured at 25.2 MHz on a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 70 eV and 100~150°C using the direct probe insert. The purity of all compounds was established spectroscopically and by tlc. Microanalytical results are reported for those compounds which could be freed of solvent. Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh. Ratios for chromatography solvents are expressed by volume. Solvents were evaporated under reduced pressure on a rotary evaporator.

1,2′-Di-*N*-benzyloxycarbonylfortimicin B (7)

To a stirring, ice bath-cooled solution prepared from 10 g of fortimicin B (**3**), 150 ml of water, 300 ml of methanol and 4.95 ml of glacial acetic acid was added 15.7 g of *N*-(benzyloxycarbonyloxy)-succinimide. Stirring was continued in the cold for 1.5 hours and then at room temperature for 25 hours. The solution was concentrated to approximately one-third volume and extracted with chloroform. The chloroform extract was washed with 1% aqueous sodium bicarbonate and dried ($MgSO_4$). Evaporation of the chloroform gave 19 g of solid. The solid was chromatographed on a column (7.0×80 cm) of silica gel prepared and eluted with chloroform - methanol - concentrated ammonium hydroxide (750: 150: 7). Evaporation of the fractions containing only the major component gave 4.2 g of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**): $[\alpha]_D^{22}$ +43° (*c* 1.0, methanol); ir ($CDCl_3$) 3562, 3432 and 1712 cm^{-1}: pmr ($CDCl_3$) δ 0.81 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 2.32 (s, NCH_3), 3.41 (s, OCH_3).

Anal. Calcd. for $C_{31}H_{44}N_4O_9$: C, 60.38; H, 7.19; N, 9.08.
Found: C, 60.25; H, 7.39; N, 9.04.

1,2′-Di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methyl-4,5-N,O-methylenefortimicin B (**9**)

A solution prepared from 3.02 g of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**), 5 ml of 37% formalin, and 195 ml of methanol was hydrogenated under three atmospheres of hydrogen for 4.5 hours in the presence of 1.5 g of 5% platinum on carbon. The catalyst was removed by filtration and the filtrate was evaporated to give 3.04 g of product. Tlc examination of the product indicated the reaction was incomplete. The product was dissolved in a mixture of 5 ml of formalin and 195 ml of methanol and further hydrogenated for 6.5 hours under three atmospheres of hydrogen in the presence of 3 g of 5% platinum on carbon. The catalyst was removed by filtration and the filtrate was evaporated to give 2.43 g of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methyl-4,5-N,O-methylenefortimicin B (**9**): pmr ($CDCl_3$) δ 0.83 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.8 Hz), 2.21, 2.32 (s, NCH_3), 3.48 (s, OCH_3), doublet between 3.79~3.86, 4.50 (d, OCH_2NCH_3, J_{AB}=3.0 Hz).

1,2′-Di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**)

A solution prepared from 2.37 g of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methyl-4,5-N,O-methylenefortimicin B (**9**), 0.840 g of hydroxylamine hydrochloride, 2.3 ml of acetic acid and 150 ml of methanol was heated under reflux for 0.5 hour. After the major portion of the methanol was evaporated the remaining solution was shaken with a mixture of dilute ammonium hydroxide and chloroform. The chloroform layer was separated and washed with saturated aqueous sodium chloride. The chloroform portion was dried ($MgSO_4$) and evaporated to give 2.30 g of product. The product was chromatographed on a column of silica gel. Elution with methylene chloride - methanol - concentrated ammonium hydroxide (14: 6: 0.2) gave fractions yielding 1.90 g of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**): $[\alpha]_D^{22}$ +44° (*c* 1.0, methanol); ir ($CDCl_3$) 3557, 3423, 3348 and 1695 cm^{-1}; pmr ($CDCl_3$) δ 0.84 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7 Hz), 2.18 [s, $N(CH_3)_2$], 2.37 (s, NCH_3), 3.43 (s, OCH_3).

Anal. Calcd. for $C_{33}H_{48}N_4O_9$: C, 61.47; H, 7.50; N, 8.69.
Found: C, 61.57; H, 7.77; N, 8.71.

1,2′,2″-Tri-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin A (**11**)

To a stirring, ice bath-cooled solution prepared from 0.626 g of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**) and 9 ml of tetrahydrofuran was added 0.4016 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Stirring was continued in the cold for 3 hours and then at room temperature for 22 hours. The reaction solution was shaken with a mixture of 5% aqueous sodium bicarbonate and chloroform. The chloroform layer was washed with water and taken to dryness to give 0.8619 g of white glass. The glass was chromatographed on a column (2.4×40 cm) of silica gel prepared and eluted with a solvent system of ethyl acetate - methanol - triethylamine (22: 2: 0.3) to give 0.7356 g of 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin A (**11**): $[\alpha]_D^{22}$ +67° (*c* 1.0, methanol); ir ($CDCl_3$) 3552, 3412, 1700 and 1628 cm^{-1}; pmr ($CDCl_3$) δ 0.90 (d, $C_{6'}$-CH_3, $J_{6',7'}$= 6.7 Hz), 2.22 (s, $C_{6'}$-NCH_3), 3.35 (s, C_4-NCH_3), 3.31 (s, OCH_3).

Anal. Calcd. for $C_{48}H_{57}N_5O_{12}\cdot H_2O$: C, 60.44; H, 7.11; N, 7.87.
Found: C, 60.44; H, 6.96; N, 8.20.

1,2′-Di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**)

(a) A solution prepared from 3.0 g of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**), 1.2 ml of benzaldehyde and 30 ml of methanol was heated under reflux for 1 hour. The reaction mixture was cooled in an ice bath and immediately treated in the cold with stirring with a freshly prepared solution of 0.518 g of sodium borohydride in 3.0 ml of water. Stirring was continued in the cold for 1 hour and then at room temperature for 3 hours. The reaction solution was shaken with a mixture of 5% aqueous sodium bicarbonate and chloroform. The chloroform layer was washed with 5% aqueous sodium chloride and dried. Evaporation of the chloroform gave 3.45 g of product. A portion of the product (1.02 g) was chromatographed on a column (2.4×60 cm) of silica gel prepared and eluted with chloroform - methanol - concentrated ammonium hydroxide (19: 1: 0.2) to give 0.68 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**): $[\alpha]_D^{23}$ +43° (*c* 1.0, methanol); ir ($CDCl_3$) 3552, 3434, 3420 and 1698 cm^{-1}; pmr ($CDCl_3$) δ 0.90 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.37 (s, NCH_3), 3.39 (s, OCH_3).

Anal. Calcd. for $C_{38}H_{50}N_4O_9$: C, 64.57; H, 7.13; N, 7.93.

Found: C, 63.99; H, 7.25; N, 7.82.

(b) A solution prepared from 2.035 g of 1,2′-di-*N*-benzyloxycarbonylfortimicin B (**7**), 0.81 ml of benzaldehyde and 20 ml of methanol was heated under reflux for 1 hour and left at room temperature for 22 hours. The solution was cooled in an ice bath and treated with stirring with a freshly prepared solution of 0.35 g of sodium borohydride in 2.0 ml of water. Stirring was continued in the cold for 1 hour and then at room temperature for 3 hours. Chloroform extraction gave 2.52 g of product. A cmr single-frequency, off-resonance, decoupled spectrum of the product in deuteriochloroform showed a doublet at 99.7 ppm. Tlc examination of the product indicated the presence of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) and a second, faster moving product in a ratio of 1: 3. A portion of the product (1.0 g) was chromatographed on a column of silica gel prepared and eluted with chloroform - methanol - concentrated ammonium hydroxide (19: 1: 0.2). The early fractions gave 0.68 g of a mixture of benzyl alcohol and a faster moving product. Later fractions gave 0.25 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) identical with that prepared in Part (a) above.

A 1.26 g sample of the product, prepared above, was dissolved in a solution of 0.4 N hydrochloric acid and 40 ml of tetrahydrofuran and allowed to stand at room temperature for 2 hours. The reaction mixture was added to a solution prepared from 1: 2 concentrated ammonium hydroxide - water and extracted with chloroform. The chloroform extract was washed with water, dried ($MgSO_4$) and evaporated to give 1.22 g of product. Tlc examination of the product showed the absence of the less polar material mentioned above. The product was chromatographed on a column of silica gel prepared and eluted with chloroform - methanol - concentrated ammonium hydroxide (19: 1: 0.2) to give 0.81 g of 1,2-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**).

1,2′-Di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzyl-4,5-N,O-methylenefortimicin B (**14**)

A solution of 3 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-benzylfortimicin B (**12**) in 220 ml of methanol, in the presence of 10 ml of 37% formalin and 3 g of 5% platinum on carbon, was catalytically hydrogenated under three atmospheres of hydrogen for 6.5 hours. The catalyst was removed by filtration and the filtrate evaporated to leave 2.71 g of product. A 2.4 g sample of the product was chromatographed on a column (3.4×50 cm) of silica gel prepared and eluted with methylene chloride - methanol - 37% formalin (17.5: 2.5: 0.5) to give 1.79 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzyl-4,5-N,O-methylenefortimicin B (**14**): pmr ($CDCl_3$) δ 0.86 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.8 Hz), 2.18, 2.30 (NCH_3), 3.47 (s, OCH_3), 3.83, 4.63 (d, OCH_2N, J_{AB}=2.5 Hz).

1,2′-Di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**)

A solution prepared from 1.74 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzyl-4,5-N,O-methylenefortimicin B (**14**), 0.57 g of hydroxylamine hydrochloride, 1.5 ml of glacial acetic acid and 100 ml of methanol was heated under reflux for 0.5 hour. After the major portion of the methanol was evaporated the remaining solution was shaken with a mixture of dilute ammonium hydroxide and chloroform. The chloroform portion was separated and washed with saturated aqueous sodium chloride. The chloroform extract was dried ($MgSO_4$) and evaporated to give 1.69 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**): $[\alpha]_D^{24}$ +39° (*c* 1.0, methanol); ir ($CDCl_3$) 3550, 3420 and 1700 cm^{-1}; pmr ($CDCl_3$) δ 0.93 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.15, 2.31 (s, NCH_3), 3.42 (s, OCH_3).

Anal. Calcd. for $C_{39}H_{52}N_4O_9$: C, 64.92; H, 7.27; N, 7.77.
Found: C, 64.47; H, 7.35; N, 7.60.

1,2′,2″-Tri-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin A (**16**)

A stirred, ice bath cooled solution prepared from 0.706 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**) and 9.0 ml of tetrahydrofuran was treated with 0.416 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Stirring was continued in the cold for 3 hours and then at room temperature for 20 hours. The reaction solution was shaken with a mixture of 5% aqueous sodium bicarbonate and chloroform. The chloroform layer was separated, washed with water and evaporated to leave 1.00 g of colorless glass. A 0.97 g sample of the glass was chromatographed on a column (2.4×49 cm) of silica gel prepared and eluted with ethyl acetate - triethylamine (19.8: 0.2) to

give 0.768 g of 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin A (**16**): $[\alpha]_D^{23}+53°$ (*c* 1.0, methanol); ir ($CDCl_3$) 3550, 3410, 1702 and 1627 cm^{-1}; pmr ($CDCl_3$) δ 1.01 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.5$ Hz), 2.17 (s, $C_{6'}$-NCH_3), 2.78 (s, C_4-NCH_3), 3.28 (s, OCH_3).

Anal. Calcd. for $C_{49}H_{61}N_5O_{12}$: C, 64.52; H, 6.74; N, 7.68.
Found: C, 63.95; H, 7.04; N, 7.25.

6′,6′-Di-*N*-methylfortimicin B (**4**)

A solution prepared from 0.407 g of 1,2′-di-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin B (**10**) and 50 ml of 0.2 N hydrochloric acid in methanol was hydrogenated in the presence of 0.40 g of 5% palladium on carbon for 4 hours under three atmospheres of hydrogen. Work up as before gave 0.304 g of 6′,6′-di-*N*-methylfortimicin B (**4**) isolated as the tetrahydrochloride: $[\alpha]_D^{24}+84°$ (*c* 1.0, methanol); pmr (D_2O) δ 1.76 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.6$ Hz), 3.29, 3.38 [s, $C_{6'}$-$N(CH_3)_2$], 3.38 (s, C_4-NCH_3), 3.95 (s, OCH_3); mass spectrum, *m/z* 376.2691 (M+·), calcd. for $C_{17}H_{36}N_4O_5$ 376.2686; sugar fragment *m/z* 189.1609, calcd. for $C_9H_{21}N_2O_2$ 189.1603; cyclitol fragment *m/z* 189.1246, calcd. for $C_8H_{17}N_2O_3$ 189.1239; sugar fragment *m/z* 171.1492, calcd. for $C_9H_{19}N_2O$ 171.1497.

6′,6′-Di-*N*-methylfortimicin A (**3**)

A solution prepared from 0.350 g of 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′,6′-di-*N*-methylfortimicin A (**11**), 33.5 ml of 0.2 N hydrochloric acid in methanol, and 15 ml of methanol was hydrogenated in the presence of 0.350 g of palladium on carbon for 4 hours under three atmospheres of hydrogen. The usual work up gave 0.2445 g of 6′,6′-di-*N*-methylfortimicin A (**3**) isolated as the tetrahydrochloride: $[\alpha]_D^{23}+77°$ (*c* 1.0, methanol), ir (KBr) 1634 cm^{-1}; pmr (D_2O) δ 1.76 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.4$ Hz), 3.36, 3.37 [$C_{6'}$-$N(CH_3)_2$], 3.58 (s, C_4-NCH_3), 3.94 (s, OCH_3); mass spectrum, *m/z* 433.2894 (M+·), calcd. for $C_{19}H_{39}N_5O_6$ 433.2900; cyclitol fragment *m/z* 246.1454, calcd. for $C_{10}H_{20}N_3O_4$ 246.1454; sugar fragment *m/z* 171.1497, calcd. for $C_9H_{19}N_2O$ 171.1497.

6′-*N*-Methylfortimicin B (**6**)

A solution prepared from 0.260 g of 1,2′-di-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin B (**15**), 36 ml of 0.2 N hydrochloric acid in methanol, and 14 ml of methanol was hydrogenated in the presence of 0.260 g of 5% palladium on carbon for 4 hours under three atmospheres of hydrogen. Work up as before gave 0.172 g of 6′-*N*-methylfortimicin B (**6**) isolated as the tetrahydrochloride: $[\alpha]_D^{23}+81°$ (*c* 1.0, methanol); pmr (D_2O) δ 1.80 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.8$ Hz), 3.21, 3.28 (s, C_4- and $C_{6'}$-NCH_3), 3.95 (s, OCH_3); mass spectrum, *m/z* 362.2522 (M+·), calcd. for $C_{16}H_{34}N_4O_5$ 362.2529; cyclitol fragment *m/z* 189.1246, calcd. for $C_8H_{17}N_2O_3$ 189.1239, sugar fragment *m/z* 157.1349, calcd. for $C_8H_{17}N_2O$ 157.1341.

6′-*N*-Methylfortimicin A (**5**)

A solution prepared from 0.432 g of 1,2′,2″-tri-*N*-benzyloxycarbonyl-6′-*N*-methyl-6′-*N*-benzylfortimicin A (**16**), 28.4 ml of 0.2 N hydrochloric acid in methanol, and 6.6 ml of methanol was hydrogenated in the presence of 0.430 g of 5% palladium on carbon for 4 hours under three atmospheres of hydrogen. The catalyst was removed by filtration and the filtrate evaporated. Excess hydrochloric acid was removed by repeated co-distillation with methanol to give 0.259 g of 6′-*N*-methylfortimicin A (**5**) isolated as the tetrahydrochloride: $[\alpha]_D^{23}+81°$ (*c* 1.0, methanol); ir (KBr) 1630 cm^{-1}; pmr (D_2O) δ 1.81 (d, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 3.20 (s, $C_{6'}$-NCH_3), 3.58 (s, C_4-NCH_3), 3.95 (s, OCH_3); mass spectrum, *m/z* 419.2731 (M+1), calcd. for $C_{18}H_{37}N_5O_6$ 419.2744; cyclitol fragment *m/z* 246.1459, calcd. for $C_{10}H_{20}N_3O_4$ 246.1454; sugar fragment *m/z* 157.1348, calcd. for $C_8H_{17}N_2O$ 157.1341.

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