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FORTIMICIN B CYCLIC CARBAMATES

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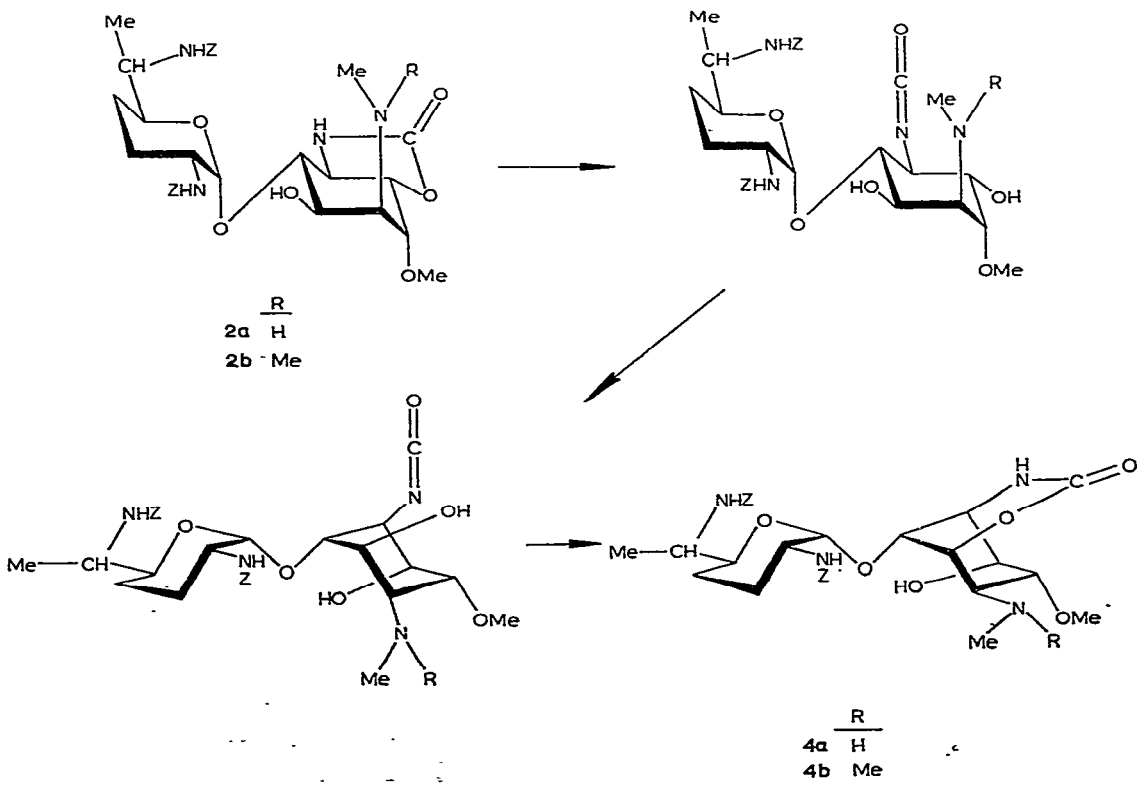
ABSTRACT

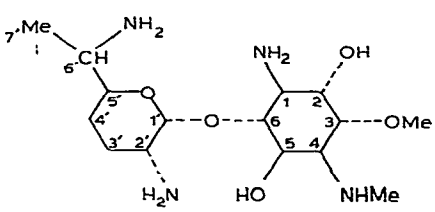
Preparations are described of fortimicin B 1,2;4,5-biscarbamate and the three possible monocyclic carbamates of fortimicin B that involve the amino and hydroxyl groups of the cyclitol ring. Rearrangement of fortimicin B 1,2-carbamates to fortimicin B 1,5-carbamates has been found to be a convenient route to the latter. Nitrous acid cleavage of fortimicin B 1,2;4,5-biscarbamate gave fortamine 1,2;4,5-biscarbamate, which is an intermediate of potential value for glycosylation at O-6.



DISCUSSION

Selective chemical reactions of polyfunctional amino alcohols frequently require protection of hydroxyl groups as well as both primary and secondary amino groups. Simultaneous protection of vicinal hydroxyl and amino groups as cyclic carbamates has been of considerable synthetic utility in the aminocyclitol field[1]. To facilitate a program of chemical modification of the fortimicin antibiotics[2], we undertook the preparation of a number of fortimicin cyclic carbamates. In the present work, the general method employed for carbamate formation was the base-catalyzed cyclization of neighboring alkoxycarbonylamino and hydroxyl groups (Scheme 1).

The stereochemical relationships among the two amino groups and the two hydroxyl groups of the cyclitol ring of fortimicin B (**1**) allow the preparation of four types of fortimicin cyclic carbamate derivatives; *trans*-1,2-carbamates (**2**), *cis*-4,5-carbamates (**3**), 1,5-carbamates (**4**), and 1,2;4,5-biscarbamates (**5**). Formation of five-membered fortimicin B *cis*-4,5-carbamates **3** is relatively straightforward and has been accomplished by cyclization of a 4-*N*-alkoxycarbonyl group with the vicinal



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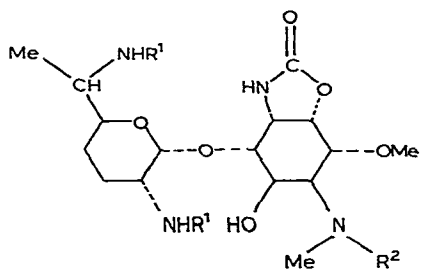

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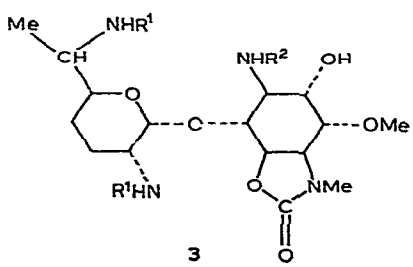
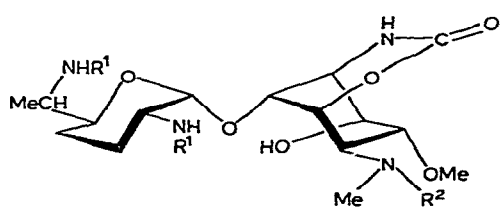
	R^1	R^2
a	Z	H
b	Z	Me
c	Z	CO_2Et
d	H	H
e	H	Me



3

	R^1	R^2
a	Z	Z
b	Z	CO_2Me
c	Z	H
d	H	H

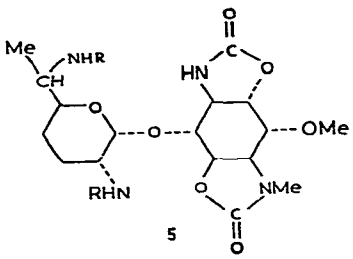


4

	R^1	R^2
a	Z	$COCH_2NHZ$
b	Z	Me
c	Z	$CO_2CH_2CCl_3$
d	Z	H
e	H	H

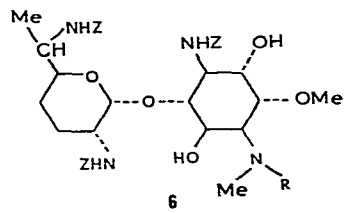
5-hydroxyl group in refluxing aqueous methanol in the presence of sodium hydrogencarbonate[2b,2c]. In contrast, cyclization of a 1-*N*-alkoxycarbonyl group may occur with either the vicinal hydroxyl group to form a five-membered, *trans*-1,2-carbamate **2**, or with the 5-hydroxyl group to form a six-membered, 1,5-carbamate **4**. The present report describes methods for conversion of 1-*N*-(benzyloxycarbonyl)fortimicin B derivatives **6** into both fortimicin B 1,2-carbamates **2** and fortimicin B 1,5-carbamates (**4**), and the rearrangements of the former (**2**) to the latter (**4**). In addition, preparation of fortimicin B 1,2;4,5-biscarbamate (**5a**) has been accomplished by cyclizations of both 4-*N*-(ethoxycarbonyl)fortimicin B 1,2-carbamate (**2c**) and 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate (**3a**).

Treatment of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (ref. 2a) (**6a**) with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) in refluxing benzene gave a mixture of products that were not separated. In contrast, treatment of both 1,2′,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A[2a] (**6b**); and 1,2′,6′-tri-*N*-benzyloxycarbonyl-4-*N*-methyl-fortimicin B (**6c**), (the latter prepared from **6a** by reductive methylation with



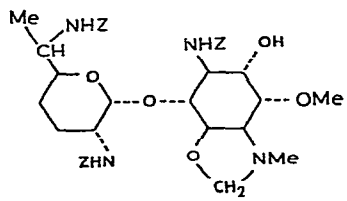
5

	R
a	Z
b	H



6

	R
a	H
b	$COCH_2NHZ$
c	Me



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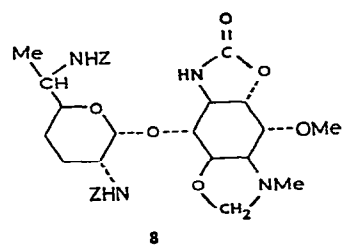

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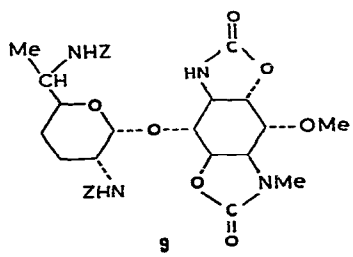

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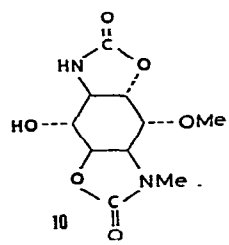

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formaldehyde and sodium cyanoborohydride) gave the 1,5-carbamates **4a** and **4b**, respectively, in conversions of 25 and 38%, together with recovered starting materials. *N*-Demethylation of **4b** with 2,2,2-trichloroethoxycarbonyl chloride in refluxing benzene[3] gave 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-fortimicin B 1,5-carbamate (**4c**). Treatment of the latter (**4c**) with zinc and acetic acid gave 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,5-carbamate **4d**. Acylation of **4d** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave 2′,6′,2″-tri-*N*-(benzyloxycarbonyl)fortimicin A 1,5-carbamate (**4a**), identical with that prepared from 1,2′,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A (**6b**) as already described.

Preparation of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**) was accomplished in a sequence initiated by protection of both the 4-methylamino group and the 5-hydroxyl group as the cyclic 4,5-methylene oxazolidine derivative **7**, which was prepared from 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**) and formaldehyde in aqueous methanol. Treatment of **7** with either DBU in refluxing benzene, or sodium hydride in *N*,*N*-dimethylformamide gave the 1,2-carbamate 4,5-oxazolidine derivative **8**. The latter (**8**) was converted into **2a** by mild, acid-catalyzed hydrolysis in the presence of hydroxylamine as a formaldehyde scavenger.

In contrast to the reactions with DBU in benzene just described, 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**) and 1,2′,6′-tri-*N*-benzyloxycarbonyl-4-*N*-methylfortimicin B (**6c**), on treatment with sodium hydride in *N*,*N*-dimethylformamide, gave the 1,2-carbamates **2a** and **2b**, respectively. Both t.l.c. and ^{1}H-n.m.r. of the total crude products showed the absence of all but minor amounts (<5%) of starting materials and the related 1,5-carbamates **4d** and **4b**. The 1,2-carbamates **2a** and **2b** rearranged almost completely in aqueous, methanolic ammonium hydroxide to the 1,5-carbamates **4d** and **4b**, respectively.

The steric constraint of the 1,2-carbamate rings of **2a** and **2b** prevents direct attack of the equatorial 5-hydroxyl group on the carbonyl group of the 1,2-carbamate. The rearrangement of the 1,2-carbamates to the 1,5-carbamates **4d** and **4b** must therefore occur *via* initial opening of the 1,2-carbamate ring to an intermediate having that cyclitol chair form in which the C-1 and C-5 substituents have a 1,3-diaxial relationship. A probable course for the rearrangements involves tautomerization of the 1,2-carbamates to the 1-isocyanates, followed by cyclization of the C-5-hydroxyl and the 1-isocyanate groups from that cyclitol conformation in which they are 1,3-diaxial (Scheme 1).

2′,6′-Di-*N*-(benzyloxycarbonyl)fortimicin B 1,2;4,5-biscarbamate (**5a**) was prepared both by direct cyclization of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate[2b] (**3a**) with sodium hydride in *N*,*N*-dimethylformamide, and by conversion of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**) into the 4-*N*-ethoxycarbonyl derivative **2c**, followed by cyclization of the latter with DBU in refluxing benzene.

Attempted conversion of 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*-(ethoxycarbonyl)-fortimicin B 1,2-carbamate (**2c**) into the biscarbamate **5a** with sodium hydrogencarbonate in refluxing methanol gave 2′,6′-di-*N*-benzyloxycarbonyl-1-*N*-(methoxy-

carbonyl)fortimicin B 4,5-carbamate (**3b**). The same product (**3b**) was formed from the biscarbamate **5a** under identical conditions. The lability of the *trans*-1,2-carbamate ring of **5a** contrasts with the stability of the *cis*-carbamate ring of 2′,6′-di-*N*-benzyloxycarbonyl-2-*epi*-fortimicin B 1,2;4,5-biscarbamate (**9**), which is stable to conditions of mild basic hydrolysis[2b].

Selective hydrolysis of the *trans*-1,2-carbamate ring of the biscarbamate **5a** was accomplished with potassium hydroxide in aqueous 1,2-dimethoxyethane to give 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate (**3c**). The latter, on treatment with methoxycarbonyl chloride, was converted into 2′,6′-di-*N*-benzyloxycarbonyl-1-*N*-(methoxycarbonyl)fortimicin B 4,5-carbamate (**3b**), identical with that prepared from **2c** and **5a** as already described.

Catalytic hydrogenolysis of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**), 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate (**3a**), 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,5-carbamate (**4d**), and 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2;4,5-biscarbamate (**5a**) removed the *N*-benzyloxycarbonyl protecting-groups to give fortimicin B 1,2-carbamate (**2d**), fortimicin B 4,5-carbamate (**3d**), fortimicin B 1,5-carbamate (**4e**), and fortimicin B 1,2;4,5-biscarbamate (**5b**), respectively, isolated as their perhydrochloride salts.

Treatment of fortimicin B 1,2;4,5-biscarbamate (**5b**) with sodium nitrite in aqueous acetic acid gave fortamine 1,2;4,5-biscarbamate (**10**). The latter (**10**) has only the 6-hydroxyl group available for reaction, and thus is an intermediate of potential value for preparation, by means of 6-*O*-glycosylation reactions*, of fortimicin derivatives containing modified sugar moieties.

Infrared spectra. — The i.r. spectra ($CDCl_3$) of all of the *N*-benzyloxycarbonyl-protected, fortimicin B five-membered, monocyclic carbamates and 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2;4,5-biscarbamate (**5a**) were characterized by carbonyl absorptions at $\sim$1750 cm^{-1}. Whereas the perhydrochloric acid salts of fortimicin B 1,2-carbamate (**2d**) and fortimicin B 4,5-carbamate (**3d**) had single carbonyl absorptions at 1750 and 1744 cm^{-1} (KBr), respectively, the dihydrochloride salt of fortimicin B 1,2;4,5-biscarbamate (**5b**) showed carbonyl absorptions at both 1722 and 1732 cm^{-1} (KBr).

The *N*-benzyloxycarbonyl-protected fortimicin B 1,5-carbamates **4a**, **4b**, **4c**, and **4d**, which were related by chemical interconversions as described here, all had their six-membered, cyclic-carbamate carbonyl absorptions masked by the carbonyl absorptions of the *N*-benzyloxycarbonyl protecting-groups. The trihydrochloride salt of fortimicin B 1,5-carbamate **4c**, prepared from 2′,6′-di-*N*-benzyloxycarbonyl-fortimicin B 1,5-carbamate (**4d**), showed carbonyl absorption (KBr) at 1702 cm^{-1}, which confirmed the presence of the six-membered carbamate ring.

*After submission of our manuscript for publication, a paper[4] appeared which reported the synthesis of **5b** from fortamine, and the utilization of **5b** for 6-*O*-glycosylation.

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 determined at 100 MHz with a Varian Associates HA-100 spectrometer. Chemical shifts determined with D_2O solutions are reported from internal sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4. Chemical shifts determined with $CDCl_3$ solutions are reported from internal Me_4Si. Mass spectra were obtained with an AEI MS-902 spectrometer at 70 eV and 100–150° by means of a direct-probe insert. Silica gel for column chromatography was that of Merck (Darmstadt), 70–230 mesh. Ratios for chromatography solvents are expressed by volume. Isolations by chloroform extraction were performed by shaking the solutions or mixtures with mixtures of chloroform and 5% aqueous sodium hydrogencarbonate. The chloroform extracts were separated and dried (magnesium sulfate) and the solvent was evaporated under diminished pressure by using a rotary evaporator.

1,2′,6′-Tri-N-*benzyloxycarbonyl-4*-N,*5*-O-*methylenefortimicin B* (**7**). — A solution of 16.0 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B^{2b} (**6a**), 8 mL of 37% aqueous formaldehyde, and 400 mL of methanol was kept overnight. Solvent was evaporated off and residual water was removed by evaporation of benzene from the residue to give 16.3 g of the oxazolidine **7** as a white glass: ^{1}H-n.m.r. ($CDCl_3$): δ 1.00 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.24 (s, NCH_3), 3.48 (s, OCH_3), 3.81, and 4.63 (d, OCH_2N, J 2.3 Hz).

2′,6′-Di-N-*benzyloxycarbonyl-4*-N,*5*-O-*methylenefortimicin B* (**8**). — A solution of compound **7** (16.3 g), 16.3 g of 1,5-diazabicyclo[5.4.0]undec-5-ene, and 815 mL of benzene was boiled for 96 h under reflux. After cooling, 250 mL of water was added and the mixture was stirred for 1 h. The mixture was shaken with 700 mL of 5% aqueous sodium hydrogencarbonate. The aqueous phase was separated and extracted with benzene. The benzene solutions were washed with saturated aqueous sodium chloride and dried. Evaporation gave 15.3 g of compound **8**; $\tilde{\nu}_{max}$ ($CDCl_3$) 3459, 1762, and 1709 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.17 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.28 (s, NCH_3), 2.80 (q, $J_{3,4}$ 6.5, $J_{4,5}$ 2.0 Hz, H-4), 3.5 (s, OCH_3), 3.81 and 4.60 (d, OCH_2N, J 2.3 Hz).

2′,6′-Di-N-(*benzyloxycarbonyl*)*fortimicin B 1,2-carbamate* (**2a**). — *A*. A solution of compound **8** (15.3 g), 14.5 mL of acetic acid, 5.2 g of hydroxylamine hydrochloride, and 900 mL of methanol was boiled for 1 h under reflux. The solution was cooled and the major portion of the methanol was evaporated. The residue was shaken with a mixture of chloroform (1 L) and 1:1 (v/v) concentrated ammonium hydroxide–water (1 L). The chloroform phase was washed with water and dried. Evaporation gave 14.8 g of residue which was chromatographed on a column of 850 g of silica gel with 39:1 chloroform–methanol to yield 7.9 g of **2a**; $[\alpha]_D^{21}$ +27° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3439, 3411, 3353, 1765, and 1705 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.14 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.39 (s, NCH_3), and 3.45 (s, OCH_3).

Anal. Calc. for $C_{32}H_{43}N_4O_{10}$: C, 59.70; H, 6.73; N, 8.70. Found: C, 59.56; H, 6.68; N, 8.65.

B. To a stirred, ice bath-cooled solution of 8.0 g of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B^{2b} (**6a**) and 160 mL of *N*,*N*-dimethylformamide in a nitrogen atmosphere was added 2.34 g of a 57% oil dispersion of sodium hydride. The resulting suspension was kept for 1 h in the cold and then for 21 h at room temperature. The mixture was cooled in an ice bath and cautiously treated with a solution prepared from 10 mL of acetic acid and 20 mL of water. The solution was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The chloroform extract was washed with 5% aqueous sodium chloride, dried and evaporated. Residual *N*,*N*-dimethylformamide was removed by evaporation of toluene from the residue to give 6.69 g of crude compound **2a**. A sample (1.01 g) of the crude **2a** was chromatographed on a column of 100 g of silica gel with 37:3 1,2-dichloroethane–2-propanol to give 0.355 g of pure **2a**, identical in all respects with that prepared by route *A*.

C. To a stirred, ice bath-cooled solution of 8.15 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-4-*N*,5-*O*-methylenefortimicin B (**7**) and 160 mL of *N*,*N*-dimethylformamide in a nitrogen atmosphere was added 2.32 g of a 57% oil dispersion of sodium hydride. The resulting suspension was kept for 1 h in the cold and then for 21 h at room temperature. The solution was cooled in an ice bath and cautiously treated with a solution prepared from 10 mL of acetic acid and 20 mL of water. The mixture was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The chloroform extract was dried and evaporated. Residual *N*,*N*-dimethylformamide was removed by repeated evaporation of toluene from the residue to give 7.86 g of crude 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*,5-*O*-methylenefortimicin B 1,2-carbamate (**3**). A portion of the crude **3** (7.81 g), 2.64 g of hydroxylamine hydrochloride, 7.35 mL of acetic acid, and 456 mL of methanol was boiled for 1 h under reflux. Most of the methanol was evaporated and the remaining residue was shaken with a mixture of 400 mL of 1:1 concentrated ammonium hydroxide–water and 200 mL of chloroform. The chloroform phase was washed with 5% aqueous sodium chloride, dried, and evaporated to give 7.72 g of residue. A portion of the residue (1.01 g) was chromatographed on a column of 100 g of silica gel using 37:3 1,2-dichloroethane–2-propanol. Evaporation of appropriate fractions gave 0.539 g of compound **2a**, identical in all respects with that prepared by the preceding methods.

1,2′,6′-Tri-N-*benzyloxycarbonyl-4*-N-*methylfortimicin B* (**6c**). — A solution of 10 g of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**), 750 mL of methanol, 225 mL of Sorensen's pH 6 buffer, and 25 mL of formalin was stirred for 50 min at room temperature. The solution was treated with 2.54 g of sodium cyanoborohydride and stirring was continued for 20 h at room temperature. The major portion of the methanol was evaporated and the remaining solution was diluted with 5% aqueous sodium hydrogencarbonate. Extraction with chloroform gave 10.2 g of **6c**; ^{1}H-n.m.r. ($CDCl_3$): δ 1.00 (d, $J_{6',7'}$ 6.7 Hz, 6′-CH_3), 2.36 [s, $N(CH_3)_2$], and 3.39 (s, OCH_3).

2′,6′-Di-N-*benzyloxycarbonyl-4*-N-*methyl-fortimicin B 1,2-carbamate* (**2b**). — To a stirred, ice bath-cooled solution of 1.01 g of compound **6c** and 20 mL of N,N-

dimethylformamide in a nitrogen atmosphere was added 0.2843 g of a 57% oil dispersion of sodium hydride. Stirring was continued for 1 h in the cold and then for 22 h at room temperature. The resulting gel was cooled and treated with a solution of 0.8 mL of acetic acid in 2 mL of water. The resulting solution was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The chloroform was evaporated and residual *N,N*-dimethylformamide was removed by evaporation of toluene from the residue to give 0.930 g of product. Chromatography of the residue on a column of silica gel with 23.4:1.4:0.1 1,2-dichloroethane–methanol–concentrated ammonium hydroxide gave 0.384 g of **2b**; $[\alpha]_D^{25}$ +70° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3443, 3313, 1765, and 1713 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.17 (d, $J_{6',7'}$ 7.2 Hz, 6′-CH_3), 2.46 [s, $N(CH_3)_2$], and 3.47 (s, OCH_3).

Anal. Calc. for $C_{33}H_{45}N_4O_{10}$: C, 60.25; H, 6.75; N, 8.53. Found: C, 60.18; H, 6.75; N, 8.82.

*2′,6′-Di-*N*-benzyloxycarbonyl-4-*N*-methyl-fortimicin B 1,5-carbamate* (**4b**). — *A.* A solution prepared from 3.04 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-4-*N*-methylfortimicin B (**6c**), 3 g of 1,5-diazabicyclo[5.4.0]undecen-5-ene, and 150 mL of benzene was boiled for 144 h under reflux. After cooling to room temperature, water was added, and the resulting mixture was stirred for 1 h. The mixture was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform to give 2.84 g of orange residue. The residue was chromatographed on a column of silica gel with 23.4:1.4:0.1 1,2-dichloroethane–methanol–concentrated ammonium hydroxide. Early fractions gave 0.40 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-4-*N*-methylfortimicin B (**6c**). Later fractions gave 1.00 g of **4b**; $[\alpha]_D^{23}$ +83° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3518, 3438, 3318, and 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 (d, $J_{6',7'}$ 7.0 Hz, 6′-CH_3), 2.42 [s, $N(CH_3)_2$], and 3.44 (s, OCH_3).

Anal. Calc. for $C_{33}H_{45}N_4O_{10}$: C, 60.25; H, 6.75; N, 8.53. Found: C, 59.78; H, 6.68; N, 8.24.

B. A stirred solution of 9.48 g of the crude compound **2b**, 470 mL of methanol, and 235 mL of 1:4 concentrated ammonium hydroxide–water was kept for 3 days at room temperature. The product (8.69 g) was isolated by extraction with chloroform and chromatographed on a column of 450 g of silica gel packed and eluted with a solvent system composed of 36:3:0.2 ethyl acetate–ethanol–triethylamine to yield 5.39 g of pure compound **4b**, identical with that prepared as just described.

*2′,6′,2″-Tri-*N*-(benzyloxycarbonyl)fortimicin A 1,5-carbamate* (**4a**). — *A.* A solution of 4.04 g of 1,2′,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A (**6b**), 4.0 g of 1,5-diazabicyclo[5.4.0]undecen-5-ene, and 200 mL of benzene was boiled for 6 days under reflux. The solution was cooled to room temperature and water (100 mL) was added. On stirring, a benzene-insoluble material separated. The supernatant solution was diluted with 5% aqueous sodium hydrogencarbonate and extracted with benzene. The benzene-insoluble material was dissolved in chloroform and washed with water. The chloroform solution was combined with the benzene solution and evaporated to dryness to give 3.93 g of brown residue. The residue was chromatographed on a column of silica gel with 19:1 ethyl acetate–2-propanol. Early fractions

gave 0.954 g of 1,2′,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A (**6b**). Later fractions yielded the 1,5-carbamate **4a**; (0.892 g); $[\alpha]_D^{23}$ +59° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3532, 3437, 3332, 1712, and 1644 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.19 (d, $J_{6',7'}$ 6.4 Hz, 6′-CH_3), 3.00, 3.04 (NCH_3, rotamers), 3.49, and 3.51 (OCH_3, rotamers).

Anal. Calc. for $C_{42}H_{51}N_5O_{13}$: C, 60.49; H, 6.16; N, 8.40. Found: C, 60.05; H, 6.34; N, 8.38.

B. To a stirred suspension of 0.6272 g of 2′,6′-di-*N*-(benzyloxycarbonyl)-fortimicin B 1,5-carbamate (**4d**) in 8 mL of oxolane was added 0.3319 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Stirring was contined for 20 h at room temperature, giving a clear, colorless solution. The product (0.7966 g) was isolated by extraction with chloroform and chromatographed on a column of 35 g of silica gel, packed and eluted with a solvent system composed of 19:1 ethyl acetate–2-propanol to yield 0.653 g of compound **4a**, identical with that just described.

*2′,6′-Di-*N*-(benzyloxycarbonyl)fortimicin B 1,5-carbamate* (**4d**). — *A.* A solution of 7.22 g of crude 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**), prepared from **7** with DBU in benzene as already described, 360 mL of methanol, and 180 mL of 1:4 concentrated ammonium hydroxide–water was kept for 48 h at room temperature. The major proportion of the methanol was evaporated off and the remaining suspension diluted with 5% aqueous sodium hydrogencarbonate. Extraction with chloroform gave 7.0 g of solid, which was chromatographed on a column of silica gel with 20:2:0.1 dichloromethane–methanol–concentrated ammonium hydroxide to give 3.26 g of **4d**; $[\alpha]_D^{19}$ +101° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3529, 3439, 3324, and 1712 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.14 (d, $J_{6',7'}$ 6.0 Hz, 6′-CH_3), 2.36 (s, NCH_3), and 3.45 (s, OCH_3).

Anal. Calc. for $C_{32}H_{42}N_4O_{10}$: C, 59.80; H, 6.59; N, 8.72. Found: C, 59.43; H, 6.72; N, 8.63.

B. A suspension of 0.378 g of 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)fortimicin B 1,5-carbamate (**4c**), 1.21 g of zinc dust, and 7 mL of acetic acid was stirred for 6 h at room temperature. The zinc was removed by filtration and the filtrate diluted with 10% aqueous sodium chloride solution and extracted with chloroform. The chloroform extract was washed with 5% aqueous sodium hydrogencarbonate, dried, and evaporated to give 0.247 g of **4d**, identical with that prepared as just described.

*2′,6′-Di-*N*-benzyloxycarbonyl-4-*N*-ethoxycarbonylfortimicin B 1,2-carbamate* (**2c**). — To a stirred suspension of 0.750 g of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**), 37 mL of methanol, and 18.5 mL of a solution prepared from 3.0 g of sodium hydrogencarbonate in 72 mL of water was added 0.28 mL of ethoxycarbonyl chloride. After 1 h, an additional 0.3 mL of ethoxycarbonyl chloride was added and stirring was continued for 4 h. The mixture was diluted with water and extracted with chloroform. The chloroform solution was dried and evaporated to give 0.789 g of compound **2c**; ^{1}H-n.m.r. ($CDCl_3$): δ 1.15 (d, $J_{6',7'}$ 6.8 Hz, 6′-CH_3), 1.25 (t, *J* 6.6 Hz, OCH_2CH_3), 2.96 (s, NCH_3), and 3.48 (s, OCH_3).

*2′,6′-Di-*N*-benzyloxycarbonyl-1-*N*-(methoxycarbonyl)fortimicin B 4,5-carba-*

mate (**3b**). — *A*. A stirred mixture of compound **2c** (0.834 g), 0.504 g of sodium hydrogencarbonate, and 42 mL of methanol was boiled for 1 h under reflux. After cooling, the resulting mixture was diluted with water. The product was isolated by extraction with chloroform to give 0.792 g of **3b**; $[\alpha]_D^{24}$ −7.8° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3563, 3438, 1755, and 1706 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.12 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.84 (s,NC H_3), 3.45 (s, OCH_3), and 3.63 (s, $NHCO_2CH_3$).

Anal. Calc. for $C_{34}H_{44}N_4O_{12}$: C, 58.27; H, 6.33; N, 8.00. Found: C, 57.91; H, 6.54; N, 7.86.

B. A suspension prepared from 0.820 g of 2′,6′-di-*N*-(benzyloxycarbonyl)-fortimicin B 1,2;4,5-biscarbamate (**5a**), 0.515 g of sodium hydrogencarbonate, and 42 mL of methanol was boiled for 2 h under reflux. The methanol was evaporated off and 200 mL of 5% aqueous sodium hydrogencarbonate added to the residue. Extraction with chloroform gave 0.861 g of **3b**, identical with that prepared as described in method *A*.

C. A stirred solution of 0.264 g of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate (**3c**), 0.463 g of sodium hydrogencarbonate, 14 mL of methanol, and 2 mL of water was treated with 0.12 mL of methoxycarbonyl chloride. After stirring for 3.5 h, the resulting suspension was diluted with 5% aqueous sodium hydrogencarbonate. Chloroform extraction gave 0.266 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-*N*-(methoxycarbonyl)fortimicin B 4,5-carbamate (**3b**), identical with that just prepared.

2′,6′-Di-N-(*benzyloxycarbonyl)fortimicin B 1,2;4,5-biscarbamate* (**5a**). — *A*. A solution prepared from 5.36 g of 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*-(ethoxycarbonyl)-fortimicin B 1,2-carbamate (**2c**), 5.61 g of 1,5-diazabicyclo[5.4.0]undecen-5-ene, and 250 mL of benzene was boiled for 6h under reflux. After being kept overnight, the solution was diluted with water and stirred for 1 h. Extraction with chloroform gave 5.79 g of product, which was chromatographed on a column of silica gel with 10:1 1,2-dichloroethane–methanol. Evaporation of appropriate fractions gave 1.49 g of **5a**; $[\alpha]_D^{25}$ −2.3° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 3300, 1750, and 1697 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 (d, $J_{6',7'}$ 7 Hz, 6′-CH_3), 2.85 (s, NCH_3), and 3.52 (s, OCH_3).

Anal. Calc. for $C_{33}H_{41}N_4O_{11}$: C, 59.18; H, 6.17; N, 8.37. Found: C, 59.50; H, 6.06; N, 8.09.

Impure fractions from the foregoing chromatography were rechromatographed on a column of silica gel with ethyl acetate to give an additional 1.46 g of pure **5a**.

B. To a stirred, ice bath-cooled solution of 1.02 g of 1,2′,6′-tri-*N*-(benzyloxy-carbonyl)fortimicin B 4,5-carbamate (**3a**) and 20 mL of *N*,*N*-dimethylformamide in a nitrogen atmosphere was added 0.280 g of a 57% oil dispersion of sodium hydride. After stirring in the cold for 4 h, 0.8 mL of acetic acid was added to the cold suspension. The resulting solution was diluted with 5% aqueous sodium hydrogencarbonate and extracted with chloroform. The chloroform was evaporated and residual *N*,*N*-dimethylformamide removed by evaporation of toluene from the residue to give 1.05 g of product, which was dissolved in 20 mL of pyridine, and then 2 mL of acetic anhydride was added. After 24 h, the solution was diluted with 5% aqueous sodium

hydrogencarbonate. Chloroform extraction and removal of residual pyridine (toluene) gave 1.04 g of white glass. The glass was chromatographed on a column of silica gel with 7:8 1,2-dichloroethane–ethyl acetate. Initial fractions gave 0.161 g of 2-*O*-acetyl-1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate; $\tilde{\nu}_{max}$ ($CDCl_3$) 3443, 3315, 1751, and 1708 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 0.99 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.08 (s, $OCOCH_3$), 2.87 (s, NCH_3), and 3.43 (s, OCH_3).

Later fractions gave 0.594 g of residue which was rechromatographed on a column of silica gel with 3:2 dichloromethane–ethyl acetate to give 0.398 g of **5a**, identical with that prepared by route *A*.

2′,6′-Di-N-(*benzyloxycarbonyl)fortimicin B 4,5-carbamate* (**3c**). — A stirred mixture of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2;4,5-biscarbamate (**9**), 10 mL of 2M aqueous sodium hydroxide, and 30 mL of 1,2-dimethoxyethane was boiled for 2.7 h under reflux. The mixture was diluted with saturated sodium chloride solution and extracted with chloroform to give 0.857 g of a solid. The solid was chromatographed on a column of silica gel with 18.8:1.2:0.1 chloroform–methanol–concentrated ammonium hydroxide to give 0.410 g of **3c**; $[\alpha]_D^{23}$ +17° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3568, 3446, 1752, and 1702 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 (d, $J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.82 (s, NCH_3), and 3.46 (s, OCH_3).

Anal. Calc. for $C_{32}H_{42}N_4O_{10}$: C, 59.80; H, 6.59; N, 8.27. Found: C, 59.90; H, 6.73; N, 8.77.

2′,6′-Di-N-*Benzyloxycarbonyl-4*-N-(*2,2,2-trichloroethoxycarbonyl)fortimicin B 1,5-carbamate* (**4c**). — *A*. A stirred solution of 0.882 g of 2′,6′-di-*N*-benzyloxycarbonyl-4-*N*-methylfortimicin B 1,5-carbamate (**4b**), 0.306 g of anhydrous potassium carbonate, 0.410 g of 2,2,2-trichloroethoxycarbonyl chloride, and 50 mL of benzene was boiled for 6 h under reflux. The mixture was cooled to room temperature and poured into 0.5M ammonium hydroxide. Extraction with benzene gave 0.910 g of solid that was chromatographed on a column of silica gel with 23.4:1.4 dichloromethane–methanol to give 0.586 g of **4c**; $[\alpha]_D^{25}$ +62° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3530, 3438, and 1704 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.18 (d, $J_{6',7'}$ 7.0 Hz, 6′-CH_3), 3.10 (s, NCH_3), and 3.45 (s, OCH_3).

Anal. Calc. for $C_{35}H_{43}Cl_3N_4O_{12}$: C, 51.38; H, 5.30; Cl, 13.00; N, 6.85. Found: C, 51.12; H, 5.38; Cl, 12.95; N, 6.79.

B. A stirred solution of 0.400 g of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,5-carbamate (**4d**) in 6 mL of oxolane was treated with 0.198 g of *N*-(2,2,2-trichloroethoxycarbonyloxy)succinimide. After stirring for 18 h, the mixture was poured into 5% aqueous sodium hydrogencarbonate and extracted with chloroform to give 0.539 g of solid. The latter was chromatographed on a column of silica gel with ethyl acetate to give 0.411 g of compound **4c**, identical with that just described.

Fortimicin B 1,2-carbamate (**2d**). — A solution of 0.810 g of 2′,6′-di-*N*-(benzyloxycarbonyl)fortimicin B 1,2-carbamate (**2a**) in 75 mL of 0.2M hydrochloric acid in methanol was hydrogenated under 3 atm of hydrogen for 4 h in the presence of 0.810 g of 5% palladium on carbon. The catalyst was removed by filtration and the solvent evaporated. Residual hydrochloric acid was removed by evaporation of

methanol from the residue to give 0.619 g of **2d**, isolated as the trihydrochloride; $[\alpha]_D^{22}$ +43° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 1750 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.86 (d, $J_{6',7'}$ 7.0 Hz, 6'-CH_3), 3.38 (s, NCH_3), 4.08 (s, OCH_3), 6.04 (d, $J_{1',2'}$ 3.7 Hz, H-1'), m.s.: cyclitol, calc. for $C_9H_{15}N_2O_4$: *m/z* 215.1032, meas. 215.1033; diamino sugar calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1193.

Fortimicin B 4,5-carbamate (**3d**). — 1,2',6'-Tri-*N*-(benzyloxycarbonyl)fortimicin B 4,5-carbamate (**3a**, 0.400 g) was hydrogenated as already described. Conventional isolation gave 0.244 g of **3d** as the trihydrochloride; $[\alpha]_D^{22}$ +12° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 1744 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.79 (d, $J_{6',7'}$ 6.4 Hz, 6'-CH_3), 3.33 (s, NCH_3), 4.00 (s, OCH_3), and 6.12 (d, $J_{1',2'}$ 4.8 Hz, H-1'); m.s.: $M^{+\cdot}$, calc. for $C_{16}H_{30}N_4O_6$: *m/z* 374.2165, meas. 374.2139.

Fortimicin B 1,5-carbamate (**4a**). — 2',6'-Di-*N*-(benzyloxycarbonyl)fortimicin B 1,5-carbamate (**4d**) was hydrogenated as described earlier. Isolation as before gave **4a** as the trihydrochloride; $\tilde{\nu}_{max}$ (KBr) 1702 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.85 (d, $J_{6',7'}$ 6.8 Hz, 6'-CH_3), 3.39 (s, NCH_3), and 3.98 (s, OCH_3); m.s: cyclitol, calc. for $C_9H_{15}N_2O_4$: *m/z* 215.1032, meas. 215.1052; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1187.

Fortimicin B 1,2;4,5-biscarbamate (**5b**). — 2',6'-Di-*N*-(benzyloxycarbonyl)-fortimicin B 1,2;4,5-biscarbamate (**5a**, 0.500 g) was hydrogenated as already described to give **5b** as the dihydrochloride; $[\alpha]_D^{22}$ +8.8° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 1737 and 1722 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.79 (d, $J_{6',7'}$ 7.0 Hz, 6'-CH_3), 3.35 (s, NCH_3), 4.03 (s, OCH_3), 5.96 (d, $J_{1',2'}$ 3.7 Hz, H-1'); m.s.: $M^{+\cdot}$, calc. for $C_{17}H_{28}N_4O_7$: *m/z* 400.1958, meas. 400.1980.

Fortimicin A 1,5-carbamate (**4g**). — 2',6',2''-Tri-*N*-(benzyloxycarbonyl)-fortimicin A 1,5-carbamate (**4a**, 0.552 g) was hydrogenated to give 0.394 g of **4g** as the trihydrochloride; $[\alpha]_D^{23}$ −22° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 1699 and 1643 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.82 (d, $J_{6',7'}$ 6.7 Hz, 6'-CH_3), 3.57 (s, NCH_3), 3.94 (s, OCH_3), and 5.81 (d, $J_{1',2'}$ 3.5 Hz, H-1')[4]. The mass spectrum could not be obtained.

Fortamine 1,2;4,5-biscarbamate (**10**). — A solution of 9.6 g of the dihydrochloride salt of fortimicin B 1,2;4,5-biscarbamate (**5b**), 438 mL of 1:2 acetic acid–water, and 14.7 g of sodium nitrite was kept for 16 h at room temperature. The solvent was evaporated and the residue was chromatographed on a column of silica gel with the lower phase of a mixture of 1:1:1 dichloromethane–methanol–water to give 3.7 g of **10**; m.p. 232–235°, $[\alpha]_D^{24}$ −80° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 1760 and 1718 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6): δ 2.76 (s, NCH_3) and 3.45 (s, OCH_3); m.s.: $M^{+\cdot}$, calc. for $C_{10}H_{14}N_2O_6$: *m/z* 258.0852, meas. 258.0844.

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