4-*N*-(2-AMINOETHANESULFONYL)FORTIMICIN B AND 4-*N*-(*p*-AMINOBENZENESULFONYL)FORTIMICIN B

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Methods have been developed for preparation of 4-*N*-arenesulfonylfortimicins B and 4-*N*-alkanesulfonylfortimicins B from derivatives of fortimicin B-1,5-carbamate. 4-*N*-(2-Aminoethanesulfonyl)fortimicin B and 4-*N*-(*p*-aminobenzenesulfonyl)fortimicin B have been prepared and found to be devoid of antibacterial activity.

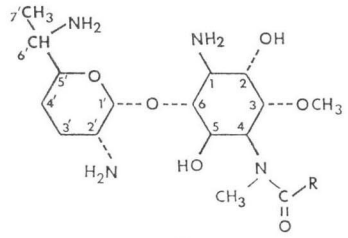
Fortimicin A (**1a**) and fortimicin B (**2**) are novel aminoglycoside antibiotics formed in fermentations by *Micromonospora olivoasterospora*.[1] Fortimicin A, which has a 4-*N*-glycyl group[2], has the much greater antibacterial activity[3]. The contrast between the antibacterial activities of fortimicin A and fortimicin B motivated the preparation of a series of 4-*N*-acylfortimicins B[4], and 4-*N*-alkylfortimicins B[5], the antibacterial activities of which further illustrated the sensitivity of the antibacterial activities of the fortimicin antibiotics to the nature of the C_4-substituent. The present work was carried out with the object of preparing 4-*N*-alkanesulfonyl- and 4-*N*-arenesulfonylfortimicins B for antibacterial evaluation. Our principle objective was the preparation of 4-*N*-(2-aminoethanesulfonyl)fortimicin B (**3a**). The latter was of interest since it is the simplest 4-*N*-aminoalkanesulfonylfortimicin B, and represents that sulfonamide derivative of fortimicin B which is related to 4-*N*-(β-alanyl)fortimicin B (**1b**). The latter was found to have about half the antibacterial activity of fortimicin A as determined by an *in vitro* assay[4].

Although 4-*N*-acylfortimicins B were prepared by 4-*N*-acylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4a**)[4] by standard methods, attempts to effect 4-*N*-sulfonylation of **4a** were unsuccessful. An attempt to effect selective 4-*N*-methanesulfonylation of **4a** with methanesulfonyl chloride in aqueous acetone in the presence of potassium carbonate gave only recovered starting material. Treatment of **4a** with methanesulfonyl chloride (two equivalents) in pyridine gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**4b**) identical with, but somewhat less pure than, material prepared by an unambiguous process[6]. In addition, attempted sulfonylation of **4a** with *p*-acetamidobenzenesulfonyl chloride in aqueous acetone in the presence of potassium carbonate gave a mixture of starting material and two more polar products (~1:1:1 by TLC and PMR) which was not separated.

Nuclear magnetic resonance studies[2] of the fortimicin antibiotics have established that whereas in aqueous solution, fortimicin A adopts that cyclitol chair conformation which has the C_4-substituent equatorial (**5**), fortimicin B adopts that cyclitol chair conformation which has the C_4-substituent axial (**6a**). This suggested that the failure to effect 4-*N*-sulfonylation of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4a**) may have been due to steric hindrance resulting from a preferred cyclitol conformation **6b** which has the C_4-methylamino group axial. It was thus hoped that steric facilitation of 4-*N*-sulfonylation would be effected with fortimicin B-1,5-carbamate derivatives[7] which have the cyclitol rings constrained by the carbamate bridge to that chair form which has the C_4-methylamino group equatorial.

Treatment of 2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**7**)[6] with *p*-acetamidobenzenesulfonyl chloride in aqueous acetone in the presence of potassium carbonate gave the desired 4-*N*-(*p*-acetamidobenzenesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**8**) in 72% yield after column chromatography. Both TLC and PMR on the total crude product showed the

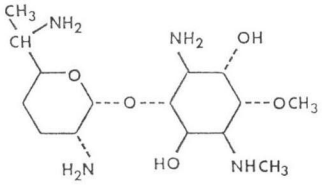
Fig. 1.



R

1a CH_2NH_2

1b $CH_2CH_2NH_2$



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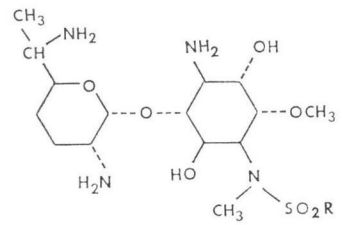
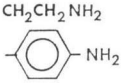
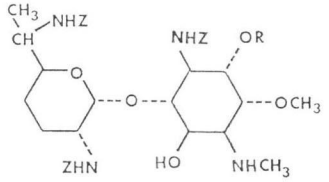


R

3a $CH_2CH_2NH_2$

3b 

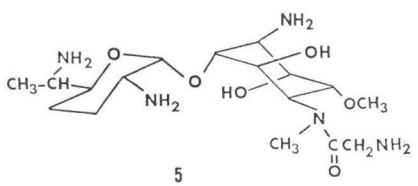


R

4a H

4b SO_2CH_3

Fig. 2.



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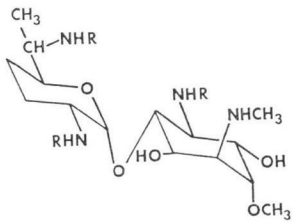
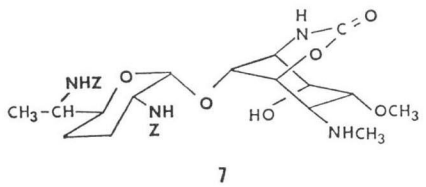


R

6a H

6b Z



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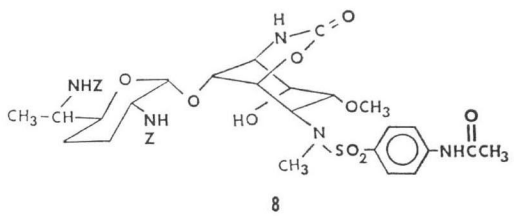

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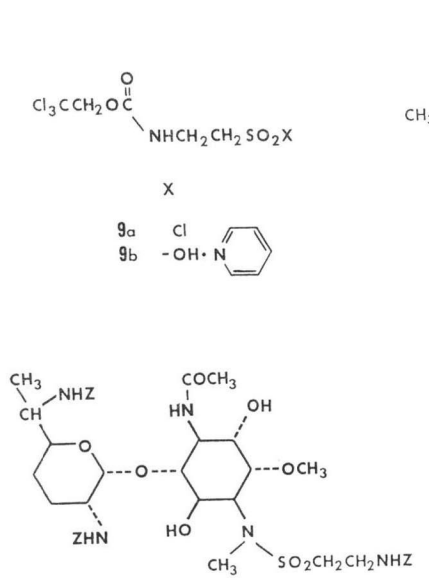
absence of starting material **7**. Catalytic hydrogenolysis of **8** followed by base-catalyzed hydrolysis gave 4-*N*-(*p*-aminobenzenesulfonyl)fortimicin B (**3b**) which was devoid of antibacterial activity as determined by an *in vitro* assay.

The preparation of 4-*N*-(2-aminoethanesulfonyl)fortimicin B (**3a**) was accomplished by a process which involved sulfonylation with 2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl chloride (**9a**). The latter was prepared by first treating a pyridine suspension of taurine with 2,2,2-trichloroethoxycarbonyl chloride to give the pyridine salt **9b** of 2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonic acid. The latter **9b** was treated with phosphorous pentachloride in chloroform, in a slight modification of the procedure of BARCO, BENETTI, POLLINI and TADDIA[8] to give the sulfonyl chloride **9a**.

An attempt to effect 4-*N*-sulfonylation of the 1,5-carbamate **7** with the sulfonyl chloride **9a** in aqueous acetone in the presence of potassium carbonate gave only recovered starting material, which is presumed to be a consequence of hydrolysis of the reagent in the aqueous medium under the reaction conditions. Preliminary attempts to effect 4-*N*-sulfonylation of **7** with **9a** in pyridine established the necessity for protection of the C_2-hydroxyl group which was accomplished as follows.

Treatment of 4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10a**)[7] with excess acetic anhydride in pyridine at room temperature gave a mixture of 2-*O*-acetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10b**) and 1-*N*,2-*O*-diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**) in yields of 56 and 35%, respectively. Acetylation of the monoacetate (**10b**) with acetic anhydride in pyridine at 60°C for 72 hours effected complete conversion to the diacetate

Fig. 3.



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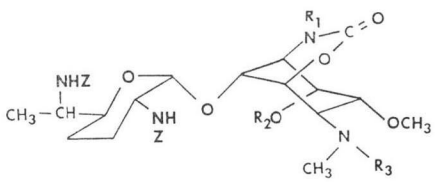
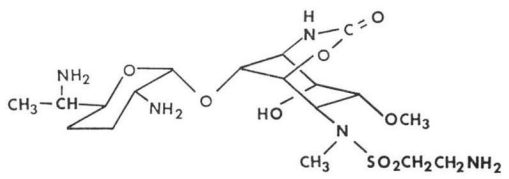


	R_1	R_2	R_3
10a	H	H	$CO_2CH_2CCl_3$
10b	H	$COCH_3$	$CO_2CH_2CCl_3$
10c	H	$COCH_3$	H
10d	$COCH_3$	$COCH_3$	$CO_2CH_2CCl_3$
10e	$COCH_3$	$COCH_3$	H
10f	$COCH_3$	$COCH_3$	$-SO_2CH_2CH_2NHCO_2CH_2CCl_3$
10g	$COCH_3$	$COCH_3$	$-SO_2CH_2CH_2NH_2$
10h	H	H	$-SO_2CH_2CH_2NHZ$
10i	H	$COCH_3$	$-SO_2CH_2CH_2NHCO_2CH_2CCl_3$



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(**10d**). Treatment of both **10b** and **10d** with zinc in acetic acid cleaved the 4-*N*-trichloroethoxycarbonyl groups to give **10c** and **10e**, respectively.

Although preliminary experiments established that 4-*N*-sulfonylations of both **10c** and **10e** with **9a** were successful, the diacetate **10e** gave the cleaner product and was thus used in subsequent steps. Treatment of the sulfonamide **10f**, prepared from **10e**, with zinc in acetic acid cleaved the trichloroethoxycarbonyl group to give **10g**. The latter proved to be water soluble, and transferred completely to the aqueous phase on attempted workup by standard chloroform - aqueous sodium bicarbonate extraction. The product **10g** was thus isolated as the acetic acid salt from the zinc - acetic acid cleavage by filtration of the excess zinc, and evaporation of the acetic acid from the filtrate. The 2-aminoethanesulfonamide **10g** thus obtained was treated with *N*-(benzyloxycarbonyloxy)succinimide and the product was hydrolyzed with aqueous, methanolic sodium bicarbonate to give a mixture of 4-*N*-[2-(*N*-benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10h**) and 1-*N*-acetyl-4-*N*-[2-(*N*-benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B (**11**) which was separated chromatographically.

Catalytic hydrogenolysis of **10h** removed the *N*-benzyloxycarbonyl protecting groups to give 4-*N*-(2-aminoethanesulfonyl)fortimicin B-1,5-carbamate (**12**). Base-catalyzed hydrolysis of **12** gave the desired 4-*N*-(2-aminoethanesulfonyl)fortimicin B (**3a**). The latter **3a** was virtually devoid of antibacterial activity as determined by an *in vitro* antibacterial assay.

CMR chemical shifts of 4-*N*-(2-aminoethanesulfonyl)fortimicin B (**3a**), 4-*N*-(*p*-aminobenzenesulfonyl)fortimicin B (**3b**), fortimicin A (**1a**), and fortimicin B (**2**) are listed in Table 1.

Table 1. CMR chemical shifts.*

	1a pD 1.0	**2** pD 1.0	**3a** pD 4.66	**3b** pD 1.96
1	51.7	53.5	53.9	53.7
2	66.3	65.5	66.5	66.8
3	71.6	74.1	74.3	74.2
4	54.1	58.1	54.5	54.7
5	71.6	66.6	72.3	73.0
6	74.5	74.2	74.6	74.9
1′	95.4	96.0	95.4	95.4
2′	51.7	51.9	51.8	51.8
3′	21.6	21.5	21.5	21.5
4′	26.3	26.3	26.3	26.3
5′	70.9	71.0	70.9	70.9
6′	49.4	49.4	49.4	49.5
7′	15.0	15.0	15.0	15.0

* ^{13}C-Fourier transform spectra were recorded on a JEOL-FX-90Q spectrometer at 22.50 MHz. 1,4-Dioxane was used as an internal reference. Solutions were about 10 % (w/v) in D_2O.

After completion of this work, a patent appeared reporting a series of 1-*N*-(ω-aminoalkanesulfonyl) derivatives of some aminoglycoside antibiotics including the 1-*N*-(2-aminoalkanesulfonyl) derivatives of the kanamycins A and B, ribostamycin, 3′-deoxykanamycin B, and 3′,4′-dideoxykanamycin B using 2-(trifluoroacetylamino)ethanesulfonyl chloride as the sulfonylating agent. The products were reported to have substantial antibacterial activities, with enhanced activities against resistant strains.

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 spectrometer. Chemical shifts determined with D_2O solutions are reported from internal sodium 3-trimethylsilylpropionate 2,2,3,3-d_4 (TSP). Chemical shifts determined with $CDCl_3$ solutions are reported from internal TMS. Mass spectra were obtained on an A.E.I.,

M.S. 902 spectrometer at 70 eV and 150~200°C using the direct probe insert. Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh. Ratios for chromatography solvents are expressed by volume. Workups by chloroform extraction were carried out by shaking the reaction solutions or reaction mixtures with mixtures of chloroform and 5% aqueous $NaHCO_3$. The chloroform extracts were separated and dried ($MgSO_4$) and the solvent was evaporated under diminished pressure using a rotary evaporator. The *in vitro* antibacterial activities of the sulfate salts of **3a** and **3b** were determined by the serial dilution method using MUELLER-HINTON agar.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**4b**)

To a stirred solution of 0.5047 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**4a**) in 5 ml of pyridine, cooled in an ice bath, was added 0.053 ml of methanesulfonyl chloride. Stirring was continued with cooling for 2 hours and then at ambient temperature for 20 hours. An additional 0.053 ml of methanesulfonyl chloride was added and stirring was continued at room temperature for 21 hours. The product (0.528 g) was isolated by chloroform extraction. PMR and TLC showed that the product, although less pure, was identical with 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-methanesulfonylfortimicin B (**4b**) prepared by an unambiguous method[6].

4-*N*-(*p*-Acetamidobenzenesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**8**)

A stirred solution of 1.46 g of 2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**7**)[6], 1.05 g of *p*-acetamidobenzenesulfonyl chloride, 0.620 g of K_2CO_3, 15 ml of water and 42 ml of acetone was kept overnight at room temperature. The product (1.68 g) was isolated by chloroform extraction and chromatographed on a column of 120 g of silica gel prepared and eluted with a solvent system composed of ethyl acetate - ethanol (9: 1) to yield 1.38 g of 4-*N*-(*p*-acetamidobenzenesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**8**): $[\alpha]_D^{21}+100°$ (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ ($CDCl_3$) 3530, 3430, 3318, 1708, 1594 cm^{-1}; PMR ($CDCl_3$) δ 1.14 d ($J_{6',7'}$ 6.5 Hz, $C_{6'}$-CH_3), 2.14 ($COCH_3$), 2.83 (NCH_3), 3.12 (OCH_3); ν(Hz) 749, 759, 764, 773 (SO_2-*Ar*-N).*

Anal. Calcd. for $C_{40}H_{49}N_5O_{13}S$ ($\frac{1}{2}$ H_2O): C, 56.59; H, 5.94; N, 8.25.
Found: C, 56.39; H, 5.91; N, 8.16.

4-*N*-(*p*-Aminobenzenesulfonyl)fortimicin B (**3b**)

Catalytic hydrogenation of 4-*N*-(*p*-acetamidobenzenesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (1.19 g, **8**) was carried out in the usual manner[4] to yield 0.846 g of product** as the perhydrochloride. A solution of 0.820 g of the latter product in 35 ml of 2 N NaOH, under a nitrogen atmosphere was heated at 90°C overnight. The resulting solution was brought to pH 6 by addition of 2 N HCl. The major portion of the solvent was evaporated under reduced pressure, and residual solvent was removed by co-distillation with ethanol under reduced pressure. The residue was triturated with 25 ml of methanol and the insoluble salts were removed by filtration. The filter cake was washed with methanol, and the combined filtrates were evaporated under reduced pressure to leave 1.95 g of residue. The latter was chromatographed on a column of 60 g of silica gel prepared and eluted with a solvent system composed of chloroform - methanol - concentrated ammonium hydroxide (5: 5: 1) to yield 0.544 g of **3b**. The latter product (0.510 g) was converted to the trihydrochloride with ethanolic hydrogen chloride, and the salt passed through a column of AG1-X2 (SO_4) resin in aqueous solution to give 0.518 g of the sulfate: $[\alpha]_D^{22}+84°$ (*c* 1.0, H_2O); PMR (D_2O) δ 1.35 d ($J_{6',7'}$ 6.7 Hz, $C_{6'}$-CH_3), 2.96 (NCH_3), 3.16 (OCH_3), 5.40 d ($J_{1',2'}$ 2.3 Hz $C_{1'}$-H); ν(Hz) 700, 709, 770, 779 (SO_2-*Ar*-N).*** (M+H) Calcd. for $C_{21}H_{38}N_5O_7S$: 504.2492, Meas. 504.2491; (Cyclitol-fragment) Calcd. for $C_{14}H_{24}N_3O_6S$: 362.1386, Meas. 362.1394; (Diaminosugar fragment) Calcd. for $C_7H_{15}N_2O$: 143.1184, Meas. 143.1189.

Anal. Calcd. for $C_{21}H_{37}N_5O_7S \cdot 1.5\ H_2SO_4 \cdot 4H_2O$: C, 34.89; H, 6.69; N, 9.69; S, 11.09.
Found: C, 35.03; H, 6.21; N, 9.48; S, 11.58.

* Observed peak positions of the AA′ BB′ system.

** Both TLC and PMR showed the presence of two components in about a 4: 1 ratio. The PMR spectrum suggested that the major product was the expected 4-*N*-(*p*-acetamidobenzenesulfonyl)fortimicin B-1,5-carbamate and that the minor component was 4-*N*-(*p*-aminobenzenesulfonyl)fortimicin B-1,5-carbamate.

*** Observed peak positions of the AA′ BB′ system.

2-*N*-(2,2,2-Trichloroethoxycarbonyl)aminoethanesulfonyl chloride (**9a**)

To a stirred suspension of 12.5 g of taurine in 100 ml of pyridine, cooled in an ice bath, was added 2,2,2-trichloroethoxycarbonyl chloride until the reaction mixture became thick and stirring stopped. The reaction mixture was removed from the ice bath and the addition of 2,2,2-trichloroethoxycarbonyl chloride was continued until a total of 15.1 ml had been added. The resulting mixture was kept at room temperature overnight giving a dark red solution. The major portion of the pyridine was evaporated under reduced pressure. Residual pyridine was removed by co-distillation with 1,2-dichloroethane under reduced pressure leaving 54 g of the pyridine salt **9b** of 2-*N*-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonic acid as a dark, red oil. To a stirred solution of the latter **9b** in 200 ml of chloroform was added slowly, portionwise, 25 g of PCl_5. After the addition was complete, stirring was continued at room temperature for 15 minutes, and the resulting solution was then heated under reflux for 2 hours. The resulting solution was cooled to room temperature, diluted with 200 ml of benzene, and the resulting solution washed with water and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and residual $POCl_3$ was removed by co-distillation with toluene under reduced pressure. The residue was triturated with hexane and the product (30.4 g), 2-*N*-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl chloride (**9a**), a white crystalline solid, was collected by filtration: mp 103~105°C; $\tilde{\nu}_{max}$ ($CDCl_3$) 3441, 3320 (s), 1740 cm^{-1}.

Anal. Calcd. for $C_5H_7NO_4SCl_4$: C, 18.82; H, 2.21; N, 4.39; Cl, 44.46.
Found: C, 19.13; H, 2.30; N, 4.55; Cl, 42.58.

2-*O*-Acetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10b**) and 1-*N*,2-*O*-Diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**)

A stirred solution of 1.65 g 4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate, 3 ml of acetic anhydride and 25 ml of pyridine was kept overnight at room temperature. The product (1.74 g) was isolated by chloroform extraction and chromatographed on a column of 120 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane (7: 3). Earlier fractions gave 0.632 g of 1-*N*,2-*O*-diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**): $[\alpha]_D^{24}$ +46° (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$($CDCl_3$) 3438, 3373, 1748, 1715 cm^{-1}; δ($CDCl_3$) 1.16 d ($J_{6',7'}$ 6.6 Hz, $C_{6'}$-CH_3), 2.11 ($OCOCH_3$), 2.52 ($NCOCH_3$), 3.07 (NCH_3), 3.36 (OCH_3).

Anal. Calcd. for $C_{39}H_{47}N_4O_{14}Cl_3$: C, 51.92; H, 5.25; N, 6.21; Cl, 11.79.
Found: C, 51.78; H, 5.37; N, 6.16; Cl, 11.48.

Later fractions gave 0.966 g of 2-*O*-acetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10b**): $[\alpha]_D^{24}$ +41° (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ ($CDCl_3$) 3437, 3363, 1718 cm^{-1}; δ ($CDCl_3$) 1.13 d ($J_{6',7'}$ 6.9 Hz, $C_{6'}$-CH_3), 2.07 ($OCOCH_3$), 3.07 (NCH_3), 3.37 (OCH_3).

Anal. Calcd. for $C_{37}H_{45}N_4O_{13}Cl_3$: C, 51.66; H, 5.27; N, 6.52; Cl, 12.37.
Found: C, 51.28; H, 5.41; N, 6.45; Cl, 12.28.

1-*N*,2-*O*-Diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**)

A solution of 5.66 g of 2-*O*-acetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10b**), 27 ml of acetic anhydride and 84 ml of pyridine was heated at 60°C for three days. The product (6.00 g) was isolated by chloroform extraction. Chromatography of 6.31 g of product thus prepared on a column of 450 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane (7: 3) gave 5.94 g of 1-*N*,2-*O*-diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**) identical with that prepared as described above.

2-*O*-Acetyl-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10c**) and 2-*O*-Acetyl-4-*N*-[2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10i**)

A stirred suspension of 0.838 g of 2-*O*-acetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10b**), 2.2 g of zinc dust and 15 ml of acetic acid was stirred over-

night at room temperature and then filtered. The filter cake was washed with ethanol. The combined filtrate and washings was poured into 500 ml of water, and the aqueous suspension was washed twice with 250 ml portions of chloroform. The chloroform extracts were combined, washed with 5% aqueous $NaHCO_3$, and dried ($MgSO_4$). Evaporation of solvent under reduced pressure left 0.499 g of 2-*O*-acetyl-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10c**): δ ($CDCl_3$) 1.15 d ($J_{6',7'}$ 6.5 Hz, $C_{6'}$-CH_3), 2.08 ($OCOCH_3$), 2.37 (NCH_3), 3.39 (OCH_3).

To a stirred solution of 470 mg of **10c**, prepared as described above, in 10 ml of pyridine, cooled in an ice bath, was added 876 mg of 2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl chloride (**9a**). Stirring was continued with cooling for 1 hour and then at ambient temperature overnight. The product (712 mg) of dark brown glass was isolated by chloroform extraction and chromatographed on a column of 70 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane (9: 1) to give 222 mg of 2-*O*-acetyl-4-*N*-[2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10i**): $[\alpha]_D^{24}+49°$ (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ 3436, 3313, 1718 cm^{-1}; δ($CDCl_3$) 1.15 d ($J_{6',7'}$ 6.5 Hz, $C_{6'}$-CH_3), 2.09 ($O\overset{O}{\overset{\|}{C}}CH_3$), 3.02 ($NCH_3$), 3.43 ($OCH_3$), 4.70 ($CO\overset{O}{\overset{\|}{C}}H_2CCl_3$).

Anal. Calcd. for $C_{39}H_{50}Cl_3N_5O_{15}S$: C, 48.42; H, 5.21; N, 7.24; Cl, 11.00; S, 3.31.
Found: C, 49.24; H, 5.35; N, 6.96; Cl, 10.44; S, 3.03.

1-*N*,2-*O*-Diacetyl-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10e**) and 1-*N*,2-*O*-Diacetyl-4-*N*-[2-(2, 2, 2-trichloroethoxycarbonyl)aminoethanesulfonyl]-2′, 6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10f**)

A stirred suspension of 0.400 g of 1-*N*,2-*O*-diacetyl-4-*N*-(2,2,2-trichloroethoxycarbonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10d**), 1.1 g of zinc dust and 8 ml of acetic acid was kept at room temperature overnight. The mixture was filtered and the filter cake was washed with acetic acid. The combined filtrate and washings were poured into 500 ml of water, and the aqueous suspension was shaken with two 150-ml portions of chloroform. The chloroform extracts were combined, washed with aqueous $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure left 0.326 g of 1-*N*,2-*O*-diacetyl-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10e**): $\tilde{\nu}_{max}$ ($CDCl_3$) 3450, 3345, 1745, 1715 cm^{-1}; δ($CDCl_3$) 1.15 d ($J_{6',7'}$ 6.6 Hz, $C_{6'}$-CH_3), 2.09 ($OCOCH_3$), 2.39 (NCH_3), 2.60 ($NCOCH_3$), 3.36 (OCH_3).

To a stirred solution of 3.94 g of **10e**, prepared as described above, in 100-ml of pyridine, cooled in an ice bath, was added 6.92 g of 2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl chloride (**9a**). Stirring was continued with cooling for 1 hour and then at ambient temperature overnight. The product (7.04 g) of dark brown glass was isolated by chloroform extraction and chromatographed on a column of 450 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane (4: 1) to give 3.47 g of 1-*N*,2-*O*-diacetyl-4-*N*-[2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10f**): $[\alpha]_D^{22}+56°$ (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ 3438, 3343, 1748, 1738, 1718 cm^{-1}; δ($CDCl_3$) 1.14 d ($J_{6',7'}$ 6.8 Hz, $C_{6'}$-CH_3), 2.11 ($OCOCH_3$), 2.60 ($NCOCH_3$), 3.01 (NCH_3), 3.40 (OCH_3), 4.69 (s) ($COCH_2CCl_3$).

Anal. Calcd. for $C_{41}H_{52}N_5O_{16}SCl_3$: C, 48.79; H, 5.19; N, 6.94; S, 3.18; Cl, 10.54.
Found: C, 49.03; H, 5.29; N, 6.99; S, 3.35; Cl, 10.68.

1-*N*,2-*O*-Diacetyl-4-*N*-(2-aminoethanesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10g**), 4-*N*-[2-(*N*-Benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10h**), and 1-*N*-Acetyl-4-*N*-[2-(*N*-benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B (**11**)

A stirred suspension of 0.764 g of 1-*N*,2-*O*-diacetyl-4-*N*-[2-(2,2,2-trichloroethoxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10f**), 2.4 g of zinc dust, and 15 ml of acetic acid was kept at room temperature overnight. The resulting suspension was filtered, and the filter cake was washed with acetic acid. The filtrate and washings were combined, and the major portion of the acetic acid was evaporated under reduced pressure. Residual acetic acid was removed

by co-distillation with chloroform under reduced pressure leaving 0.846 g of 1-*N*,2-*O*-diacetyl-4-*N*-(2-aminoethanesulfonyl)-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (**10g**) as the acetic acid salt: $\tilde{\nu}_{max}$ ($CDCl_3$) 3434, 1752, 1714 cm^{-1}.

A stirred solution of 0.800 g of the latter product, 0.283 g of *N*-(benzyloxycarbonyloxy)succinimide, 0.2 ml of triethylamine, and 30 ml of pyridine was kept at room temperature overnight. The product was isolated by chloroform extraction*. A sample (1.81 g) of product prepared as thus described was dissolved in a solution prepared from 20 ml of 5% aqueous $NaHCO_3$ and 100 ml of methanol, and the resulting solution was kept at room temperature overnight. The solvent was evaporated under reduced pressure, and residual water was removed by co-distillation with ethanol under reduced pressure. The residue was triturated several times with chloroform. The chloroform supernatants were combined and the chloroform was evaporated under reduced pressure to leave 1.61 g of residue. The latter was chromatographed on a column of 130 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - ethanol (19: 1) to give in the earlier fractions 0.821 g of 4-*N*-[2-(*N*-benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate(**10h**): $[\alpha]_D^{22}$ +78° (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ ($CDCl_3$) 3540, 3435, 1715 cm^{-1}; δ($CDCl_3$) 1.16 d ($J_{6',7'}$ 6.9 Hz, $C_{6'}$-CH_3), 3.01 (NCH_3), 3.44 (OCH_3).

Anal. Calcd. for $C_{42}H_{53}N_5O_{14}S$: C, 57.06; H, 6.04; N, 7.92; S, 3.63.
Found: C, 56.51; H, 6.25; N, 8.19; S, 3.36.

Further elution with ethyl acetate - ethanol (7: 3) gave 0.350 g of 1-*N*-acetyl-4-*N*-[2-(*N*-benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B (**11**): $[\alpha]_D^{22}$ +82° (*c* 1.0, CH_3OH); $\tilde{\nu}_{max}$ 3520 (s), 3430, 1715, 1670 cm^{-1}; δ($CDCl_3$) 1.17 d ($J_{6',7'}$ 6.6 Hz, $C_{6'}$-CH_3), 1.88 ($COCH_3$), 3.04 (NCH_3), 3.47 (OCH_3).

4-*N*-(2-Aminoethanesulfonyl)fortimicin B-1,5-carbamate (**12**) and 4-*N*-(2-aminoethanesulfonyl) fortimicin B (**3a**)

4-*N*-[2-(*N*-Benzyloxycarbonyl)aminoethanesulfonyl]-2′,6′-di-*N*-benzyloxycarbonylfortimicin B-1,5-carbamate (0.611 g, **10h**) was catalytically hydrogenated in the usual manner[4] to give 0.399 g of the trihydrochloride salt of 4-*N*-(2-aminoethanesulfonyl)fortimicin B-1,5-carbamate (**12**): $\tilde{\nu}_{max}$ (KBr) 1700 cm^{-1}, δ(D_2O) 1.33 d ($J_{6',7'}$ 6.9 Hz, $C_{6'}$-CH_3), 3.08 (NCH_3), 3.37 (OCH_3), 5.31 d ($J_{1',2'}$ 3.0 Hz, $C_{1'}$-H).

The latter product (0.350 g) was dissolved in 35 ml of 1 N NaOH and the resulting stirred solution was heated at 80°C for 4 hours under nitrogen. The solution was cooled to room temperature and brought to pH 6 by addition of 1 N hydrochloric acid. The major portion of the solvent was evaporated under reduced pressure, and residual water was removed by co-distillation with ethanol under reduced pressure. The residue was triturated with methanol and the suspension was filtered. The methanol was evaporated from the filtrate under reduced pressure leaving 1.05 g of residue. The latter was chromatographed on a column of 40 g of silica gel packed and eluted with a solvent system composed of chloroform - methanol - concentrated ammonium hydroxide (10: 10: 1) to give 0.316 g of residue. The latter (0.250 g) was converted to the tetrahydrochloride with excess 0.2 N hydrochloric acid in methanol. An aqueous solution of the resulting salt was passed over a column of AG1-X2 (SO_4) resin to give 0.262 g of the disulfate of 4-*N*-(2-aminoethanesulfonyl)fortimicin B (**3a**): $[\alpha]_D^{22}$ +60° (*c* 1.0, H_2O), δ(D_2O) 1.34 d ($J_{6',7'}$ 6.6 Hz, $C_{6'}$-CH_3), 3.12 (NCH_3), 3.51 (OCH_3), 5.30 d ($J_{1',2'}$ 3.5 Hz, $C_{1'}$-H), (M+H) Calcd. for $C_{17}H_{38}N_5O_7S$: 456.2492, Meas. 456.2466; (Cyclitol fragment) Calcd. for $C_{10}H_{24}N_3O_6S$: 314.1386, Meas. 314.1403; (Diaminosugar fragment) Calcd. for $C_7H_{15}N_2O$: 143.1184, Meas. 143.1178.

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* TLC assays on aliquots of the *N*-benzyloxycarbonylation suggested partial hydrolysis of one or both of the acetyl groups under the reaction conditions.

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