Carbohydrate Research, 96 (1981) 185–203
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

2-SUBSTITUTED FORTIMICINS BY RING OPENING OF 2-DEOXY-1,2-EPIMINO-2-*epi*-FORTIMICIN B AND BY NUCLEOPHILIC DISPLACEMENTS OF 2-*O*-(METHYLSULFONYL)FORTIMICIN DERIVATIVES

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(Received August 21st, 1980; accepted for publication in revised form, December 28th, 1980)

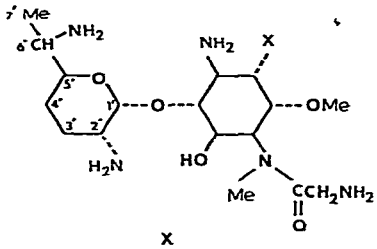
ABSTRACT

Opening of the aziridine ring of 2-deoxy-1,2-epimino-2-*epi*-fortimicin B (**10**) has been effected with both chloride and azide. The reactions are both stereo- and regiospecific and give 2-chloro-2-deoxyfortimicin B (**2c**) and 2-azido-2-deoxyfortimicin B (**2d**). The nucleophilic displacements of the methanesulfonate groups of some 1-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin derivatives with chloride, azide, and cyanide in *N*,*N*-dimethylformamide are dependent both on the nature of the nucleophile and the specific 1-*N*-benzyloxycarbonyl-2-methanesulfonate employed as the substrate. Striking differences in the stereochemistry of the azide displacements with different 2-methanesulfonates are believed to have a conformational basis. 2-Amino-2-deoxyfortimicin A (**1c**) and both of the 2-epimeric 2-chloro-2-deoxyfortimicins A (**1b**) and (**5**) were prepared for antibacterial assay and the *in vitro* results are reported.

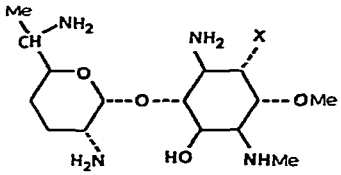
DISCUSSION

Fortimicin A (**1a**) and fortimicin B (**2a**) are aminocyclitol antibiotics formed in fermentations by *Micromonospora olivoasterospora*[1]. Fortimicin A, which has the 4-*N*-glycyl group, has significant antibacterial activity, whereas fortimicin B is virtually devoid of activity[2]. In the context of a program in our laboratories devoted to the preparation of chemically modified fortimicins for antibacterial assay, we have prepared some cyclitol-modified fortimicins from 2-*O*-(methylsulfonyl)fortimicin derivatives. Previously we had used 2-*O*-(methylsulfonyl)fortimicins to prepare[3] the 2-deoxyfortimicins A (**3a**) and B (**3b**), and the 2-*epi*-fortimicins A[4] (**4a**) and B[5] (**4b**). In the present work, we describe nucleophilic displacements of 2-*O*-(methylsulfonyl)-fortimicins with azide and chloride ions which led to the preparation of 2-amino-2-deoxyfortimicin A (**1c**) and the 2-epimeric 2-chloro-2-deoxyfortimicins A (**1b** and **5**) for biological evaluation.

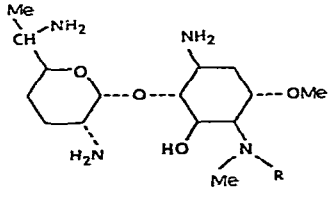
We reported elsewhere[3] the preparation of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**) by a process in which the first step was conversion



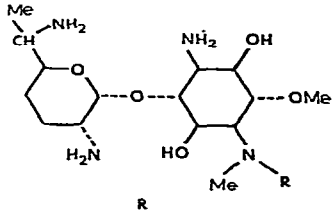
	X
1a	OH
1b	Cl
1c	NH_2
1d	OMs



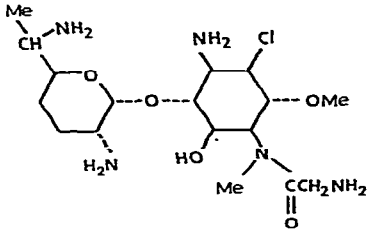
	X
2a	OH
2b	OMs
2c	Cl
2d	N_3
2e	NH_2



	R
3a	$COCH_2NH_2$
3b	H



	R
4a	$COCH_2NH_2$
4b	H



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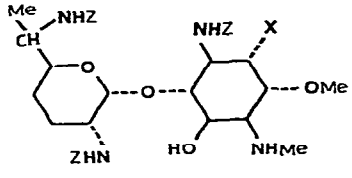
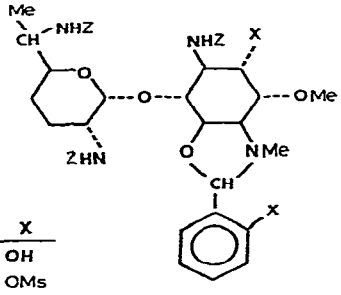
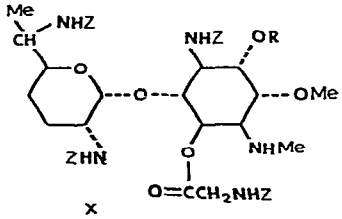


	X
6a	OH
6b	OMs
6c	Cl
6d	N_3



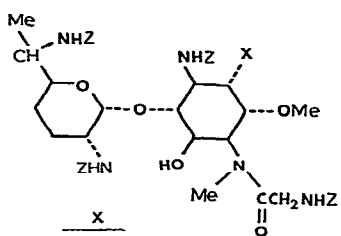
	X
7a	OH
7b	OMs



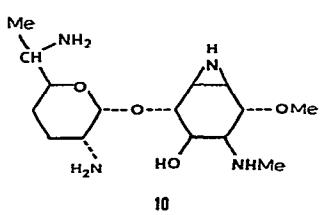
	X
8a	OMs
8b	H

of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**) into the 4,5-salicylaldehyde oxazolidine **7a**. Treatment of the latter with methanesulfonyl chloride in pyridine gave the dimethanesulfonate **7b**, which on mild, acid-catalyzed hydrolysis gave **6b**. We have now found that when the acid-catalyzed hydrolysis of **7b** is conducted in the presence of Girard's reagent T as an aldehyde scavenger, the methanesulfonate **6b** may be prepared from 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**) in essentially quantitative yield, without purification after any step, to give a product of adequate purity for further reactions.

Treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**) with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide gave a mixture of two products in a ratio of ~1:5, as estimated by t.l.c. These were partially separated by column chromatography. The minor, less polar product was shown to be 1,2′,6′-tri-*N*-benzyloxycarbonyl-5-*O*-(*N*-benzyloxycarbonyl)glycyl-2-*O*-(methylsulfonyl)fortimicin B (**8a**) by its ^{1}H-n.m.r. spectrum, which showed that the chemical shift (δ 2.33) of the NCH_3 protons was characteristic of that of a free $NHCH_3$ group (as for 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**), δ C-4-$NHCH_3$ = 2.32)[6]. The 5-*O*-(*N*-benzyloxycarbonyl)glycyl derivative **8a** rearranged quantitatively by 5-*O* to 4-*N*-acyl migration to the major product, 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) in aqueous oxolane containing triethylamine. Preparatively, it was advantageous to treat the mixture of **8a** and **9a** with triethylamine in aqueous oxolane to convert **8a** into **9a** prior to column chromatography, which gave **9a** from **6b** in ~88% yield.



	X
9a	OMs
9b	OH
9c	Cl
9d	N_3



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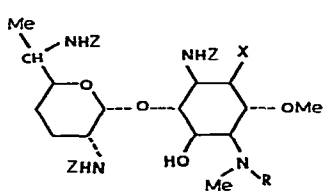
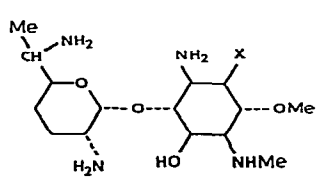


	X	R
11a	Cl	$COCH_2NHZ$
11b	N_3	$COCH_2NHZ$
11c	Cl	H
11d	N_3	H



	X
12a	Cl
12b	N_3

Catalytic hydrogenolysis of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) gave 2-*O*-(methylsulfonyl)fortimicin A (**1d**), which was isolated as the tetrahydrochloride salt.

It should be noted that reinvestigation of the acylation of 1,2′,6′-tri-*N*-(benzyloxycarbonyl)fortimicin B (**6a**) with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, which was reported previously[6], showed that the isolated product was 1,2′,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A (**1a**), with no evidence of the 5-*O*-acylated product **8b**.

We have reported that catalytic hydrogenolysis of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**) in the presence of an excess of hydrochloric acid gave 2-*O*-(methylsulfonyl)fortimicin B (**2b**), which was isolated as the tetrahydrochloride salt[3]. Treatment of an aqueous solution of the latter with AG2-X8 (OH) resin gave[3] 2-deoxy-1,2-epimino-2-*epi*-fortimicin B (**10**). Treatment of **10** with 0.2M aqueous hydrochloric acid gave 2-chloro-2-deoxyfortimicin B (**2c**). When an aqueous solution of the latter, as the free base, was heated for 4 days at 60°, the chloride **2c** reverted back to the epimine **10**, which is consistent with the *trans* relationship between the 1-amino group and the 2-chloro group of **2c**.

When an aqueous solution of the epimine **10**, containing an excess of sodium azide, was brought to pH 6 by addition of hydrochloric acid, opening of the epimine ring occurred to give 2-azido-2-deoxyfortimicin B (**2d**). Both 2-chloro-2-deoxyfortimicin B (**2c**) and 2-azido-2-deoxyfortimicin B (**2d**) were converted into the corresponding 1,2′,6′-tri-*N*-benzyloxycarbonyl derivatives **6c** and **6d**. The chloride **6c** was acylated with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, and the crude product treated with triethylamine in aqueous oxolane to give 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A (**9c**). Catalytic hydrogenolysis of **9c** gave 2-chloro-2-deoxyfortimicin A (**1b**), isolated as the tetrahydrochloride salt.

Displacement of the methanesulfonate group of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) with lithium chloride in *N*,*N*-dimethylformamide gave 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-chloro-2-*epi*-fortimicin A (**11a**). Catalytic hydrogenolysis of **11a** gave 2-chloro-2-deoxy-2-*epi*-fortimicin A (**5**), isolated as the tetrahydrochloride salt. Mild, base-catalyzed hydrolysis of **11a** gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-chloro-2-*epi*-fortimicin B (**11c**). Catalytic hydrogenation of **11c** gave 2-chloro-2-*epi*-fortimicin B (**12a**), used for the ^{13}C-n.m.r. structural studies described later.

In contrast to the reaction of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) with lithium chloride in *N*,*N*-dimethylformamide, which occurred with stereospecific inversion of configuration at C-2, the reaction of **9a** with sodium azide in *N*,*N*-dimethylformamide proceeded with stereospecific retention of configuration to give 2-azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**9d**). Mild, base-catalyzed hydrolysis of **9d** gave 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**6d**), identical with that prepared *via* the epimine **10** as already described. Base-catalyzed hydrolysis of **6d** with potassium hydroxide in

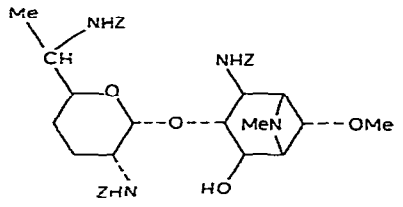
aqueous ethanol gave 2-azido-2-deoxyfortimicin B (**2d**), identical with that prepared from the epimine **10**.

A control experiment established that 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A (**1b**) was stable to treatment with lithium chloride in *N*,*N*-dimethylformamide under conditions that effect complete conversion of the methanesulfonate **9a** into 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxy-2-*epi*-fortimicin A (**11a**). The conversion of the methanesulfonate **9a** into the 2-*epi* chloride **11** is thus consistent with a direct displacement-reaction (S_N2). In contrast, the displacement of the methanesulfonate group of **9a** with sodium azide in *N*,*N*-dimethylformamide, which occurs with retention of configuration at C-2, is believed to be the result of intramolecular participation of the 1-*N*-benzyloxycarbonyl group of **9a**, which leads to retention of configuration *via* a bridged intermediate. Our studies that led to the preparation of 2-*epi*-fortimicin B (**4d**) showed that participation of a 1-*N*-benzyloxycarbonyl group in solvolysis of 1-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicins may occur with participation of either the nitrogen or the carbonyl oxygen atom of the 1-*N*-benzyloxycarbonyl group[5]. A study of the mechanism of the reaction of **9a** with sodium azide in *N*,*N*-dimethylformamide will be reported in a forthcoming paper from these laboratories[7].

Catalytic hydrogenolysis of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**6d**) and 2-azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**9d**) gave 2-amino-2-deoxyfortimicin B (**2e**) and 2-amino-2-deoxyfortimicin A (**1c**).

In contrast to the reaction of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) with sodium azide in *N*,*N*-dimethylformamide, which occurred with stereospecific retention at C-2, similar treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**) occurred with stereospecific inversion of configuration at C-2 to give 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**) in 12% yield, together with 22% of the azetidine (**13**). The azide **11d** was converted into 2-azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin A (**11b**) on acylation with *N*-(*N*-benzyloxycarbonylglycyloxy)-succinimide followed by treatment with triethylamine in aqueous oxolane. Attempted catalytic hydrogenolysis of **11b** with 5% palladium-on-carbon, which was successful in the conversion of **9d** into **1c**, gave a complex mixture of products that was not resolved.

Attempted base-catalyzed hydrolysis of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**) to 2-azido-2-deoxy-2-*epi*-fortimicin B (**12b**) gave instead 2-azido-2-deoxy-2-*epi*-fortimicin B 1,4-urea (**14**). The latter was stable to conditions of basic hydrolysis (6M aqueous potassium hydroxide, 110°, three days) more vigorous than those adequate for conversion of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**6d**) into 2-azido-2-deoxyfortimicin B (**2d**). Two factors may be considered to account for the contrast between the base-catalyzed hydrolyses of the tri-*N*-benzyloxycarbonyl-2-normal azide **6d** and the tri-*N*-benzyloxycarbonyl-2-*epi*-azide **11d**. First, the *cis*-relationship of the 1-*N*-benzyloxycarbonyl group and the 2-azido group of the 2-*epi*-azide **11d** might be expected to sterically hinder base-



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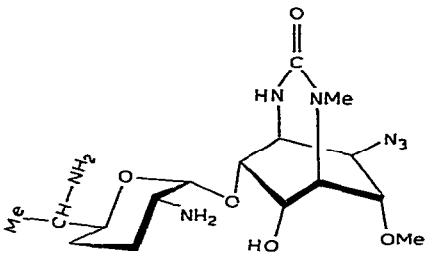

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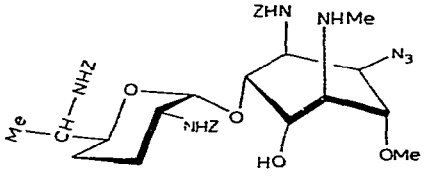

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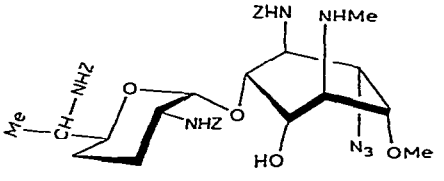

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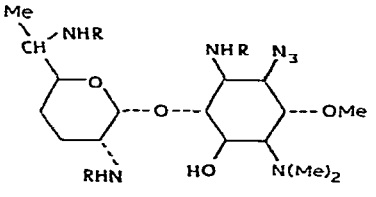
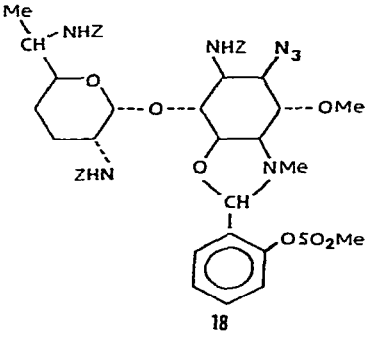


	R
17a	Z
17b	H



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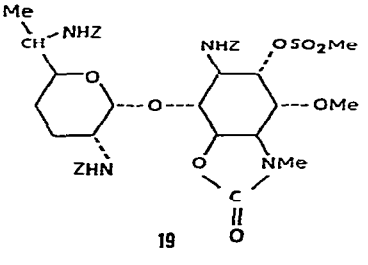

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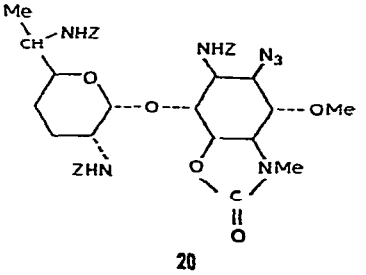

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catalyzed hydrolysis of the 1-*N*-benzyloxycarbonyl group, relative to hydrolysis of the 1-*N*-benzyloxycarbonyl group, of the 2-normal azide in which the corresponding groups have a *trans* relationship. Second, the boat (or skew) conformation of the cyclitol ring of the 2-*epi*-azide **15**, which must be assumed in order to effect cyclization to the urea **14**, has the 2-azido and the 3-methoxy groups in a gauche relationship. In contrast, the corresponding boat conformation of the 2-normal azide **16** has the 2-azido and the 3-methoxy groups in the energetically unfavorable, eclipsed relationship.

In order to avoid formation of the urea, with the object of converting the tri-*N*-benzyloxycarbonyl-2-*epi*-azide **11d** into a derivative suitable for the ^{13}C-n.m.r. structural studies described next, **11d** was converted into 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-4-*N*-methyl-2-*epi*-fortimicin B (**17a**) with sodium cyanoborohydride and formalin. Base-catalyzed hydrolysis of **17a** gave 2-azido-2-deoxy-4-*N*-methyl-2-*epi*-fortimicin B (**17b**).

The striking contrast in the stereochemistry of the displacement of the methanesulfonate groups of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) and 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**) with sodium azide in *N*,*N*-dimethylformamide led us to study similar displacements with additional derivatives of 1-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl) fortimicins. Treatment of the tri-*N*-benzyloxycarbonyl-di-*O*-(methylsulfonyl)fortimicin B salicylaldehyde oxazolidine[3] **7b** with sodium azide in *N*,*N*-dimethylformamide gave the 2-*epi*-azide **18**. Mild, acid-catalyzed hydrolysis of **18** in the presence of Girard's reagent T gave 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**) in essentially quantitative yield from **7b**. Treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate[5] (**19**) with sodium azide in *N*,*N*-dimethylformamide gave 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B 4,5-carbamate (**20**). Base-catalyzed hydrolysis of **20** gave the 1,4-urea **14**, identical with that formed by hydrolysis of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**).

The contrast between the azide displacements of the methanesulfonates **6b**, **7b**, and **19** on the one hand (inversion) and the methanesulfonate **9a** on the other (retention) is believed to be conformationally derived. Fortimicin A (**1a**) has been shown to exist as the free base in aqueous solution with that cyclitol chair-conformation **21a** having the C-1 and C-2 substituents in a *trans* diaxial relationship[8]. With a similar cyclitol chair-conformation (see structure **21b**) the methanesulfonate **9a** would have the 1-*N*-benzyloxycarbonylamino group and the 2-methanesulfonate group in a *trans* diaxial relationship, which is optimal for neighboring-group participation and formation of a bridged intermediate that would open to give net retention of configuration. Fortimicin B (**2a**), however, has been shown to exist as the free base in aqueous solution with that cyclitol chair-conformation **22a** having the 1-amino and the 2-hydroxyl groups in a *trans*-diequatorial relationship[8]. With fortimicin B-like cyclitol conformations (see structures **22b**, **23**, and **24**) the methanesulfonates **6b**, **7b**, and **19** would have the 1-*N*-benzyloxycarbonylamino groups and the 2-methane-

TABLE I

CARBON-13 MAGNETIC RESONANCE PARAMETERS[a]

Carbon atom	*Fortimicin B*[8] (2a)		2-epi-*Fortimicin B*[8] (4b)		*2-Azido-2-deoxy-fortimicin B* (2d)		*2-Chloro-2-deoxyfortimicin B* (2c)		*2-Azido-2-deoxy-4-N-methyl-2-epi-fortimicin B* (17b)		*2-Chloro-2-deoxy-2-epi-fortimicin B* (12a)	
	pD 1.8	*β-Shift*	*pD 3.16*	*β-Shift*	*pD 1.4*	*β-Shift*	*pD 1.2*	*β-Shift*	*pD 1.17*	*β-Shift*	*pD 2.22*	*β-Shift*
1′	96.0	6.5	95.8	5.4	96.6	5.6	97.1	5.5	95.4	4.2	95.7	4.5
2′	51.9		51.9		51.9		51.9		51.7		51.7	
3′	21.5	5.5	21.5	5.8	21.5	5.3	21.5	5.5	21.5	5.6	21.5	5.9
4′	26.3		26.2		26.4		26.4		26.2		26.2	
5′	71.0	4.1	71.1	3.9	71.1	3.4	71.3	3.0	71.1	3.2	71.1	4.1
6′	49.4		49.3		49.4		49.3		49.1		49.8	
7′	15.1	3.4	15.2	3.6	15.2	3.1	15.1	3.4	15.2	3.4	15.4	3.4
1	53.5		54.9		52.1		54.1		53.6		55.7	
2	65.5	5.7	65.6	3.9	58.3	4.8	54.5	8.4	62.3	2.0	62.1	3.5
3	74.1	5.5	76.6	2.4	73.9	4.7	72.7	7.2	74.7	2.7	77.0	3.2
4	58.1		61.2		57.3		57.2		64.9		60.6	
5	66.6	4.6	68.8	3.7	66.0	4.8	66.4	4.5	66.2	4.1	64.8	3.4
6	74.2	9.9	72.9	6.5	74.1	9.3	74.8	9.5	72.5	3.2	72.9	4.0

[a] ^{13}C Fourier-transform spectra were recorded with a Varian XL-100-15 spectrometer at 25.16 MHz with a Nicolet TT-100 computer and a JEOL FX-90Q spectrometer at 22.50 MHz. All samples were dissolved in D_2O and titrated with D_2SO_4 from basic to acidic pD. 1,4-Dioxane was used as an internal reference. A flip angle of $\sim 60°$ was used. All solutions were $\sim 10\%$ (w/v).

sulfonate groups in the *trans*-diequatorial relationship unfavorable for participation of the 1-*N*-benzyloxycarbonyl group, thus favoring direct displacement.

The contrast in the stereochemistry of the displacement of the methanesulfonate **9a** with lithium chloride in *N,N*-dimethylformamide (inversion) and sodium azide in *N,N*-dimethylformamide (retention) is believed to be a consequence of the difference between the basicities of the media. This will be discussed further in a forthcoming paper dealing with the mechanism of the azide displacement[7].

An attempt to displace the methanesulfonate group of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) with sodium cyanide in *N,N*-dimethylformamide led to extensive decomposition. Similar treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**22b**) gave 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-1,2-epimino-2-*epi*-fortimicin B 4,5-carbamate (**25**). The latter (**25**) was formed in essentially quantitative yield on treatment of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate[5] (**19**) with potassium carbonate in refluxing aqueous methanol.

Formation of **25** from **22b** with sodium cyanide in *N,N*-dimethylformamide may be formulated (Scheme 1) as occurring by initial formation of the acyl-aziridine **27** *via* the enolate anion **26**. Attack of cyanide on **26** would give the epimine **28** and benzyloxycarbonyl cyanide. 4-*N*-Acylation of **28** with benzyloxycarbonyl cyanide would give the 4-*N*-benzyloxycarbonyl derivative **29**, which on cyclization would give **25**. Alternatively, **29** might be formed from **27** by an intramolecular rearrangement.

Structural assignments. — The positions of the newly introduced substituents of the 2-substituted fortimicins were established by means of ^{13}C-n.m.r. spectroscopy. Titration studies with both of the 2-epimeric 2-chloro-2-deoxyfortimicins B **2c** and

TABLE II

PROTON MAGNETIC RESONANCE PARAMETERS

Proton (δ) or coupling (Hz)	*Fortimicin B*[8] (**2a**)	*2-Chloro-2-deoxyfortimicin B* (**2c**)	*2-Azido-2-deoxyfortimicin B* (**2d**)
1	3.43	3.61	3.60
2	4.17	4.65	4.08
3	4.11	4.30	4.37
4	3.54	3.64	3.59
5	4.44	4.53	4.52
6	3.93	4.00	4.08
$J_{1,2}$	9.5	10.5	9.5
$J_{2,3}$	3.0	3.0	3.0
$J_{3,4}$	3.0	4.5	4.0
$J_{4,5}$	4.5	4.5	4.0
$J_{5,6}$	9.5	9.0	9.0
$J_{1,6}$	9.5	9.0	9.0

TABLE III

In vitro ANTIBACTERIAL ACTIVITIES[a]

Organism	*Fortimicin A* (1a)	*2-Chloro-2-deoxy-fortimicin A* (1b)	*2-Amino-2-deoxy-fortimicin A* (1c)	*2-Chloro-2-deoxy-2-epi-fortimicin A* (5)	*2-O-(Methylsulfonyl)-fortimicin A* (1d)
Staphylococcus aureus Smith	0.78	6.2	1.56	0.78	12.5
Streptococcus faecalis 10541	25	100	100	12.5	>100
Enterobacter aerogenes 13048	3.1	50	12.5	6.2	>100
Escherichia coli Juhl	6.2	25	12.5	6.2	50
Escherichia coli BL-3676 (Res)	25	50	25	12.5	>100
Escherichia coli 76-2	3.1	12.5	6.2	3.1	50
Klebsiella pneumoniae 10031	1.56	50	6.2	12.5	>100
Klebsiella pneumoniae KY 4262	6.2	>100	12.5	25	>100
Providencia 1577	1.56	100	12.5	6.2	>100
Pseudomonas aeruginosa BMH no. 10	0.78	6.2	1.56	0.78	25
Pseudomonas aeruginosa KY-8512	12.5	100	25	50	>100
Pseudomonas aeruginosa KY-8516	6.2	50	100	25	>100
Pseudomonas aeruginosa 209	>100	>100	>100	>100	>100
Pseudomonas aeruginosa 27853	25	>100	>100	100	>100
Salmonella typhimurium Ed. no. 9	6.2	12.5	6.2	3.1	100
Serratia marcescens 4003	1.56	12.5	6.2	3.1	100
Shigella sonnei 9290	6.2	25	12.5	6.2	100
Proteus rettgeri U6333	12.5	>100		100	>100
Proteus vulgaris JJ	6.2	50	6.2	6.2	100
Proteus mirabilis Fin. no. 9	6.2	50	12.5	12.5	100

[a]The *in vitro* activities were determined by the serial dilution method using Mueller–Hinton Agar with the per-sulfate salts. Activities are expressed as minimum inhibitory concentrations of free base in μg per mL.

12a, 2-azido-2-deoxyfortimicin B (**2d**), and 2-azido-2-deoxy-4-*N*-methyl-2-*epi*-fortimicin B (**17b**) showed that the resonances of four of the cyclitol carbon atoms of each were subject to β-shifts on protonation of the cyclitol amino groups (Table I), which is compatible with 1,4-diaminocyclitols, as was the case for both fortimicin B[8] (**2a**) and 2-*epi*-fortimicin B[5] (**4b**). It may be noted that the 2-normal epimers **2c** and **2d**, like fortimicin B (**2a**), show relatively large β-shifts for the C-6 atoms.

The configurations at C-2 of the 2-substituted fortimicins were established by ^{1}H-n.m.r. studies. The ^{1}H-n.m.r. spectral data of the free bases of 2-chloro-2-deoxyfortimicin B (**2c**), 2-azido-2-deoxyfortimicin B (**2d**), and fortimicin B (**2a**) are listed in Table II. The coupling constants of the cyclitol protons of **2a**, **2c**, and **2d** are all compatible with those cyclitol chair conformations in which the 4-substituents are axial. In addition, the coupling constants of each ($J_{1,2}$ ~10 Hz) are consistent with the axial orientations of H-2 and thus the equatorial and normal orientations of the 2-substituents.

In vitro *antibacterial activities*. — The *in vitro* antibacterial activities of 2-*O*-(methylsulfonyl)fortimicin A (**1d**), 2-amino-2-deoxyfortimicin A (**1c**), the 2-epimeric 2-chloro-2-deoxyfortimicins A (**1b** and **5**), and fortimicin A (**1a**) are listed in Table III. Although none of the new fortimicins has activity equal to that of fortimicin A, it is of interest that the activity of 2-chloro-2-deoxy-2-*epi*-fortimicin A (**5**), about half that of fortimicin A (**1a**), is significantly greater than the activities of the other 2-substituted fortimicins A, including the 2-epimeric chloride **1b**.

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. ^{13}C-N.m.r. spectra were measured with a Varian Associates–Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal 1,4-dioxane (67.4 p.p.m.) and are reported in p.p.m. downfield from Me_4Si. Mass spectra were obtained with an AEI MS-902 spectrometer at 70 eV and 100–150° using a direct-probe insert. Silica gel for chromatography refers to that of Merck (Darmstadt) 70–230 mesh. Microanalytical results are reported for those compounds which could be freed of solvent. Unless otherwise specified, chloroform extractions were performed by shaking mixtures or solutions with mixtures of chloroform and 5% aqueous sodium hydrogencarbonate. The chloroform solutions were separated, washed and dried (magnesium sulfate), and evaporated under diminished pressure.

2-Azido-2-deoxyfortimicin B (**2d**). — *A*. To a stirred solution of 1.70 g of 1,2-epimino-2-deoxy-2-*epi*-fortimicin B (**10**) and 6.92 g of sodium azide in 20 mL of water, cooled in an ice bath, was added dropwise 6M hydrochloric acid until the

solution was brought to pH 6. Stirring was continued with cooling for 1 h, and then for 43 h at room temperature. The solvent was evaporated under diminished pressure, and residual water was removed by evaporation of methanol under diminished pressure. The residue was triturated 4 times with 50-mL portions of methanol. The supernatant solutions were combined and the methanol was evaporated leaving 6.17 g of residue. The latter was chromatographed on a column of 200 g of silica gel packed and eluted with the lower phase of 2:2:1:1 dichloromethane–methanol–concentrated ammonium hydroxide–water to yield 1.33 g of **2d**. The latter was converted into the tetrahydrochloride salt with methanolic hydrochloric acid. Evaporation of the solvent left 1.82 g of the salt; $[\alpha]_D$ +82° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ (KBr) 2113 cm^{-1}; ^{1}H-n.m.r. (D_2O, tetrahydrochloride): δ 1.37 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.83 (NCH_3), 3.58 (OCH_3), and 5.36 d ($J_{1',2'}$ 3.1 Hz, 1'-H); m.s. (M+H), calc. for $C_{15}H_{32}N_7O_4$: *m/z* 374.2516, meas. 374.2528; cyclitol, calc. for $C_8H_{18}N_5O_3$: *m/z* 232.1410, meas. 232.1412; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1178.

B. A stirred solution of 1.02 g of 2-azido-1,2',6'-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**6d**), 8 mL of 6M aqueous potassium hydroxide, and 16 mL of ethanol was heated for 2 days at 85° under nitrogen. The resulting solution was cooled, diluted with 50 mL of ethanol and brought to pH 5 by addition of 2M aqueous hydrochloric acid. The solvent was evaporated and residual water removed by evaporation of methanol from the residue. The residue was triturated with methanol. The methanolic supernatant was separated and the methanol evaporated. The residue was chromatographed on a column of 40 g of silica gel packed and eluted with the lower phase of a 2:2:1:1 mixture of dichloromethane–methanol–concentrated ammonium hydroxide–water to give 0.132 g of **2d**, identical with that prepared by method *A*, just described.

1,2',6'-Tri-N-*benzyloxycarbonyl-5*-O-(N-*benzyloxycarbonyl*)*glycyl-2*-O-(*methylsulfonyl*)*fortimicin A* (**8a**) *and 1,2',6',2''-tetra*-N-*benzyloxycarbonyl-2*-O-)*methylsulfonyl*)*fortimicin A* (**9a**). — A stirred solution of 14.9 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**), 6.09 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, and 140 mL of oxolane was kept for 50 min at 0° and then overnight at room temperature. The product (18.4 g, two components by t.l.c., 5:1 major/minor) was isolated by extraction with chloroform and chromatographed on a column of 600 g of silica gel packed and eluted with ethyl acetate. Early fractions contained 2.4 g of compound **8a**; $[\alpha]_D^{25}$ +29° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3420, 3317, and 1714 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.03 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.33 (NCH_3), 2.86 (OSO_2CH_3), and 3.55 (OCH_3).

Anal. Calc. for $C_{50}H_{61}N_5O_{16}S \cdot H_2O$: C, 57.84; H, 6.12; N, 6.75; S, 3.09. Found: C, 57.72; H, 6.18; N, 6.80; S, 3.60.

Further elution gave 9.5 g of a mixture of **8a** and compound **9a**. Later fractions gave 4.3 g of pure **9a**; $[\alpha]_D^{24}$ +41° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3427, 1710, and 1635 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.14 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.81 (NCH_3), 3.02 (OSO_2CH_3), and 3.34 (OCH_3).

Anal. Calc. for $C_{50}H_{61}N_5O_{16}S$: C, 58.87; H, 6.03; N, 6.87; S, 3.14. Found: C, 58.70; H, 6.04; N, 6.62; S, 2.89.

*Rearrangement of 1,2',6'-tri-*N*-benzyloxycarbonyl-5-*O*-(*N*-benzyloxycarbonyl)-glycyl-2-*O*-(methylsulfonyl)fortimicin B* (**8a**) *to 1,2',6',2"-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A* (**9a**). — A solution of 0.4 g of compound **8a** in 8 mL of 4:1:0.5 oxolane–water–triethylamine was kept for 3 h at room temperature. Triethylamine (1.0 mL) was added and the resulting solution kept for an additional 3 h at room temperature. Extraction with chloroform gave 0.4 g of compound **9a**, identical with that already described.

*2-Azido-1,2',6',2"-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A* (**9d**). — A stirred solution of 7.6 g of compound **9a**, 7.6 g of sodium azide, and 420 mL of *N,N*-dimethylformamide was heated overnight at 93°. The product (7.32 g) was isolated by extraction with chloroform followed by evaporation of chloroform and removal of residual *N,N*-dimethylformamide by evaporation of toluene from the residue. Chromatography of the crude product on a column of 700 g of silica gel packed and eluted with 9:1 ethyl acetate–hexane gave 5.55 g of **9d**; $[\alpha]_D^{25}$ +38° (*c* 1.5, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3480–3170, 2955, 2112, 1715, and 1640 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.15 d ($J_{6',7'}$ 6.1 Hz, 6'-CH_3), 2.76 (NCH_3), and 3.32 (OCH_3).

Anal. Calc. for $C_{49}H_{58}N_8O_{13}$: C, 60.86; H, 6.05; N, 11.59. Found: C, 61.14; H, 6.32; N, 11.62.

*2-Azido-1,2',6'-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B* (**6d**). — *A.* To a stirred solution of 0.400 g of the tetrahydrochloride salt of 2-azido-2-deoxyfortimicin B (**2d**) prepared from 2-deoxy-1,2-epimino-2-*epi*-fortimicin B (**10**), 0.312 g of triethylamine, 2.2 mL of water, and 8.8 mL of methanol, cooled in an ice bath, was added 0.633 g of *N*-(benzyloxycarbonyl)succinimide. Stirring was continued with cooling for 3 h and then overnight at room temperature. The product (0.571 g) was isolated by extraction with chloroform and chromatographed on a column of 60 g of silica gel with 100:1 ethyl acetate–triethylamine to yield 0.367 g of **6d**, identical with that prepared from 2-azido-1,2',6',2"-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**9d**) as described next.

B. A stirred suspension of 5.15 g of compound **9d**, prepared from 1,2',6',2"-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**), 25 mL of 5% aqueous sodium hydrogencarbonate, and 250 mL of methanol was boiled for 2.5 h under reflux. The product (4.10 g) was isolated by extraction with chloroform. A sample (0.600 g) of the product was chromatographed on a column of 60 g of silica gel packed and eluted with 100:1 ethyl acetate–triethylamine to yield 0.412 g of compound **6d**; $[\alpha]_D^{23}$ +20° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CHCl_3$) 3421, 3345, 2105, and 1713 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 d ($J_{6',7'}$ 6.0 Hz, 6'-CH_3), 2.32 (NCH_3), and 3.43 (OCH_3).

Anal. Calc. for $C_{39}H_{59}N_7O_{10}$: C, 60.37; H, 6.37; N, 12.64. Found: C, 60.28; H, 6.43; N, 12.40.

*2-Azido-1,2',6'-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B* (**11d**) *and 1,2',6'-Tri-*N*-benzyloxycarbonyl-2-deoxy-2,4-epiminofortimicin B* (**13**). — A stirred

solution of 2.0 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B (**6b**), 2.0 g of sodium azide and 111 mL of *N*,*N*-dimethylformamide was heated for 18 h at 93°. The product was isolated by extraction with chloroform and chromatographed on a column of silica gel packed and eluted with 9.5:0.5:0.05 ethyl acetate–95% ethanol–concentrated ammonium hydroxide. Earlier fractions gave 0.225 g of **11d**; $[\alpha]_D^{25}$ +55° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 2105, 1710, and 1500 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.11 d ($J_{6',7'}$ 6.0 Hz, 6′-CH_3), 2.30 (NCH_3), and 3.53 (OCH_3).

Anal. Calc. for $C_{39}H_{49}N_7O_{10}$: C, 60.38; H, 6.37; N, 12.64. Found: C, 60.11; H, 6.35; N, 12.31.

Later fractions gave 0.381 g of compound **13**; $[\alpha]_D^{23}$ +41° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3439 and 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 d ($J_{6',7'}$ 6.0 Hz, 6′-CH_3), 2.54 (NCH_3), and 3.23 (OCH_3).

Anal. Calc. for $C_{39}H_{48}N_4O_{10}$: C, 63.92; H, 6.60; N, 7.65. Found: C, 63.91; H, 6.55; N, 7.90.

*2-Azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B* (**11d**). — A solution of 8.9 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)-4-*N*,5-*O*-[(2-*O*-methylsulfonyl)salicylidene]fortimicin B (**7b**), 7.83 g of sodium azide, and 400 mL of *N*,*N*-dimethylformamide was heated for 22 h at 93°. Conventional extraction with chloroform gave 7.86 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-4-*N*,5-*O*-[(methylsulfonyl)salicylidene]-2-*epi*-fortimicin B (**18**); $\tilde{\nu}_{max}$ ($CHCl_3$) 3440, 3304, 2104, and 1714 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.01 d ($J_{6',7'}$ 6.7 Hz, 6′-CH_3), 2.28 (NCH_3), 3.02 (OSO_2CH_3), and 3.53 (OCH_3).

A solution of compound **18**, 4.03 g of Girard's reagent T, 3.6 mL of acetic acid, and 180 mL of methanol was boiled for 2 h under reflux. Extraction with chloroform gave 6.30 g of compound **11d**, identical with that described in the preceding experiment.

*2-Azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B 4,5-carbamate* (**20**). — A solution of 2.49 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin B 4,5-carbamate (**19**), 2.49 g of sodium azide, and 62 mL of *N*,*N*-dimethylformamide was heated for 21 h at 90°. The product (2.12 g) was isolated by conventional extraction with chloroform and chromatographed on a column of 210 g of silica gel, packed and eluted with 4:1 ethyl acetate–hexane to yield 1.70 g of **20**; $[\alpha]_D^{23}$ +22° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3443, 3297, 2104, 1762, and 1712 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 d ($J_{6',7'}$ 6.7 Hz, 6′-CH_3), 2.88 (NCH_3), and 3.50 (OCH_3).

Anal. Calc. for $C_{40}H_{47}N_7O_{11}$: C, 59.91; H, 5.91; N, 12.23. Found: C, 60.16; H, 6.03; N, 11.85.

*2-Azido-1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin A* (**11b**). — A stirred solution of 0.596 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**) and 0.285 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide in 10 mL of oxolane was kept for 1 h at 0° and then overnight at room temperature. The product (0.798 g) was isolated by extraction with chloroform and chromatographed on a column of 30 g of silica gel packed and eluted with ethyl acetate to yield 0.606 g of **11b**; $[\alpha]_D^{25}$ +37° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CHCl_3$) 3435, 3317, 2106, 1717,

and 1642 cm^{-1}, ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 d ($J_{6',7'}$ 7.1 Hz, 6'-CH_3), 2.82 (NCH_3), and 3.46 (OCH_3).

Anal. Calc. for $C_{49}H_{58}N_8O_{13}$: C, 60.86; H, 6.05; N, 11.59. Found: C, 60.83; H, 6.13; N, 11.24.

1,2',6',2''-Tetra-N-*benzyloxycarbonyl-2-chloro-2-deoxy-2*-epi-*fortimicin A* (**11a**). — A stirred solution of 14.0 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**), 14.0 g of lithium chloride, and 730 mL of *N,N*-dimethylformamide was heated for 117 h at 93°. The product (11.8 g) was isolated by extraction with chloroform. Chromatography of 5.9 g of the product on a column of 700 g of silica gel with 1:1 ethyl acetate–toluene gave 1.72 g of **11a**; $[\alpha]_D^{24}$ +37° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 1710, and 1638 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.17 d ($J_{6',7'}$ 7.0 Hz, 6'-CH_3), 2.85 (NCH_3), and 3.46 (OCH_3).

Anal. Calc. for $C_{49}H_{58}ClN_5O_{13}$: C, 61.28; H, 6.09; Cl, 3.69; N, 7.29. Found: C, 61.36; H, 6.21; Cl, 3.97; N, 7.00.

1,2',6'-Tri-N-*benzyloxycarbonyl-2-chloro-2-deoxy-2*-epi-*fortimicin B* (**11c**). — A solution of 1.50 g of compound **11a**, 7.3 mL of 5% aqueous sodium hydrogencarbonate, and 73 mL of methanol was boiled for 4.5 h under reflux. The product (1.21 g) was isolated by extraction with chloroform, and chromatographed on a column of 75 g of silica gel packed and eluted with 4:1 ethyl acetate–hexane to yield 0.729 g of compound **11c**; $[\alpha]_D^{25}$ +62° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3442 and 1712 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.14 d ($J_{6',7'}$ 6.2 Hz, 6'-CH_3), 2.32 (NCH_3), and 3.55 (OCH_3).

Anal. Calc. for $C_{39}H_{49}ClN_4O_{10}$: C, 60.89; H, 6.42; Cl, 4.61; N, 7.28. Found: C, 61.09; H, 6.60; Cl, 5.36; N, 7.15.

2-Chloro-2-deoxy-2-epi-*fortimicin B* (**12a**). — Compound **11c** (0.500 g) in 52 mL of 0.2M hydrochloric acid in methanol was hydrogenated for 2.5 h in the presence of 0.50 g of 5% palladium-on-carbon to yield 0.351 g of **12a** as the tetrahydrochloride salt; $[\alpha]_D^{25}$ +72° (*c* 1.0, methanol); ^{1}H-n.m.r. (D_2O, tetrahydrochloride): δ 1.37 d ($J_{6',7'}$ 6.7 Hz, 6'-CH_3), 2.83 (NCH_3), 3.72 (OCH_3), and 5.43 d ($J_{1',2'}$ 3.4 Hz, 1'-H); m.s. (M+H), calc. for $C_{15}H_{32}ClN_4O_4$: *m/z* 367.2112, meas. 367.2107; cyclitol, calc. for $C_8H_{18}ClN_2O_3$: *m/z* 225.1006, meas. 225.1009; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1181.

2-Amino-2-deoxyfortimicin A (**1c**). — 2-Azido-1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-deoxyfortimicin A (**9d**, 2.54 g) in 263 mL of 0.2M hydrochloric acid in methanol was hydrogenated under 3 atm of hydrogen for 5 h in the presence of 2.54 g of 5% palladium-on-carbon to give 1.6 g of compound **1c** as the pentahydrochloride salt. An aqueous solution of the latter was passed through a column containing an excess of AG1-X2(SO_4^{2-}) resin. The eluates containing product were combined, and the solvent evaporated to give the persulfate salt; $[\alpha]_D^{25}$ +69° (*c* 1.0, water); $\tilde{\nu}_{max}$ (KBr) 1635 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.47 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 3.27 (NCH_3), 3.68 (OCH_3), and 5.48 d ($J_{1',2'}$ 3 Hz, 1'-H); m.s. (free base, $M^{+\cdot}$), calc. for $C_{17}H_{36}N_6O_5$: *m/z* 404.2747, meas. 404.2733; cyclitol, calc. for $C_{10}H_{21}N_4O_3$:

m/z 245.1614, meas. 245.1617; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1179.

Anal. Calc. for $C_{17}H_{36}N_6O_5 \cdot 5/2\ H_2SO_4 \cdot 3/2\ H_2O$: C, 30.17; H, 6.55; N, 12.42; S, 11.18. Found: C, 29.97; H, 6.45; N, 12.33; S, 11.44.

2-Azido-2-deoxy-2-epi-*fortimicin B 1,4-urea* (**14**). — *A.* A stirred suspension of 0.8300 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B 4,5-carbamate (**20**), 8 mL of 6M aqueous potassium hydroxide, and 16 mL of ethanol was heated for 8 h at 85° under nitrogen. The crude product was isolated and chromatographed as described next in part *B*, to give 0.148 g of compound **14**; $[\alpha]_D^{25}$ +19° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3442-3047, 2102, and 1647 cm^{-1}; ^{1}H-n.m.r. (D_2O, free base): δ 1.07 d ($J_{6',7}$ 6.5 Hz, 6′-CH_3), 3.06 (NCH_3); 3.54 (OCH_3), and 5.06 d ($J_{1',2'}$ 3.8 Hz, 1′-H); m.s. (M+H), calc. for $C_{16}H_{30}N_7O_5$: *m/z* 400.2309, meas. 400.2315; (cyclitol-H_2O) calc. for $C_9H_{14}N_5O_3$: *m/z* 240.1097, meas. 240.1091; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1181.

B. A stirred solution of 1.00 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**), 6 mL of 6M aqueous potassium hydroxide, and 12 mL of ethanol was heated under nitrogen for 18 h at 93°. The product was isolated conventionally and chromatographed on a column of 50 g of silica gel packed and eluted with 1:1 dichloromethane–methanol and then 1:1:0.1 dichloromethane–methanol–concentrated ammonium hydroxide to give 0.210 g of compound **14**, identical with that just described. The product was recovered unchanged after treatment with 6M aqueous potassium hydroxide for 3 days at 110° under nitrogen.

*2-Azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-4-*N*-methyl-2-*epi*-fortimicin B* (**17a**). — A stirred solution of 1.01 g of 2-azido-1,2′,6′-tri-*N*-benzyloxycarbonyl-2-deoxy-2-*epi*-fortimicin B (**11d**), 0.262 g of sodium cyanoborohydride, 0.4 mL of acetic acid, 1.0 mL of 37% formalin, and 10 mL of methanol was kept overnight at room temperature. Isolation by extraction with chloroform gave 1.07 g of **17a**; $[\alpha]_D^{24}$ +57° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CHCl_3$) 3440, 3343, 2103, and 1715 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 d ($J_{6',7'}$ 6.5 Hz, 6′-CH_3), 2.41 (NCH_3), and 3.59 (OCH_3).

Anal. Calc. for $C_{40}H_{51}N_7O_{10}$: C, 60.83; H, 6.51; N, 12.41. Found: C, 60.12; H, 6.65; N, 11.35.

*2-Azido-2-deoxy-4-*N*-methyl-2-*epi*-fortimicin B* (**17b**). — A stirred solution of compound **17a**, 10 mL of 6M aqueous potassium hydroxide, and 20 mL of ethanol was heated for 18 h at 85° under nitrogen. The product was isolated conventionally and chromatographed on a column of 40 g of silica gel. Elution with the lower phase of a 2:2:1:1 mixture of dichloromethane–methanol–concentrated ammonium hydroxide–water gave 0.354 g of **17b**; $[\alpha]_D^{24}$ +123° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CHCl_3$) 3378, 3242, and 2106 cm^{-1}; ^{1}H-n.m.r. (D_2O, tetrahydrochloride): δ 1.31 d ($J_{6',7'}$ 6.7 Hz, 6′-CH_3), 3.07 (NCH_3), 3.81 (OCH_3), and 5.41 d ($J_{1',2'}$ 3.6 Hz, 1′-H); m.s. (M+H), calc. for $C_{16}H_{34}N_7O_4$: *m/z* 388.2672, meas. 388.2659; cyclitol, calc. for $C_9H_{20}N_5O_3$: *m/z* 246.1566, meas. 246.1568; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1182.

2-Chloro-2-deoxyfortimicin B (**2c**). — A solution of 1.91 g of 2-deoxy-1,2-

epimino-2-*epi*-fortimicin B (**10**) in 132 mL of 0.2M methanolic hydrochloric acid was kept for 25 h at room temperature. Solvent was evaporated leaving 2.92 g of **2c** as the tetrahydrochloride salt; $[\alpha]_D^{25}$ +98° (*c* 1.0, methanol); ^{1}H-n.m.r. (D_2O, tetrahydrochloride): δ 1.37 d ($J_{6',7'}$ 6.6 Hz, 6'-CH_3), 2.83 (NCH_3), 3.52 (OCH_3), and 5.39 d ($J_{1',2'}$ 3.5 Hz, 1'-H), m.s. (M+H), calc. for $C_{15}H_{32}ClN_4O_4$: *m/z* 367.2112, meas. 367.2100.

*2-Deoxy-1,2-epimino-2-*epi-*fortimicin B* (**10**). — An aqueous solution of 0.155 g of the tetrahydrochloride salt of compound **2c** was passed over a column containing an excess of AG2-X8(OH^-) resin. Basic eluates were combined and the solvent was evaporated leaving 0.111 g of residue. The latter was dissolved in 20 mL of water, and the resulting aqueous solution heated for 4 days at 60°, and then passed over a column of excess AG2-X8(OH^-) resin. Basic fractions were combined and the solvent evaporated. The residue was chromatographed on a column of 20 g of silica gel with the lower phase of a 2:2:1:1 mixture of dichloromethane–methanol–concentrated ammonium hydroxide–water to give 33.9 mg of the epimine **10**, identical with that prepared from 2-*O*-(methylsulfonyl)fortimicin B (**2b**) as previously described[3].

*1,2',6'-Tri-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin B* (**6c**). — A stirred solution of 2.3 g of the tetrahydrochloride salt of 2-chloro-2-deoxyfortimicin B (**2c**), 3.74 g of *N*-(benzyloxycarbonyloxy)succinimide, 12 mL of water, 48 mL of methanol, and 2.5 mL of triethylamine was kept for 2 h at 0° and then for 20 h at room temperature. The product (3.84 g) was isolated by extraction with chloroform, and chromatographed on a column of 380 g of silica gel packed and eluted with 5:2 ethyl acetate–hexane to give 1.15 g of **6c**; $[\alpha]_D^{25}$ +15° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3420, 3327, and 1712 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.08 d ($J_{6',7'}$ 6.0 Hz, 6'-CH_3), 2.32 (NCH_3), and 3.43 (OCH_3).

Anal. Calc. for $C_{39}H_{48}ClN_4O_9 \cdot H_2O$: C, 60.81; H, 6.80; Cl. 4.60; N, 7.27. Found: C, 60.67; H, 6.46; Cl, 4.82; N, 7.12.

*1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A* (**9c**). — A solution of 1.09 of compound **6c**, 1.70 g of *N*-(*N*-benzyloxycarbonylglycyloxy)-succinimide, and 14 mL of oxolane was stirred for 1 h at 0° and then overnight at room temperature. The product (1.47 g) was isolated by extraction with chloroform and treated for 3 h with 20 mL of 8:2:1 oxolane–water–triethylamine. The product was isolated by extraction with chloroform and chromatographed on a column of 175 g of silica gel packed and eluted with 2:1 ethyl acetate–hexane to give 0.9734 g of **9c**; $[\alpha]_D^{24}$ +41° (*c* 1.0, methanol); $\tilde{\nu}_{max}$ ($CDCl_3$) 3438, 1716, and 1638 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.18 d ($J_{6',7'}$ 6.5 Hz, 6'-CH_3), 2.77 (NCH_3), and 3.28 (OCH_3).

Anal. Calc. for $C_{49}H_{58}ClN_5O_{13}$: C, 61.28; H, 6.09; Cl, 3.69; N, 7.29. Found: C, 61.31; H, 6.28; Cl, 4.07; N, 7.35.

*Treatment of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A* (**9c**) *with lithium chloride in* N,N-*dimethylformamide*. — 1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A (**9c**, 0.3017 g) on treatment with 0.327 g of lithium chloride in *N*,*N*-dimethylformamide for 72 h at 90° was recovered (0.284 g) unchanged.

2-Chloro-2-deoxyfortimicin A (**1b**). — A sample (0.303 g) of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-chloro-2-deoxyfortimicin A (**9c**) was catalytically hydrogenated in the presence of 5% palladium-on-carbon in 0.2M methanolic hydrochloric acid to give 2-chloro-2-deoxyfortimicin A as the tetrahydrochloride salt* which was converted conventionally into the disulfate salt; $[\alpha]_D^{25}$ +80° (*c* 1.0, H_2O); ^{1}H-n.m.r. (D_2O): δ 1.34 d ($J_{6',7'}$ 6.6 Hz, 6′-CH_3), 3.13 (NCH_3), 3.49 (OCH_3), and 5.35 d ($J_{1',2'}$ 4.0 Hz, 1′-H).

Anal. Calc. for $C_{17}H_{38}ClN_5O_{13}S_2 \cdot 4H_2O$: C, 29.50; H, 6.70; Cl, 5.12; N, 10.12; S, 9.26. Found: C, 29.19; H, 6.17; Cl, 5.44; N, 10.01; S, 10.73.

2-Amino-2-deoxyfortimicin B (**2e**). — A sample (0.8167 g) of 2-azido-1,2′6′-tri-*N*-benzyloxycarbonyl-2-deoxyfortimicin B (**6d**) in 0.2M methanolic hydrochloric acid was hydrogenated conventionally in the presence of 5% palladium-on-carbon to give **2e** as the pentahydrochloride salt; $[\alpha]_D^{23}$ +89° (*c* 1.0, methanol); ^{1}H-n.m.r. (D_2O, free base): δ 1.04 d ($J_{6',7'}$ 6.7 Hz, 6′-CH_3), 2.39 (NCH_3), 3.47 (OCH_3), and 5.03 d ($J_{1',2'}$ 3.7 Hz, 1′-H); m.s. $(M+H)^+$, calc. for $C_{15}H_{34}N_5O_4$: *m/z* 348.2611, meas. 348.2597; cyclitol, calc. for $C_8H_{20}N_2O_3$: *m/z* 206.1505, meas. 206.1522; diamino sugar, calc. for $C_7H_{15}N_2O$: *m/z* 143.1184, meas. 143.1181.

2-O-(Methylsulfonyl)fortimicin A (**1d**). — A sample (1.01 g) of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-*O*-(methylsulfonyl)fortimicin A (**9a**) was catalytically hydrogenated to give 0.623 g of **1d** as the tetrahydrochloride salt*. The latter was converted into the disulfate salt with AG1-X2 (SO_4^{2-}) resin; $[\alpha]_D^{22}$ +80° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O): δ 1.32 d ($J_{6',7'}$ 7.0 Hz, 6′-CH_3), 3.13 (NCH_3), 3.35 (OSO_2CH_3), 3.59 (OCH_3), and 5.34 d ($J_{1',2'}$ 3.5 Hz, 1′-H).

Anal. Calc. for $C_{18}H_{41}N_5O_{15}S_3 \cdot 2H_2O$: C, 30.28; H, 6.33; N, 9.79. Found: C, 30.08; H, 6.13; N, 9.64.

ACKNOWLEDGMENTS

The authors thank Ms. S. Mueller for mass spectra, Mr. W. H. Washburn for i.r. spectra, Dr. R. Girolami for antibacterial assays, Ms. Julie Hood for microanalysis, and Mr. D. A. Dunnigan and Mr. G. Nemeth for catalytic hydrogenations.

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