DIASTEREOMERIC FORTIMICIN 1,2-EPOXIDES

THE PREPARATION OF THE 1-DEAMINO-2-DEOXYFORTIMICINS A AND B AND THE 1,2-DI-*EPI*-FORTIMICINS A AND B

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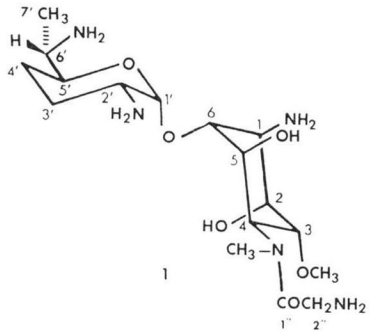
The preparation of 1,2-anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**) and its conversion to the two diastereomeric 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-1,2-epoxyfortimicin B-4,5-carbamates **7** and **13** are described. The olefin **4** was used for preparation of 1-deamino-2-deoxyfortimicin A (**6d**) while the β-epoxide **13** was used for the preparation of 1,2-di-*epi*-fortimicin A (**17b**) and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**). The *in vitro* antibacterial activities of **6d**, **17b** and **19c** are reported.

The fortimicins comprise a group of pseudodisaccharides having 1,4-diaminocyclitol moieties.[1] Of the large group of naturally occurring fortimicins,[2,3,4] fortimicin A (**1**), the first member isolated, has high, broad spectrum antibacterial activity[5,6] while the 4-*N*-deglycyl derivative, fortimicin B (**2**) has only weak antibacterial activity. Fortimicin A is of particular note because of its apparent lack of ototoxicity.[7] We are systematically modifying the fortimicins in an effort to further improve their therapeutic effectiveness. We have previously reported the preparation of several cyclitol modified fortimicins, *e.g.*, 2-*epi*-fortimicins,[8] 2-deoxyfortimicins[9] and 3-*O*-demethylfortimicins.[10] We now report the preparation of some fortimicin derivatives modified at C-1 and C-2.

2′,6′-Di-*N*-benzyloxycarbonyl-1,2(*R*)-epiminofortimicin B (**3**) was prepared from fortimicin B (**2**) as previously described.[11] Treatment of **3** with sodium nitrite in aqueous acetic acid gave 1,2-anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**) in quantitative yield. Catalytic hydrogenation of **4** in the presence of 5% Pd/C afforded **5** which on alkaline hydrolysis of the carbamate ring gave 1-deamino-2-deoxyfortimicin B (**6a**).

Treatment of the olefin **4** with *m*-chloroperbenzoic acid gave almost exclusively the α-epoxide **7**. The latter **7** was converted to a 1 : 1 mixture of the isomeric hydroxyazides **8** and **9** with sodium azide.[13] The azides **8** and **9** were readily separated by silica gel column chromatography. Catalytic hydrogenation of **8** and **9** followed by removal of the carbamate group afforded the isomeric aminoalcohols **2** and **10** respectively. The identity of one of the products as fortimicin B (**2**)[11] established the stereochemistry of the epoxide ring of **7** and the structure of the isomer as 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B (**10**).

The CMR spectrum (Table 1) of **10** was consistent with the assigned structure and titration experiments revealed the three β-shifts necessary to support the 1,3-diaminocyclitol structure. The 220 MHz PMR spectrum of **10** shows, in addition to the resonances associated with the 6-*epi*-purpurosamine sugar, six separate multiplets from the cyclitol ring protons (Table 2). Spin decoupling experiments, performed at 100 MHz, determined coupling patterns and coupling constants. The magnitude of the coupling



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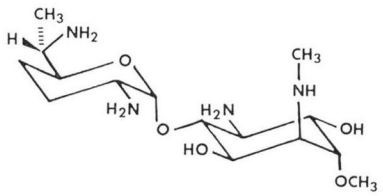

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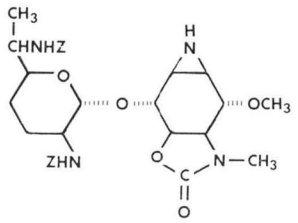

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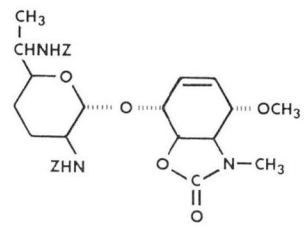

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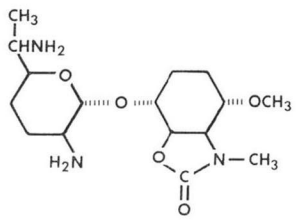

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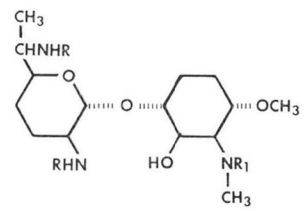


	R	R_1
6a	H	H
6b	Z	H
6c	Z	$COCH_2NHZ$
6d	H	$COCH_2NH_2$

constants indicated that protons H-1, H-2, H-3 and H-4 are axial and H-5 and H-6 are equatorial. If the cyclitol ring of **10** existed in the fortimicin B (**2**) conformation[1] there should be four equatorial and two axial protons. Thus, to account for the observed coupling constants the cyclitol ring of **10** must exist in the flipped conformation determined for fortimicin A (**1**).[1]

Treatment of the olefin **4** with *N*-bromosuccinimide gave a mixture of bromohydrins **11** and **12** which was converted to the β-epoxide **13** with 1,5-diazabicyclo[5.4.0]undec-5-ene in refluxing benzene. Azidolysis of **13** with sodium azide in dimethylformamide in the presence of boric acid[13] afforded a 1:1 mixture of the isomeric hydroxyl azides **14** and **15**. The mixture was catalytically hydrogenated and subsequently hydrolyzed to give a mixture of the isomeric fortimicins **16a** and **18** which were separated by ion exchange chromatography.

As two azido compounds were produced from **13** on azidolysis the structures of the two hydroxyl azides may be formulated as **14** and **15**. The CMR spectral data of the reduced and deblocked products **16a** and **18** compared with fortimicin B (**2**) is shown in Table 1. Protonation of the amine groups of **18** produced β-shifts in three cyclitol ring carbons and hence consistent with a 1,3-diamino structure. These

Table 1. 100 MHz CMR parameters for fortimicin B (**2**), 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B (**10**), 1,2-di-*epi*-fortimicin B (**16a**) and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**) free bases in D_2O solution.

Carbon number	**2**		**10**		**16a**		**18**	
	pD 10.7 (ppm)	β-Shift	pD 12.2 (ppm)	β-Shift	pD 10.5 (ppm)	β-Shift	pD 10.4 (ppm)	β-Shift
1	53.8		71.0	4.2	52.7		71.4	2.5
2	71.1	5.7	54.6		75.6	4.4	52.7	
3	79.9	5.8	83.4	6.3	86.6	3.6	81.5	
4	60.8		60.2		60.6		60.9	
5	71.1	4.6	64.7	0.8	64.4		73.2	3.8
6	84.1	9.9	75.6		76.5	5.3	80.0	7.0
NCH_3	35.4	3.1	33.3		33.3		35.2	
OCH_3	59.2		60.2		60.3		59.2	
1′	102.5	6.5	97.6	2.9	97.6	6.1	100.8	
2′	50.6		50.3		50.9		51.0	
3′	27.0	5.5	27.0	5.2	26.7	5.3	26.6	4.7
4′	27.1		27.2		27.3		27.1	
5′	75.1	4.1	74.4	4.2	74.0	3.3	73.7	3.5
6′	50.4		50.3		50.4		50.4	
7′	18.5	3.4	18.7	3.6	19.7	2.9	17.6	2.5

observations suggest that **16a** must have a 1,4-diaminocyclitol. Consistent with this proposal are the four β-shifts seen on protonation.

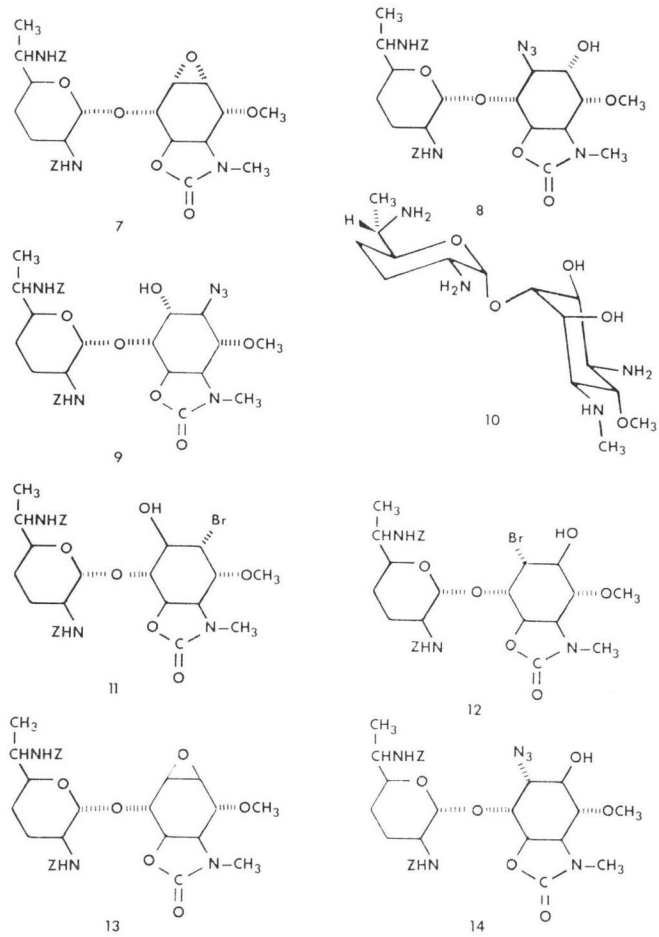
The cyclitol ring conformations of **16a** and **18** were assigned on the basis of PMR studies (Table 2). In both compounds the 220 MHz PMR spectra allowed measurement of all cyclitol ring protons and spin decoupling experiments at 100 MHz gave coupling patterns. Spin decoupling experiments revealed that three axial-axial couplings were exhibited by the cyclitol ring protons of **16a**. If the cyclitol ring of **16a** existed in the fortimicin B conformation only one axial-axial relationship (H-5 and H-6) would be evident. The proposed inverted conformation shown for **16a**, previously seen in fortimicin A (**1**), would require the requisite number of large couplings.

The proton coupling constants obtained for 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**) shows that the cyclitol ring has the same conformation as fortimicin B (**2**). Spin decoupling experiments determined the coupling constants of **18** (Table 2) and revealed that H-1, H-2, H-5 and H-6 are axial and H-3 and H-4 are equatorial. These data are consistent with the cyclitol conformation shown for **18**.

As introduction of a 4-*N*-glycyl group greatly enhanced the antibiotic activity of fortimicin B (**2**),[1] we found it desirable to prepare the 4-*N*-glycyl derivatives of **6a**, **10**, **16a** and **18**. Treatment of **6a**, **16a** and **18** with *N*-(benzyloxycarbonyloxy)succinimide under conditions previously described[12] gave respectively the *N*-benzyloxycarbonyl derivatives **6b**, **16b** and **19a**. Treatment of **10** with *N*-(benzyloxycarbonyloxy)succinimide under identical conditions gave a complex mixture of benzyloxycarbonyl derivatives which was not pursued further. Glycylation of **6b**, **16b** and **19a** with *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide led respectively to **6c**, **17a** and **19b** which on hydrogenolysis over Pd/C gave 1-deamino-2-deoxyfortimicin A (**6d**), 1,2-di-*epi*-fortimicin A (**17b**), and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**) respectively.

Table 2. 100 MHz PMR parameters for fortimicin B (**2**), 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B (**10**), 1,2-di-*epi*-fortimicin B (**16a**) and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**) free bases in D_2O solution.

2				**10**				**16a**				**18**			
Chemical shift (ppm)		Coupling constant (Hz)		Chemical shift (ppm)		Coupling constant (Hz)		Chemical shift (ppm)		Coupling constant (Hz)		Chemical shift (ppm)		Coupling constant (Hz)	
H-1′	5.50	$J_{1',2'}$	3.8	H-1′	5.31	$J_{1',2'}$	3.3	H-1′	5.37	$J_{1',2'}$	3.3	H-1′	5.55	$J_{1',2'}$	3.5
H-2′	~3.4	$J_{5',6'}$	6.8	H-2′	3.22	$J_{5',6'}$	6.6	H-2′	~3.4	J_{6',CH_3}	6.7	H-2′	3.34	J_{6',CH_3}	6.6
CH_2-3′,4′	1.8~2.7	$J_{1,2}$	9.5	CH_2-3′,4′	1.4~2.4	$J_{1,2}$	10.0	CH_2-3′,4′	1.5~2.5	$J_{1,2}$	10.3	CH_2-3′,4′	1.7~2.5	$J_{1,2}$	9.7
H-6′	3.27	$J_{2,3}$	~3.0	H-6′	3.22	$J_{2,3}$	10.0	H-6′	3.4	$J_{2,3}$	~9.2	H-6′	~3.3	$J_{2,3}$	3.5
CH_3-6′	1.49	$J_{3,4}$	3.0	CH_3-6′	1.47	$J_{3,4}$	10.0	CH_3-6′	1.55	$J_{3,4}$	10.5	CH_3-6′	1.50	$J_{3,4}$	~3.5
H-1	3.43	$J_{4,5}$	4.5	H-1	4.12	$J_{4,5}$	3.0	H-1	3.41	$J_{4,5}$	3.0	H-1	3.88	$J_{4,5}$	4.5
H-2	4.17	$J_{5,6}$	9.5	H-2	3.57	$J_{5,6}$	3.9	H-2	4.03	$J_{5,6}$	3.7	H-2	3.34	$J_{5,6}$	9.3
H-3	4.11	$J_{6,1}$	9.5	H-3	3.57	$J_{6,1}$	3.5	H-3	3.70	$J_{6,1}$	3.0	H-3	4.12	$J_{6,1}$	9.3
H-4	3.54			H-4	3.10			H-4	3.10			H-4	3.53		
H-5	4.44			H-5	4.73			H-5	4.79			H-5	4.44		
H-6	3.93			H-6	4.45			H-6	4.46			H-6	4.08		
OCH_3	3.92			OCH_3	3.98			OCH_3	4.05			OCH_3	3.93		
NCH_3	2.85			NCH_3	2.80			NCH_3	2.85			NCH_3	2.86		

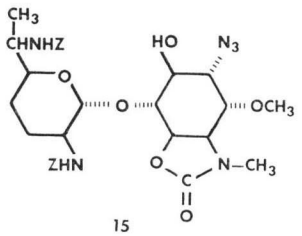


The *in vitro* antibacterial activities of 1-deamino-2-deoxyfortimicin A (**6d**), 1,2-di-*epi*-fortimicin A (**17b**), and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**) are shown in Table 3. 1,2-Di-*epi*-fortimicin A (**17b**) was approximately half as active as fortimicin A (**1**). 1-Deamino-2-deoxyfortimicin A (**6d**) and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**) were inactive at the levels tested.

Experimental

General Methods

Optical rotations were determined with a Perkin-Elmer Model 241 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 or at 220 MHz with a Varian Associates HR-200 spectrometer. Chemical shifts are reported in ppm downfield from external TMS contained in a co-axial capillary in the sample tube. CMR spectra were measured on a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. Mass spectra were determined on an A.E.I. MS-902 spectrometer at 70 eV and 100~150°C using the direct probe insert. Silica gel for chromatography refers to that of Merck (Darmstadt) 70~230 mesh. All evaporations were carried out under diminished pressure. Microanalytical results are reported for those compounds which could be freed of solvent.



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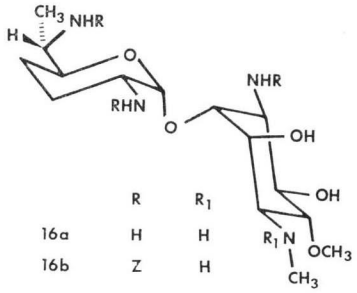
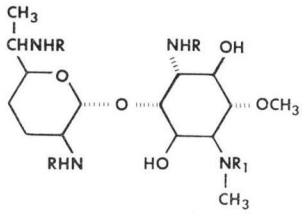
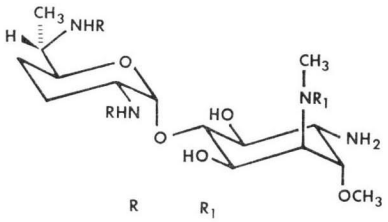


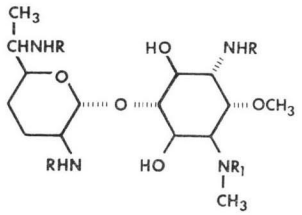
	R	R_1
16a	H	H
16b	Z	H



	R	R_1
17a	Z	$COCH_2NHZ$
17b	H	$COCH_2NH_2$



	R	R_1
18	H	H



	R	R_1
19a	Z	H
19b	H	$COCH_2NHZ$
19c	H	$COCH_2NH_2$

1,2-Anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**)

A stirring solution of 10.5 g of 2′,6′-di-*N*-benzyloxycarbonyl-2-deoxy-1,2(*R*)-epiminofortimicin B-4,5-carbamate (**3**), prepared as previously described,[11] and 487 ml of glacial acetic acid was treated dropwise with 6.75 g of sodium nitrite in 445 ml of water. The reaction mixture was stirred for 0.5 hour and then adjusted to pH 9.0 with 4 N sodium hydroxide. The product was isolated by chloroform extraction to give 9.58 g of 1,2-anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**): $[\alpha]_D^{36}+32°$ (*c* 0.71, $CHCl_3$); PMR ($CDCl_3$) δ 1.16 (d, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 2.94 (s, NCH_3), 3.43 (s, OCH_3), 7.35 (m, Cbz-aromatic).

Anal. Calcd. for $C_{32}H_{39}N_3O_9$: C 63.04, H 6.45, N 6.89.
Found: C 63.15, H 6.53, N 6.74.

1-Deamino-2-deoxyfortimicin B-4,5-carbamate (**5**)

A solution of 1.142 g of 2′,6′-di-*N*-benzyloxycarbonyl-1,2-anhydro-1-deaminofortimicin B-4,5-carbamate (**4**) and 70 ml of 0.2 N hydrochloric acid in methanol was hydrogenated in the presence of 1.2 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The catalyst was collected on a filter and the filtrate was taken to dryness. Excess hydrochloric acid was removed by repeated co-distillation with methanol to give 0.765 g of 1-deamino-2-deoxyfortimicin B-4,5-carbamate (**5**) isolated as the trihydrochloride: IR (KBr) 1729 cm^{-1}; PMR (D_2O) δ 1.77 (d, $C_{6'}$-CH_3, $J_{6',7'}=7.0$ Hz), 3.31 (s, NCH_3), 3.87 (s, OCH_3), 5.78 (d, $H_{1'}$, $J_{1',2'}=4.3$ Hz).

Table 3. *In vitro* antibacterial activity of 1-deamino-2-deoxyfortimicin A (**6d**), 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**), and 1,2-di-*epi*-fortimicin A (**17b**) compared with fortimicin A (**1**)†.

Organism	Minimum inhibitory concentration (mcg/ml)*			
	1	**6d**	**17b**	**19c**
Staphylococcus aureus Smith	1.56	>100	1.56	>100
Streptococcus faecalis 10541	50	>100	>100	>100
Enterobacter aerogenes 13048	6.2	>100	12.5	>100
Escherichia coli Juhl	6.2	>100	12.5	>100
Escherichia coli BL-3676	25	>100	50	>100
Klebsiella pneumoniae 10031	3.1	>100	12.5	>100
Providencia sp. 1577	1.56	>100	6.2	>100
Pseudomonas aeruginosa KY-8512	25	>100	25	>100
Pseudomonas aeruginosa BMH #10	0.78	>100	1.56	>100
Pseudomonas aeruginosa 209	>100	>100	>100	>100
Salmonella typhimurium Ed #9	6.2	>100	6.2	>100
Serratia marcescens 4003	3.1	>100	3.1	>100
Shigella sonnei 9290	6.2	>100	12.5	>100
Proteus rettgeri U6333	12.5	>100	25	>100
Proteus vulgaris JJ	6.2	>100	6.2	>100

† Compounds were tested as hydrochlorides on a weight basis.
* Antibiotic activity was determined by serial two-fold dilution in MUELLER-HINTON agar.

1-Deamino-2-deoxyfortimicin B (**6a**)

A stirring suspension of 0.502 g of 1-deamino-2-deoxyfortimicin B-4,5-carbamate (**5**), 160 ml of water and 0.882 g of barium hydroxide octahydrate was heated at 70°C for 96 hours. The reaction mixture was filtered and carbon dioxide was passed through the filtrate. Precipitated barium carbonate was removed by centrifugation to give a solid which was chromatographed on a column of silica gel using the lower phase of a mixture of chloroform - methanol - concentrated ammonium hydroxide (1 : 1 : 1). Appropriate fractions were taken to dryness to give 0.290 g of 1-deamino-2-deoxyfortimicin B (**6a**): PMR (D_2O) δ 1.46 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.78 (s, NCH_3), 3.82 (s, OCH_3), 5.31 (d, $H_{1'}$, $J_{1',2'}$= 4.0 Hz); mass spectrum, *m/z* 317.2286 (M^+), calcd. for $C_{15}H_{31}N_3O_4$ 317.2314.

2′,6′-Di-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin B (**6b**)

To a stirring, ice-bath cooled solution of 0.290 g of 1-deamino-2-deoxyfortimicin B (**6a**), 4.5 ml of water and 9.0 ml of methanol was added 0.468 g of *N*-(benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 3 hours and then at room temperature for 22 hours. Chloroform extraction gave 0.433 g of solid which was chromatographed on a column of silica gel using chloroform - methanol - concentrated ammonium hydroxide (23.4: 1.4: 0.1). Concentration of appropriate fractions gave 0.173 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin B (**6b**): PMR ($CDCl_3$) δ 1.17 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 2.29 (s, NCH_3), 3.34 (s, NCH_3), 4.88 (d, $H_{1'}$, $J_{1',2'}$=4.0 Hz), 7.38 (m, Cbz-aromatic).

2′,6′,2″-Tri-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin A (**6c**)

A solution of 0.173 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin B (**6b**) and 3.0 ml of tetrahydrofuran was treated with 0.095 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide. Stirring was continued for 18 hours at room temperature. Evaporation gave 0.391 g of solid which was chromatographed on a column of silica gel using benzene - methanol - 95% ethanol - concentrated ammonium hydroxide (23.5: 1.5: 1.9: 0.2) to give 0.227 g of 2′,6′,2″-tri-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin A (**6c**): PMR ($CDCl_3$) δ 1.17 (d, $C_{6'}$–CH_3, $J_{6',7'}$=6.5 Hz, rotamer present), 2.89 (s, NCH_3, rotamer present), 3.28 (s, OCH_3), 4.82 (d, $H_{1'}$, $J_{1',2'}$=3.5 Hz), 7.32 (m, Cbz-aromatic).

1-Deamino-2-deoxyfortimicin A (**6d**)

A solution of 0.253 g of 2′,6′,2″-tri-*N*-benzyloxycarbonyl-1-deamino-2-deoxyfortimicin A (**6c**), 30 ml of methanol and 20 ml of 0.2 N hydrochloric acid in methanol was hydrogenated over 0.250 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. Work up as before gave 0.131 g of 1-deamino-2-deoxyfortimicin A (**6d**) isolated as the trihydrochloride: PMR (D_2O) δ 1.76 (d, $C_{6'}$-CH_3, $J_{6',7'}$= 7.0 Hz), 3.5 (s, NCH_3), 3.48 (s, OCH_3), 5.69 (d, $H_{1'}$, $J_{1',2'}$=3.5 Hz); mass spectrum, *m/z* 374.2539 (M^+), calcd. for $C_{17}H_{34}N_4O_5$ 374.2529.

2′,6′-Di-*N*-benzyloxycarbonyl-1-deamino-1,2(*S*)-epoxyfortimicin B-4,5-carbamate (**7**)

A stirring solution of 0.288 g of 1,2-anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**) in 6 ml of methylene chloride was treated dropwise by the addition of 0.490 g of *m*-chloroperoxybenzoic acid in 8 ml of methylene chloride. The reaction was stirred at room temperature for 20 hours. After the addition of 5% aqueous sodium bicarbonate, chloroform extraction gave 0.531 g of residue. The residue was chromatographed on a column of silica gel with ethyl acetate - dichloroethane (9: 1) to give 0.143 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-1,2(*S*)-epoxyfortimicin B-4,5-carbamate (**7**): $[\alpha]_D^{36}$+6° (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 1752, 1713 and 1503 cm^{-1}; PMR ($CDCl_3$) δ 1.17 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 2.88 (s, NCH_3), 3.46 (s, OCH_3), 7.35 (m, Cbz-aromatic).

2′,6′-Di-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-azido-1-*epi*-hydroxyfortimicin B-4,5-carbamate (**9**) and 1-Azido-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**8**)

A solution prepared from 1.40 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-1,2(*S*)-epoxyfortimicin B-4,5-carbamate (**7**), 1.397 g of boric acid, 1.397 g of sodium azide and 53 ml of dimethylformamide was refluxed for 2.5 hours. The reaction mixture was diluted with 5% aqueous sodium bicarbonate and extracted with chloroform. The chloroform extract was washed with water and evaporated. Residual dimethylformamide was removed by repeated codistillation with toluene to leave a residue which was chromatographed on a column of silica gel with ethyl acetate - hexane (9: 1). Initial fractions gave 0.663 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-azido-1-*epi*-hydroxyfortimicin B-4,5-carbamate (**9**): $[\alpha]_D^{30}$+75° (*c* 1.0, CH_3OH); IR ($CDCl_3$) 3438, 3345, 2940, 2110, 1755, 1712 and 1508 cm^{-1}; PMR ($CDCl_3$) δ 1.16 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 7.33 (m, Cbz-aromatic).

Anal. Calcd. for $C_{32}H_{40}N_6O_{10}$: C 57.48, H 6.03, N 12.57.
Found: C 57.56, H 6.30, N 12.23.

Further elution gave 0.530 g of 1-azido-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**8**): $[\alpha]_D^{36}$+11° (*c*, 1.0, CH_3OH); IR ($CDCl_3$) 3555, 3435, 2435, 2112, 1762, 1710 and 1503 cm^{-1}; PMR ($CDCl_3$) δ 1.19 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.0 Hz), 2.79 (s, NCH_3), 3.47 (s, OCH_3), 5.26 (d, $H_{1'}$, $J_{1',2'}$=4.0 Hz), 7.33 (m, Cbz-aromatic).

Anal. Calcd. for $C_{32}H_{40}N_6O_{10}$: C 57.48, H 6.03, N 12.57.
Found: C 57.78, H 6.45, N 12.69.

1-Deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B (**10**)

A solution prepared from 0.216 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-2-*epi*-azido-1-*epi*-hydroxyfortimicin B-4,5-carbamate (**9**) and 20 ml of 0.2 N hydrochloric acid in methanol was hydrogenated over 0.22 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The usual work up gave 0.169 g of 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B-4,5-carbamate isolated as the trihydrochloride: IR (KBr) 3412, 2930, 1732, 1600 and 1493 cm^{-1}; PMR (D_2O) δ 1.79 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 3.48 (s, NCH_3), 4.05 (s, OCH_3), 5.82 (d, $H_{1'}$, $J_{1',2'}$=4.0 Hz); mass spectrum, *m/z* 374.2182 (M^+), calcd. for $C_{16}H_{30}N_4O_6$ 374.2165.

A stirring mixture prepared from 0.823 g of 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B-4,5-carbamate trihydrochloride, 3.62 g of barium hydroxide and 19.6 ml of water was heated at 70°C for 20 hours. Work up as before gave 0.080 g of 1-deamino-2-deoxy-2-*epi*-amino-1-*epi*-hydroxyfortimicin B (**10**): $[\alpha]_D^{36}$+63° (*c* 1.0, CH_3OH); IR (KBr) 1587 and 1440 cm^{-1}; PMR (See Table 2); CMR (See Table 1); mass spectrum, *m/z* 349.2428 $(M+H)^+$, calcd. for $C_{15}H_{33}N_4O_5$ 394.2451.

Fortimicin B (**2**)

A solution of 0.401 g of 1-azido-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate

(**8**) and 36 ml of 0.2 N hydrochloric acid in methanol was hydrogenated over 0.40 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The usual work up gave 0.286 g of fortimicin B-4,5-carbamate isolated as the trihydrochloride: IR ($CDCl_3$) 1742, 1600 and 1495 cm^{-1}; PMR ($CDCl_3$) δ 1.84 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 3.38 (s, NCH_3), 4.04 (s, OCH_3); mass spectrum, m/z 374.2189, (M^+), calcd. for $C_{16}H_{30}N_4O_6$ 374.2165.

A stirring mixture prepared from 0.148 g of fortimicin B-4,5-carbamate trihydrochloride, 4.36 g of barium hydroxide and 23.6 ml of water was heated at 70°C for 20 hours. The usual work up gave 0.132 g of fortimicin B (**2**) identical in all respects with an authentic sample.

2′,6′-Di-*N*-benzyloxycarbonyl-1-deamino-1,2(*R*)-epoxyfortimicin B-4,5-carbamate (**13**)

A stirring solution of 9.783g of 1,2-anhydro-2′,6′-di-*N*-benzyloxycarbonyl-1-deaminofortimicin B-4,5-carbamate (**4**), 118ml of peroxide free dioxane and 39.4ml of perchloric acid, prepared by adding 3.5ml of 60% perchloric acid to 46 ml of water, was treated with 4.2 g of freshly recrystallized bromoacetamide. After stirring for 3 hours the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed successively with aqueous solutions of 5% sodium iodide, 5% sodium thiosulfate and 5% sodium carbonate. After washing with water evaporation of the chloroform gave 9.76 g of a crude inseparable mixture of 2′,6′-di-*N*-benzyloxycarbonyl-1-bromo-1-deaminofortimicin B-4,5-carbamate (**11**) and 2′,6′-di-*N*-benzyloxycarbonyl-2-bromo-1-deamino-2-*epi*-deoxy-1-*epi*-hydroxyfortimicin B-4,5-carbamate (**12**).

A solution prepared from 14.76 g of the crude mixture of **11** and **12**, 295 ml of benzene and 21 ml of 1,5-diazabicyclo[5.4.0]undec-7-ene was stirred at room temperature for 2 hours. After the addition of 400 ml of water and 400 ml of benzene the reaction was stirred for 0.5 hour. The product was isolated by benzene extraction. The benzene extract was washed with 5% aqueous sodium bicarbonate and then with water. Evaporation of the benzene gave a solid (10.51 g) which was chromatographed on a column of silica gel using ethyl acetate - hexane (3: 1). Fractions containing only the major component were evaporated to give 3.42 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-1,2(*R*)-epoxyfortimicin B-4,5-carbamate (**13**): $[\alpha]_D^{36}+45°$ (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 1725, 1711 and 1506 cm^{-1}; PMR ($CDCl_3$) δ 1.22 (d, $C_{6'}$-CH_3, $J_{6',7'}$=6.5 Hz), 2.86 (s, NCH_3), 3.45 (s, OCH_3), 7.34 (m, Cbz-aromatic).

Anal. Calcd. for $C_{32}H_{39}N_3O_{10}$: C 61.43, H 6.28, O 6.72.
Found: C 59.19, H 6.36, O 6.35.

1,2-Di-*epi*-fortimicin B (**16a**) and 2-Amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**)

A solution prepared from 5.574 g of 2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-1,2(*R*)-epoxyfortimicin B-4,5-carbamate (**13**), 100 ml of dry dimethylformamide, 5.576 g of sodium azide and 5.576 g of boric acid was refluxed for 2.5 hours. The reaction mixture was diluted with 5% aqueous sodium bicarbonate and extracted with chloroform. The chloroform extract was washed with water and evaporated. Residual dimethylformamide was removed by repeated codistillation with toluene to give 5.82 g of solid. The solid was chromatographed on a column of silica gel with ethyl acetate - hexane (3: 1) to yield 4.825g of a mixture of 1-azido-2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-1,2-di-*epi*-fortimicin B-4,5-carbamate (**14**) and 2-azido-2′,6′-di-*N*-benzyloxycarbonyl-1-deamino-2-deoxy-1-hydroxyfortimicin B-4,5-carbamate (**15**): IR ($CDCl_3$) 2110, 1755, 1710 and 1503 cm^{-1}.

A solution prepared from 2.31 g of the mixture of **14** and **15** and 250 ml of 0.2 N hydrochloric acid in methanol was hydrogenated over 2.31 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The usual work up gave 1.635 g of a mixture of 1,2-di-*epi*-fortimicin B-4,5-carbamate and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B-4,5-carbamate isolated as the perhydrochloride: IR (KBr) 1736, 1605 and 1497 cm^{-1}.

A stirring suspension prepared from 1.635 g of the mixture prepared above, 265 ml of water and 49.3 g of barium hydroxide was heated at 70°C for 18 hours. The usual work up gave 1.248 g of a mixture of 1,2-di-*epi*-fortimicin B (**16a**) and 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**).

A sample (2.437 g) of the mixture of **16a** and **18** was chromatographed on a column of cation exchange resin (Bio-Rex 70, 100~200 mesh, NH_4^+ form) using a gradient of water to 1 N ammonium hydroxide. Fractions containing only the first component eluted gave a solid. To decompose carbonates the solid was treated with 0.2 N hydrochloric acid in methanol to give 0.555 g of 1,2-di-*epi*-

fortimicin B tetrahydrochloride. The free base was prepared by passing a water solution of the salt through an anion exchange column (AG2-X8, 100~200 mesh, OH^- form) to give 1,2-di-*epi*-fortimicin B (**16a**): IR (KBr) 1578 and 1443 cm^{-1}; PMR (See Table 2); CMR (See Table 1); mass spectrum *m/z* 349.2428 $(M+H)^+$, calcd. for $C_{15}H_{33}N_4O_5$ 349.2451.

Continued elution gives fractions which were taken to dryness to give a solid which was treated as above to decompose carbonates to yield 0.555 g of 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B hydrochloride. Treatment as above with AG2-X8 gave 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**): IR (KBr) 1585 and 1445 cm^{-1}; PMR (See Table 2); CMR (See Table 1); mass spectrum, *m/z* 349.2428 $(M+H)^+$, calcd. for $C_{15}H_{33}N_4O_5$ 349.2451.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin B (**16b**)

A stirring, ice-bath cooled solution of 0.252 g of 1,2-di-*epi*-fortimicin B (**16a**), 3.8 ml of water, and 7.6 ml of methanol was treated with 0.552 g of *N*-(benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 3 hours and then at room temperature for 22 hours. The product (0.385 g), isolated by chloroform extraction, was chromatographed on a column of silica gel using dichloroethane - 95% ethanol - concentrated ammonium hydroxide (18: 2: 0.04). Fractions containing the slowest moving component gave 0.155 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin B (**16b**): IR ($CDCl_3$) 1705 and 1505 cm^{-1}; PMR ($CDCl_3$) δ 2.42 (s, NCH_3), 3.58 (s, OCH_3), 7.32 (m, Cbz-aromatic).

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin A (**17a**)

A stirring solution prepared from 0.155 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin B (**16b**) in 2.7 ml of tetrahydrofuran was treated with 0.067 g of *N*-(*N*-benzyloxycarbonylglycyloxy)-succinimide. Stirring was continued for 17 hours at room temperature. Evaporation of the tetrahydrofuran gave a residue which was chromatographed on a column of silica gel using dichloroethane - 95% ethanol - concentrated ammonium hydroxide (18: 6: 0.04) to give 0.098 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin A (**17a**): $[\alpha]_D^{23}+41°$ (*c* 0.5, $CHCl_3$); IR ($CDCl_3$) 1697, 1637 and 1496 cm^{-1}; PMR ($CDCl_3$) δ 1.02 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.5$ Hz), 2.88 (s, NCH_3), 3.46 (s, OCH_3), 7.31 (m, Cbz-aromatic).

Anal. Calcd. for $C_{49}H_{53}N_5O_{14}$: C 62.48, H 6.31, N 7.43.
Found: C 62.95, H 6.83, N 7.00.

1,2-Di-*epi*-fortimicin A (**17b**)

A solution of 0.098 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-1,2-di-*epi*-fortimicin A (**17a**) and 9 ml of 0.2 N hydrochloric acid in methanol was hydrogenated over 0.10 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The usual work up gave 0.057 g of 1,2-di-*epi*-fortimicin A (**17b**) isolated as the tetrahydrochloride: IR (KBr) 3410, 2930, 1640 and 1490 cm^{-1}; PMR (D_2O) δ 1.47 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.5$ Hz), 3.51 (s, NCH_3), 3.83 (s, OCH_3), 5.31 (d, $H_{1'}$, $J_{1',2'}=3.0$ Hz); mass spectrum, *m/z* 405.2584 (M^+), calcd. for $C_{17}H_{35}N_5O_6$ 405.2587.

2,2′,6′-Tri-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**19a**)

A stirring, ice-bath cooled solution prepared from 0.358 g of 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**18**), 5.4 ml of water and 10.8 ml of methanol was treated with 0.784 g of *N*-(benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 3 hours and then at room temperature for 20 hours. Chloroform extraction gave 0.578 g of residue which was chromatographed on a column of silica gel using dichloroethane - 95% ethanol-concentrated ammonium hydroxide (18: 6: 0.04). Fractions containing the major product were taken to dryness and rechromatographed on a column of Sephadex LH-20 using 95% ethanol to give 0.414 g of 2,2′,6′-tri-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**19a**): $[\alpha]_D^{26}+3°$ (*c* 1.0, CH_3OH); IR ($CDCl_3$) 3439, 1702 and 1502 cm^{-1}; PMR ($CDCl_3$) δ 1.14 (d, $C_{6'}$-CH_3, $J_{6',7'}=6.5$ Hz), 2.39 (s, NCH_3), 3.34 (s, OCH_3), 4.94 (d, $H_{1'}$, $J_{1',2'}=3.7$ Hz).

Anal. Calcd. for $C_{39}H_{50}N_4O_7$: C 62.39, H 6.71, N 7.46.
Found: C 62.14, H 6.88, N 7.41.

2,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19b**)

A solution prepared from 0.177 g of 2,2′,6′-tri-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin B (**19a**), 3.7 ml of tetrahydrofuran and 0.075 g of *N*-(*N*-benzyloxycarbonylglycyl-

oxy)succinimide was stirred at room temperature for 21 hours. The tetrahydrofuran was evaporated and the residue was chromatographed on a column of silica gel using methylenechloride - methanol - concentrated ammonium hydroxide (96: 3.5: 0.05) to give 0.087 g of 2,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19b**): IR ($CDCl_3$) 1707, 1636 and 1505 cm^{-1}; PMR ($CDCl_3$) δ 1.04 (unresolved doublet, $C_{6'}$-CH_3), 2.92 (s, NCH_3), 3.34 (s, OCH_3), 7.28 (m, Cbz-aromatic).

Anal. Calcd. for $C_{49}H_{59}N_5O_{14}$: C 62.49, H 6.31, N 7.43.
Found: C 62.12, H 6.57, N 7.33.

2-Amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**)

A solution prepared from 0.087 g of 2,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19b**) and 7.5 ml of 0.2 N hydrochloric acid in methanol was hydrogenated in the presence of 0.086 g of 5% Pd/C for 4 hours under 3 atmospheres of hydrogen. The usual work up gave 0.063 g of 2-amino-1-deamino-2-deoxy-1-hydroxyfortimicin A (**19c**) isolated as the tetrahydrochloride: IR (KBr) 1645, 1590 and 1490 cm^{-1}; PMR (D_2O) δ 1.79 (d, $C_{6'}$-CH_3, $J_{6',7'}$=7.0 Hz), 3.59 (s, NCH_3), 3.87 (s, OCH_3), 5.87 (d, $H_{1'}$, $J_{1',2'}$=3.5 Hz); mass spectrum, *m/z* 405.2576 (M^+), calcd. for $C_{17}H_{35}N_5O_6$ 405.2587.

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