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Synthesis of 3-*O*-Demethylfortimicins

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Treatment of fortimicin B with lithium in ethylamine gave 3-*O*-demethylfortimicin B. The latter was converted by methodology developed with fortimicin B to 3-*O*-demethylfortimicin A, 4-*N*-sarcosyl-3-*O*-demethylfortimicin B, 4-*N*-β-alanyl-3-*O*-demethylfortimicin B, and 4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B. 3-*O*-demethylfortimicin A and the 4-*N*-acyl-3-*O*-demethylfortimicins B had appreciably higher antibacterial activities than the correponding parent fortimicins. Most significant was the increased activity of 3-*O*-demethylfortimicin A relative to fortimicin A against a variety of strains of *Pseudomonas aeruginosa*.

The fortimicins, produced by fermentation of *Micromonospora olivoasterospora* (7), are novel pseudodisaccharide antibiotics with unusual 1,4-diaminocyclitol moieties (1). Fortimicin A (Fig. 1, compound 1), the major component of the fortimicin complex, has high, broad-spectrum antibacterial activity (2, 4). In addition, fortimicin A has remarkable activity against a wide variety of microorganisms resistant to many of the pseudotrisaccharide aminoglycoside antibiotics (4).

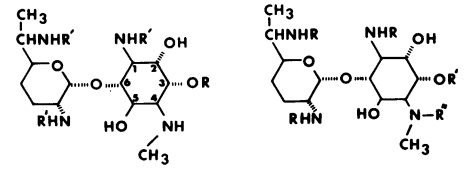
We are systematically modifying the fortimicins in an attempt to obtain derivatives with superior antimicrobial and pharmacological properties and to develop structure-activity and structure-toxicity relationships for this novel series of antibiotics. In a previous paper (9) we described the chemistry and biology of a number of fortimicin B (Fig. 1, compound 2) derivatives substituted at the C-4-methylamino function. Our interest in the preparation of 3-*O*-demethylfortimicins was stimulated in part by earlier work on several aminoglycoside antibiotics, in which the presence of an *O*-methyl group diminished antibacterial activity (10, 11; A. C. Sinclair and R. L. DeVault, personal communication). In this paper we report the synthesis and antibacterial activity of several 3-*O*-demethylfortimicins.

MATERIALS AND METHODS

General methods. Proton magnetic resonance (pmr) spectra were recorded at 100 MHz with a Varian XL-100 spectrometer. Chemical shifts are reported in parts per million downfield from external tetramethylsilane contained in a coaxial capillary in the sample tube. Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 70 eV and 100 to 150°C with direct probe insert. Thin-layer chromatography was performed on Merck (Darmstadt) precoated Silica Gel 60 plates. Unblocked fortimicins were visualized with ninhydrin; *N*-blocked fortimicins were detected with ceric sulfate reagent (50 g of ammonium molybdate and 20 g of ceric sulfate in 900 ml of water and 100 ml of concentrated sulfuric acid).

3-*O*-Demethylfortimicin B. 3-*O*-Demethylfortimicin B (Fig. 1, compound 3) was prepared from fortimicin B by the *O*-demethylation procedure of Monneret et al. (6). A stirring solution of 6.0 g of fortimicin B free base in 110 ml of ethylamine, freshly distilled immediately before use, was treated with 1.61 g of lithium wire suspended in 90 ml of ethylamine (the lithium wire was cut into small pieces in the ethylamine immediately before addition to the fortimicin B solution). The reaction mixture, which turns a deep, dark blue a few minutes after the addition of the lithium, was stirred under reflux for 2 h, and then methanol was cautiously added to consume excess lithium. The solvent was evaporated under reduced pressure, and the organic products were separated from the lithium salts by chromatography on a column (3.0 by 75 cm) of silica gel prepared and eluted with the lower phase of a mixture of methylene chloride-methanol-concentrated ammonium hydroxide (1:1:1, vol/vol). All fractions containing 3-*O*-demethylfortimicin B were collected and rechromatographed on a column of Bio Rex 70, 100–200 mesh, NH_4^+ form. Elution with a gradient of water to 1 N ammonium hydroxide gave the product which was lyophilized to give compound 3 (1.74 g): $[\alpha]_D^{24}$ + 41° (*c* 1.02, methanol); ir (KBr) 3,370 and 1,585 cm^{-1}; pmr (D_2O) δ1.50 (d, $C_{6'}$-CH_3, $J_{6',7'}$, 6.5 Hz), 2.83 (s, C_4-NCH_3), 5.53 (d, $H_{1'}$, $J_{1',2'}$ 3.8 Hz); mass spectrum, *m/e* 334.2220 (M^+), calculated for $C_{14}H_{30}N_4O_5$ 334.2216.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (Fig. 1, compound 4). To a stirring solution of 1.59 g of 3-*O*-demethylfortimicin B, 48 ml of methanol, and 24 ml of water, cooled in an ice bath, was added 3.55 g of *N*-(*N*-benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 3 h and then at room temperature for 22 h. The major portion of the methanol was evaporated under reduced pressure, and the syrup was shaken with a mixture of 400 ml of water and 200 ml of chloroform. The chloroform layer was separated, washed with water, and dried ($MgSO_4$). After evaporation of the chloroform the residue was chromatographed on a column (2.2 by 75 cm) of silica gel prepared and eluted with chloroform-methanol-concentrated ammonium hydroxide (23.4:1.4:1.0, vol/vol). Fractions containing



	R	R'		R	R'	R''
2	CH_3	H	1	H	CH_3	$COCH_2NH_2$
3	H	H	5	H	H	$COCH_2NHCH_3$
4	H	Z	6	H	H	$COCH_2CH_2NH_2$
			7	H	H	$COCH_2NH_2$
			8	Z	H	CH_2CH_2NHZ
			9	Z	H	$COCH_2NHZ$
			10	H	H	$CH_2CH_2NH_2$
			11	Z	H	$COCH_2NZCH_3$
			12	Z	H	$COCH_2CH_2NHZ$

FIG. 1. *Structures of 3-O-demethylfortimicins.*

the major product were evaporated to give compound 4 (1.70 g): $[\alpha]_D^{23}$ + 19° (*c* 1.0, methanol); pmr ($CDCl_3$) δ0.99 (d, $C_{6'}$-CH_3, $J_{6',7'}$, 5.0 Hz), 2.27 (s, C_4-NCH_3), 7.27 (m, Cbz-aromatic). Analysis Calculated for $C_{33}H_{48}N_4O_{11}$: C, 61.94; H, 6.57; N, 7.60. Found: C, 61.83; H, 6.74; N, 7.51.

1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin A (Fig. 1, compound 9). A stirring solution of 0.80 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B in 5.35 ml of tetrahydrofuran was treated with 0.399 g of the *N*-hydroxysuccinimide ester of benzyloxycarbonylglycine. Stirring was continued for 22 h at room temperature. The tetrahydrofuran was evaporated under reduced pressure to leave a white residue. The product was purified by chromatography on a column of silica gel with benzene–methanol–95% ethanol–concentrated ammonium hydroxide (23.5:1.4:2.0:0.2, vol/vol) as the eluent to give compound 9 (0.488 g): $[\alpha]_D^{24}$ + 45° (*c* 1.03, methanol); ir ($CDCl_3$) 3,425, 1,705 and 1,645 cm^{-1}; pmr ($CDCl_3$) δ1.15 (unresolved d, $C_{6'}$-CH_3), 2.90 (s, C_4-NCH_3), 7.28 (m, Cbz-aromatic). Analysis Calculated for $C_{48}H_{57}N_5O_{14}$: C, 62.13; H, 6.19; N, 7.55. Found: C, 61.80; H, 6.31; N, 7.64.

1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-4-*N*-sarcosyl-3-*O*-demethylfortimicin B (Fig. 1 compound 11). A stirring solution prepared from 0.298 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B, 0.113 g of *N*-benzyloxycarbonylsarcosine, 0.129 g of 1-hydroxybenzotriazole, and 3.0 ml of tetrahydrofuran was treated with 0.107 g of *N,N'*-dicyclohexylcarbodiimide in 3.0 ml of tetrahydrofuran. Stirring was continued for 16 h at room temperature. Insoluble dicyclohexylurea was removed by filtration, and the filtrate was evaporated to leave a pale yellow solid. The solid was chromatographed on a column of silica gel using benzene–methanol–95% ethanol–concentrated ammonium hydroxide (23.5:1.4:2.0:0.2, vol/vol) as the eluent. Fractions containing only the major product were evaporated to dryness. Other fractions containing the major product and a minor second component were rechromatographed on a column of silica gel with benzene–methanol–concentrated ammonium hydroxide (85:15:1, vol/vol) as the eluent. Fractions containing only the major component were combined with material obtained in the first column to give compound 11 (0.209 g): $[\alpha]_D^{24}$ + 43° (*c* 1.01, methanol); ir ($CDCl_3$) 3,435, 1,703, and 1,635 cm^{-1}; pmr ($CDCl_3$) δ1.17 (unresolved d, $C_{6''}$-CH_3), ~2.9 (broad s, sarcosyl-NCH_3), 2.99 (s, C_4-NCH_3), 4.83 (d, $H_{1'}$, $J_{1',2'}$, 3.5 Hz), 7.31 (m, Cbz-aromatic). Analysis Calculated for $C_{49}H_{59}N_5O_{14}$: C, 62.48; H, 6.31; N, 7.43. Found: C, 62.35; H, 6.65; N, 7.57.

1,2',6',3''-Tetra-*N*-benzyloxycarbonyl-4-*N*-β-alanyl-3-*O*-demethylfortimicin B (Fig. 1, compound 12). A stirring solution of 4.499 g of 1,2',6'-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B in 76.5 ml of tetrahydrofuran was treated with 2.897 g of *N*-hydroxy-5-norbornene-2,3-dicarboximidyl-*N*-benzyloxycarbonyl-β-alanine. Stirring was continued for 18 hat room temperature. The tetrahydrofuran was evaporated to leave 7.565 g of residue. The residue was chromatographed on a column (3.0 by 72 cm) of silica gel with dichloroethane–95% ethanol–concentrated ammonium hydroxide (20:2.0:0.04, vol/vol) as the eluent to give compound 12 (2.251 g): $[\alpha]_D^{23}$ + 43° (*c* 1.03, methanol); ir ($CDCl_3$) 1,700, 1,611, and 1,500 cm^{-1}; pmr ($CDCl_3$) δ1.16 (unresolved d, $C_{6''}$-CH_3), 2.93 (s, C_4-NCH_3), 7.29 (m, Cbz-aromatic). Analysis Calculated for $C_{49}H_{59}N_5O_{14}$: C, 62.48; H, 6.31; N, 7.43. Found: C, 62.17; H, 6.47; N, 7.38.

3-*O*-demethylfortimicin A (Fig. 1, compound 7). A solution of 0.140 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin A in 25 ml of 0.2 N hydrochloric acid in methanol was hydrogenated under 3 atmospheres (302 kPa) of hydrogen for 4 h in the presence of 0.14 g of 5% palladium on carbon. The catalyst was collected on a filter and washed with methanol. The filtrate was concentrated to dryness, and the excess hydrochloric acid was removed by repeated codistillation with methanol under reduced pressure to give compound 7 (0.071 g) as the tetrahydrochloride: $[\alpha]_D^{23}$ + 79° (*c* 1.0, methanol); ir (KBr) 3,410, 1,639, and 1,595 cm^{-1}; pmr (D_2O) δ1.81 (d, $C_{6''}$-CH_3, $J_{6',7'}$, 6.5 Hz), 3.62 (s, C_4-NCH_3), 5.79 (d, $H_{1'}$, $J_{1',2'}$, 3.5 Hz); mass spectrum, *m/e* 391.2414 ($M^{+\cdot}$), calculated for $C_{16}H_{33}N_5O_6$ 391.2431.

4-*N*-Sarcosyl-3-*O*-demethylfortimicin B (Fig. 1, compound 5). A solution of 0.125 g of 1,2',6',2''-tetra-*N*-benzyloxycarbonyl-4-*N*-sarcosyl-3-*O*-demethylfortimicin B in 25 ml of 0.2 N hydrochloric acid in methanol was hydrogenated under 3 atmospheres of hydrogen for 4 h in the presence of 0.13 g of 5% palladium on carbon. Work-up as above gave compound 5 (0.073 g) as the tetrahydrochloride: $[\alpha]_D^{24}$ + 84° (*c* 1.01, methanol); ir (KBr) 3,420 and 1,635 cm^{-1}; pmr (D_2O) δ1.80 (d, $C_{6''}$-CH_3, $J_{6',7'}$, 6.5 Hz), 3.27 (s, sarcosyl-NCH_3). 3.60 (s, C_4-NCH_3), 5.79 (d, $H_{1'}$, $J_{1',2'}$, 3.5 Hz); mass spectrum, *m/e* 405.2614 ($M^{+\cdot}$), calculated for $C_{17}H_{35}N_5O_6$ 405.2587.

4-*N*-β-alanyl-3-*O*-demethylfortimicin B (Fig. 1, compound 6). 1,2',6',3''-Tetra-*N*-benzyloxycarbonyl-4-*N*-β-alanylfortimicin B (Fig. 1, compound 12) (1.213 g) was hydrogenated as described above. The usual work-up gave compound 6 (0.754 g) as the tetrahydrochloride: $[\alpha]_D^{23}$ + 71° (*c* 0.87, methanol); ir (KBr) 3,400, 1,610, and 1,483 cm^{-1}; pmr (D_2O) δ1.83 (d, $C_{6''}$-CH_3, $J_{6',7'}$, 7.0 Hz), 3.82 (s, C_4NCH_3), 5.79 (d, $H_{1'}$, $J_{1',2'}$, 3.5 Hz); mass spectrum, *m/e* 405.2564 ($M^{+\cdot}$), calculated for $C_{17}H_{35}O_6N_5$ 405.2587.

1,2',6',2''-Tetra-*N*-benzyloxycarbonyl-4-*N*-(β-aminoethyl)-3-de-*O*-methylfortimicin B (Fig. 1,

TABLE 1. *In vitro antibacterial activity of parent and 3-O-demethylfortimicins*

Organism	Minimal inhibitory concentration (μg/ml)							
	Fortimicin A	3-*O*-Demethyl-fortimicin A	4-*N*-sarcosyl fortimicin B	4-*N*-Sarcosyl-3-*O*-demethyl-fortimicin B	4-*N*-β-alanyl fortimicin B	4-*N*-β-alanyl-3-*O*-demethyl fortimicin B	4-*N*-(β-aminoethyl)-fortimicin B	4-*N*-(β-aminoethyl)-3-*O*-demethyl fortimicin B
Staphylococcus aureus (Smith)	1.56	0.78	3.1	1.56	3.1	1.56	12.5	3.1
Streptococcus faecalis 10541	100	25	100	100	100	100	>100	>100
Enterobacter aerogenes 13048	6.2	3.1	6.2	3.1	6.2	6.2	100	6.2
Escherichia coli JUHL	6.2	3.1	6.2	6.2	6.2	12.5	50	12.5
E. coli BL-3676	25	12.5	25	25	25	25	>100	50
Klebsiella pneumoniae 10031	3.1	1.56	6.2	3.1	6.2	3.1	100	12.5
K. pneumoniae KY-4262	6.2	12.5	25	6.2	25	6.2	>100	25
Providencia sp. 1577	3.1	1.56		3.1		3.1	50	12.5
P. aeruginosa KY-8512	12.5	3.1	25	12.5	25	6.2	>100	25
P. aeruginosa KY-8516	100	25	50	50	50	50	>100	100
P. aeruginosa 209	>100	>100	>100	>100	>100	>100	>100	>100
P. aeruginosa 27853	25	12.5		12.5		12.5	>100	25
Salmonella typhimurium Ed #9	6.2	1.56	3.1	6.2	1.56	6.2	25	6.2
Serratia marcescens 4003	3.1	6.2	3.1	3.1	1.56	3.1	12.5	6.2
Shigella sonnei 9290	12.5	6.2	6.2	6.2	6.2	12.5	50	25
Proteus rettgeri U-6333	12.5	25	100	25	100	25	>100	50
Proteus vulgaris JJ	6.2	3.1	6.2	6.2	12.5	12.5	50	6.2

compound 8). A stirring, nitrogen-purged solution of 0.80 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin A and 12.5 ml of dry tetrahydrofuran was treated with 2.5 ml of a 1 M solution of diborane in tetrahydrofuran. Stirring was continued for 3 h under a nitrogen atmosphere, and then an additional 1.5 ml of the diborane solution was added. After stirring for an additional 2 h under nitrogen, excess diborane was consumed by the addition of water. The solvent was evaporated under reduced pressure, and the residue was codistilled with methanol. The product was purified by chromatography on a column (1.9 by 58 cm) of silica gel with benzene–methanol–95% ethanol–concentrated ammonium hydroxide (23.5:1.5:1.9:0.2, vol/vol) to give compound 8 (0.580 g): $[\alpha]_D^{25}$ + 40° (*c* 0.75, methanol); ir ($CDCl_3$) 1,700 and 1,500 cm^{-1}; pmr ($CDCl_3$) δ1.12 (unresolved d, $C_{6''}$-CH_3), 2.38 (s, C_4-NCH_3), 7.28 (m, Cbz-aromatic). Analysis Calculated for $C_{48}H_{59}N_5O_{13}$: C, 63.08; H, 6.51; N, 7.66. Found: C, 62.78; H, 6.91; N, 7.29.

4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B (Fig. 1, compound 10). A solution of 0.580 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B in 60 ml of 0.2 N hydrochloric acid in methanol was hydrogenated under 3 atmospheres of hydrogen for 4 h in the presence of 0.60 g of 5% palladium on carbon. After the usual work-up, 0.342 g of compound 10 was isolated as the pentahydrochloride: $[\alpha]_D^{21}$ + 70° (*c* 1.02, methanol); ir (KBr) 1,595 and 1,487 cm^{-1}; pmr (D_2O) δ1.82 (d, $C_{6''}$-CH_3, $J_{6'',7''}$, 6.5 Hz), 3.66 (s, C_4-NCH_3) 5.88 (d, $H_{1'}$, $J_{1',2'}$, 4.0 Hz); mass spectrum, *m/e* 378.2701 $(M + H)^+$, calculated for $C_{16}H_{36}N_5O_5$ 378.2717.

Determination of minimal inhibitory concentrations. Minimal inhibitory concentrations were determined by the agar dilution method with Mueller-Hinton agar, pH 7.4. An inoculum of approximately 5 × 10^4 colony-forming units was applied to the agar surface with a replicating device. The test organisms were grown at 35°C for 18 h. The minimal inhibitory concentration was defined as the lowest concentration of antibiotic which inhibited development of visible growth. A slight haze or up to three colonies was ignored.

RESULTS AND DISCUSSION

Our initial efforts were directed toward preparing 4-*N*-acyl-3-*O*-demethylfortimicins. The key intermediate, 3-*O*-demethylfortimicin B was prepared by the *O*-demethyl procedure of Monneret et al. (6). Treatment of fortimicin B with excess metallic lithium in freshly distilled ethylamine followed by removal of lithium salts by chromatography on silica gel and subsequent isolation by ion exchange chromatography gave 3-*O*-demethylfortimicin B in 30% isolated yield.

Selective *N*-blocking of the three primary amino groups of 3-*O*-demethylfortimicin B was achieved by treatment with *N*-(*N*-benzyloxycarbonyloxy)succinimide under conditions described earlier (9) to afford, after silica gel chromatography, 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B in 49% isolated yield. The *O*-demethylfortimicins, 4-*N*-sarcosyl-3-*O*-demethylfortimicin B, 4-*N*-β-alanyl-3-*O*-demethylfortimicin B, and 3-*O*-demethylfortimicin A, were readily prepared by acylation of the C_4-methylamino group of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B with the activated esters of the appropriate *N*-benzyloxycarbonyl protected amino acids. Acyl activation was accomplished using derivatives of *N*-hydroxysuccinimide, *N*-hydroxy-5-norbornene-2,3-dicarboxamide or 1-hydroxybenzotriazole, the latter formed in situ. Catalytic hydrogenolysis of the benzyloxycarbonyl groups in methanol containing excess hydrochloric acid gave the deblocked antibiotics as hydrochloride salts.

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B was prepared by the selective reduction of the corresponding per-*N*-benzyloxycarbonyl-4-*N*-acyl-3-*O*-demethylfortimicin B derivatives according to the method described by Sato and Mori (8). Treatment of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin A with an excess of diborane in tetrahydrofuran gave, after chromatography of the reaction mixture, 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B in 73% yield. The benzyloxycarbonyl groups were removed by hydrogenolysis over palladium on carbon in acidic methanol to give 4-*N*-(β-aminoethyl)-3-*O*-demethylfortimicin B isolated as the pentahydrochloride salt.

The in vitro antibacterial activities of the 3-*O*-demethylfortimicins compared with those of some of the parent fortimicins are shown in Table 1. The 4-*N*-acyl-3-*O*-demethylfortimicins all show superior activity compared with the parent fortimicins. For example, 3-*O*-demethylfortimicin A was approximately 50% more active than fortimicin A against a broad spectrum of bacteria. Of particular note was the two- to fourfold-increased activity of 3-*O*-demethylfortimicin A over fortimicin A against a variety of recent clinical isolates of *Pseudomonas aeruginosa* (Table 2). The antibacterial activity of 3-*O*-demethylfortimicin A is more fully described by Jones et al. (5) and Girolami and Stamm (3).

TABLE 2. *In vitro antipseudomonal activity of 3-O-demethylfortimicin A compared with fortimicin A*

Organism[a]	Minimal inhibitory concentration (μg/ml)	
	3-*O*-demethyl fortimicin A	Fortimicin A
P. aeruginosa 8055	6.2	25
P. aeruginosa 8977	1.6	6.2
P. aeruginosa 8764	12.5	25
P. aeruginosa 8589	12.5	25
P. aeruginosa 9286	6.2	12.5
P. aeruginosa 9246	12.5	50
P. aeruginosa 9315	6.2	12.5
P. aeruginosa 4116	6.2	25
P. aeruginosa 8406	12.5	50
P. aeruginosa 11416	25	50

[a] Randomly selected recent clinical isolates susceptible to gentamicin.

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