Synthesis on 1,4-Diaminocyclitol Antibiotics. V. Synthesis of 3′-Enofortimicin D

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Synopsis. 3′-Enofortimicin D has been prepared by condensation of 1-*O*-acetyl-2,6-bis(2,4-dinitrophenylamino)-2,3,4,6-tetradeoxy-D-*erythro*-hex-3-enopyranose with 2,5-di-*O*-benzoyl-1,4-bis-*N*-(methoxycarbonyl)fortamine B, followed by deprotection.

In the preceding paper,[1] we have reported the synthesis and antibacterial assay of 4′-hydroxyfortimicin D. In continuation of our study on modification of the amino sugar moiety of antibiotic fortimicins, introduction of a double bond into the diamino sugar moiety of fortimicins would be of interest in connection with elucidation of a role of its conformation. We now describe the synthesis and biological activity of 3′-enofortimicin D.

Methyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hex-3-enopyranoside sulfate (**1**),[2] was first converted to the bis[*N*-(2,4-dinitrophenyl)] derivative (**2**) in 79% yield. Hydrolysis of **2** with acetic acid containing 2M hydrochloric acid (1 M=1 mol dm^{-3}), followed by acetylation, gave the acetate (**3**) in 64% yield.

Condensation of **3** with 2,5-di-*O*-benzoyl-1,4-bis[*N*-(methoxycarbonyl)] fortamine B (**4**)[3] was carried out in 1,2-dichloroethane in the presence of trimethylsilyl trifluoromethanesulfonate under argon to give sole condensate **5** in 36% yield.

Deprotection of **5** with barium hydroxide in boiling aqueous dioxane in the usual way resulted in further hydrolysis of the glycosidic bond to give mainly fortamine B (**7**) (71%), together with a small amount (11%) of desired 3′-enofortamine KE (**6**). The ^{1}H NMR spectrum of **6** revealed a doublet (δ 5.26, $J_{1',2'}$=3 Hz) due to C-1′ α-anomeric proton. The ready cleavage of the α-glycosidic bond might be attributable to the presence of a double bond between C-3′ and C-4′. Similar results have been observed by Fukatsu[5] in the case of 3′-enokanamycin derivative. Several attempts to increase the yield of **6** failed.

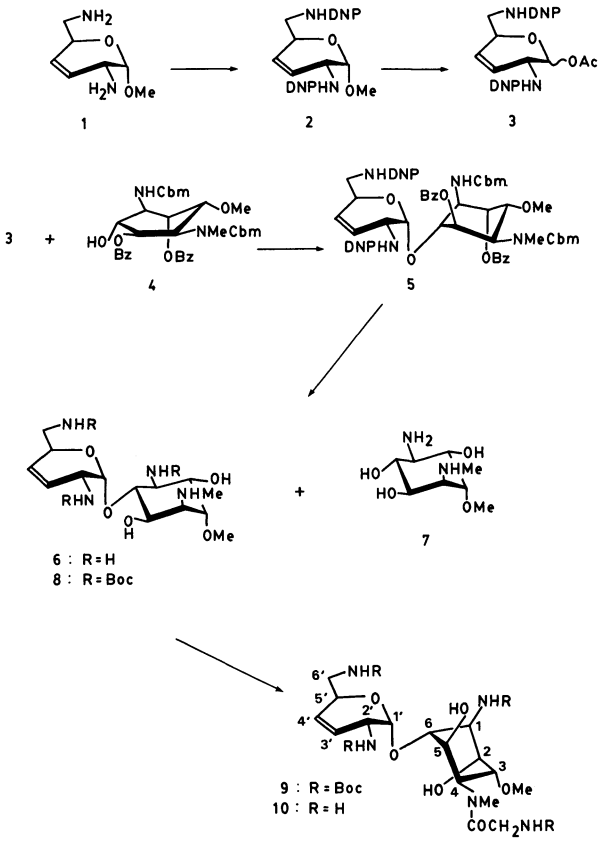
Compound **6** was treated with 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON)[6] in aqueous dioxane to give 1,2′,6′-tris[*N*-(*t*-butoxycarbonyl)] derivative (**8**) in 65% yield. The 4-[*N*-(*N*-*t*-butoxycarbonyl)glycyl] derivative (**9**) was obtained in 66% yield by treatment of **8** with activated ester of *N*-(*t*-butoxycarbonyl)glycine[7] in tetrahydrofuran. Finally, treatment of **9** with trifluoroacetic acid in chloroform gave 3′-enofortimicin D (**10**) as the trifluoroacetate.

The minimum inhibitory concentration of **10** is listed together with those of fortimicin A (Table 1). 3′-Enofortimicin D (**10**) exhibited weak activity compared to that of fortimicin A against many organisms.

Table 1. Antimicrobial Activity of **10** and Fortimicin A[a]

Test Organisms	**10**	FM A
Streptococcus faecalis KY4280	100	6.3
Pseudomonas aeruginosa KY4276	50	6.3
Staphyllococcus aureus KY4279	1.6	0.2
Escherichia coli KY4271	12.5	0.78
Bacillus subtilis KY4273	0.78	0.1
Shigela sonnei KY4281	25	1.6
Klebsiella pneumoniae KY4275	3.1	0.2

a) Minimum inhibitory concentration in μg ml^{-1}.



Experimental[8]

Methyl 2,6-Bis(2,4-dinitrophenylamino)-2,3,4,6-tetradeoxy-α-D-*erythro*-3-enopyranoside (2). A solution of methyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hex-3-enopyranoside sulfate (**1**)[2] (500 mg) in 85% aqueous methanol (10 ml) containing 1-fluoro-2,4-dinitrobenzene (0.51 ml) and triethylamine (1.1 ml) was stirred at 0 °C for 1 h, and stirring was continued at room temperature overnight. The product was purified by chromatography on silica gel with toluene–acetone (20 : 1) and crystallization from acetone–petroleum ether gave **2** (754 mg, 79%): mp 150—152 °C; $[\alpha]_D^{23}$ −143° (*c* 1.08, $CHCl_3$).

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Found: C, 46.48; H, 3.76; N, 17.04%. Calcd for $C_{19}H_{18}N_6O_{10}$: C, 46.54; H, 3.70; N, 17.14%.

1-*O*-Acetyl-2,6-bis(2,4-dinitrophenylamino)-2,3,4,6-tetradeoxy-D-*erythro*-hex-3-enopyranose (3). A solution of **2** (400 mg) in acetic acid (20 ml) containing 2M hydrochloric acid (2 ml) was stirred at 80 °C for 7 h. The mixture was extracted with chloroform (50 ml) and the extract was dried. After concentration, the residue was treated with acetic anhydride (4 ml) in pyridine (10 ml) for 12 h. The product was purified by chromatography on silica gel and crystallization from acetone-2-propanol gave **3** (273 mg, 64%): mp 92—102 °C; $[\alpha]_D^{24}$ −226° (*c* 1.07, $CHCl_3$).

Found: C, 46.28; H, 3.58; N, 16.34%. Calcd for $C_{20}H_{18}N_6O_{11}$: C, 46.34; H, 3.50; N, 16.21%.

2,5-Di-*O*-benzoyl-2′,6′-bis[*N*-(2,4-dinitrophenyl)]-1,4-bis[*N*-(methoxycarbonyl)]-3′-enofortimicin KE (5). To a solution of **3** (249 mg) and **4**[3] (271 mg) in 1,2-dichloroethane (5 ml) in the presence of powdered molecular sieve 4A (230 mg) was added trimethylsilyl trifluoromethanesulfonate (0.07 ml), and the mixture was stirred for 3.5 h under argon at room temperature. The product was purified by chromatography on silica gel with toluene-2-butanone (30 : 1) and crystallization from acetone-2-propanol gave **5** (166 mg, 36%): mp 134—140 °C; $[\alpha]_D^{20}$ −97.5° (*c* 1.02, $CHCl_3$).

Found: C, 53.22; H, 4.46; N, 11.27%. Calcd for $C_{44}H_{44}N_8O_{19}$: C, 53.44; H, 4.48; N, 11.33%.

3′-Enofortimicin KE (6). A solution of **5** (135 mg) in aqueous dioxane (2 : 1, 9 ml) in the presence of barium hydroxide octahydrate (3.0 g) was refluxed for 12 h. After cooling, carbon dioxide was introduced to the mixture, and an insoluble material was filtered. After concentration, the residue was eluted from a column of Amberlite CG-50 (NH_4^+) resin with aqueous ammonia (0—0.20 M) to give, first, fortamine B (**7**) (20 mg, 71%), the ^1H NMR spectrum of which was superimposable on that of an authentic sample.[4] The fraction eluted second contained compound **6** as a solid (5 mg, 11%): $[\alpha]_D^{25}$ −47.2° (*c* 0.23, MeOH); ^1H NMR (CD_3OD) δ=2.40 (3H, s, NMe), 3.45 (3H, s, OMe), 5.26 (1H, d, *J*=3 Hz, H-1′), 5.71 (2H, bs, H-3′, 4′); MS (SIMS) Found: *m/z*, 333. Calcd for $C_{14}H_{29}N_4O_5$: M+1, 333.

1,2′,6′-Tris[*N*-(*t*-butoxycarbonyl)]-3′-enofortimicin KE (8). To a solution of **7** (7.9 mg) in 50% aqueous dioxane (0.5 ml) was added Boc-ON (29.3 mg), and the mixture was stirred for 6 h. Triethylamine (0.03 ml) was added to it and stirring was continued overnight. The product was purified by chromatography on silica gel with chloroform-methanol (30 : 1) to give **8** as a solid (8.7 mg, 65%): $[\alpha]_D^{23}$ −32.9° (*c* 1.02 . $CHCl_3$); ^1H NMR ($CDCl_3$) δ=1.43 (27H, bs, Boc×3), 2.40 (3H, s, NMe), 3.46 (3H, s, OMe).

1,2′,6′-Tris[*N*-(*t*-butoxycarbonyl)]-4-[*N*-[*N*-(*t*-butoxycarbonyl)-glycyl]]-3′-enofortimicin KE (9). To a mixture of *N*-(*t*-butoxycarbonyl)glycine (3 mg), 1-hydroxybenzotriazole (2.4 mg) and dicyclohexylcarbodiimide (4.3 mg) in tetrahydrofuran (0.5 ml) was added a solution of **8** (8.7 mg) in tetrahydrofuran (0.5 ml), and the mixture was stirred for 3 d. The product was purified by chromatography on silica gel with chloroform-methanol (97 : 3) to give **9** as a solid (7.2 mg, 66%): $[\alpha]_D^{27}$ −6.0° (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$) δ=1.41, 1.43 (36H in total, each s, Boc×4), 3.14 (3H, s, NMe), 3.46 (3H, s, OMe); MS (SIMS) Found: *m/z*, 790. Calcd for $C_{36}H_{64}N_5O_{14}$: M+1, 790.

3′-Enofortimicin D (10). To a solution of **9** (7.2 mg) in chloroform (0.5 ml) was added trifluoroacetic acid (0.1 ml), and the mixture was stirred for 1.5 h. After concentration, the residue was washed with ether to give a solid of **10** (7.2 mg) as the trifluoroacetate: $[\alpha]_D^{26}$ −5.5° (*c* 0.44, water); ^1H NMR (200 MHz, D_2O) δ=3.12 (1H, s, NMe), 3.22 (1H, dd, *J*=6 Hz, 14 Hz, H-6′a), 3.36 (1H, dd, *J*=3.5 Hz, 14 Hz, H-6′b), 3.46 (3H, s, OMe), 5.46 (1H, d, *J*=4 Hz, H-1′), 5.92 and 6.18 (each 1H, bd, *J*=11 Hz, H-3′ and 4′); MS (SIMS) Found: *m/z*, 390. Calcd for $C_{16}H_{32}N_5O_6$: M+1, 390.

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8) The general procedures used in the present work have been described in the preceding paper.[3]