SYNTHESIS OF DACTIMICIN

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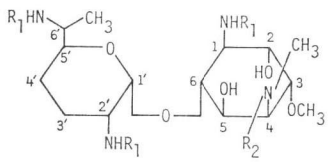
Dactimicin,* produced by *Dactylosporangium matsuzakiense*, is a new member of fortimicin-group antibiotic.[1~3] Structurally, this antibiotic is the first aminoglycoside having a formimino moiety. We wish to report here the first synthesis of dactimicin (**1**).

The starting material, 1,2′,6′-tri-*N*-Boc-fortimicin B[4] (**2**) was prepared from fortimicin B by treatment with di-*t*-butyldicarbonate in the presence of triethylamine. The 4-methylamino group of **2** was acylated with 1-hydroxybenzotriazole ester of *N*-Cbz-glycine to afford 2″-*N*-Cbz-1,2′,6′-tri-*N*-Boc-fortimicin A (**3**) (78% yield). The 2″-*N*-Cbz group of **3** was removed by catalytic hydrogenation with 10% palladium on charcoal in a mixture of ethanol and acetic acid to give 1,2′,6′-tri-*N*-Boc-fortimicin A (**4**) (92% yield). The free amino group on 2″-C was converted into a formamidine by treatment with ethyl formimidate hydrochloride[5,6] in dry ethanol to yield 1,2′,6′-tri-*N*-Boc-dactimicin** (**5**). Removal of the Boc groups in **5** with 90% trifluoroacetic acid, followed by salt-exchange with dilute sulfuric acid gave (**1**) as the disulfate trihydrate (72% yield from **4**).

The synthetic dactimicin showed IR and NMR spectra, optical rotation, retention time in HPLC and antibacterial activity identical with those of natural one.

Scheme 1.



	R_1	R_2
1	H	$COCH_2NHCH{=}NH$
2	Boc	H
3	Boc	$COCH_2NHCbz$
4	Boc	$COCH_2NH_2$
5	Boc	$COCH_2NHCH{=}NH$

Boc=*t*-butoxycarbonyl
Cbz=benzyloxycarbonyl

* Dactimicin was previously reported as substance SF-2052.[1]

Experimental

2″-*N*-Benzyloxycarbonyl-1,2′,6′-tri-*N*-*t*-butoxycarbonylfortimicin A (**3**)

To an ice-cooled solution of *N*-benzyloxycarbonylglycine 0.535 g and 1-hydroxybenzotriazole 0.345 g in THF 20 ml was added dicyclohexylcarbodiimide 0.527 g and stirred for 2 hours under ice-cooling. To the reaction mixture was added (**2**) (1.380 g) and the solution was stirred for 24 hours at room temperature. Insoluble *N*,*N*′-dicyclohexylurea was removed by filtration and the THF was evaporated under reduced pressure to give a pale yellow residue. The residue was chromatographed on silica gel column (chloroform - methanol - 17% ammonium hydroxide, 1000: 36: 5) to give **3** (1.400 g, 78% yield): NMR ($CDCl_3$) δ 1.15 (3H, d, J=7Hz, 6′-C-CH_3), 1.40 (27H, s, -O-C(CH_3)$_3$), 3.07 (3H, s, 4-N-CH_3), 3.37 (3H, s, 3-O-CH_3), 5.11 (2H, s, CH_2Ph), 7.30 (5H, s, aromatic).

1,2′,6′-Tri-*N*-*t*-butoxycarbonylfortimicin A (**4**)

To a solution of **3** (1.400 g) and acetic acid (0.5 g) in ethanol (50 ml) was added 10% palladium on charcoal (0.2 g) and stirred for 14 hours under hydrogen atmosphere at room temperature. The catalyst was removed by filtration and the ethanol was evaporated under reduced pressure. To the residue was added 7% aqueous sodium hydrogen carbonate and the aqueous mixture was extracted with three portions of chloroform. After drying the combined extracts over anhydrous potassium carbonate, the chloroform was evaporated under reduced pressure. The residue was chromatographed on silica gel column (chloroform - methanol - 17% ammonium hydroxide, 100: 10: 1) to give **4** (1.080 g, 92% yield): mp 137.0~138.5°C (dec.); $[\alpha]_D^{25}$ +54°

** The recent report[3], concerning the structural determination, adopts the numbering system based on *chiro*-inositol stereochemistry, while in this report the numbering system of the structural formulae is in accordance with that of fortimicins by convention for its simplicity.

(*c* 1, $CHCl_3$); NMR ($CDCl_3$) δ 1.13 (3H, d, J= 7Hz, 6′-C–CH_3), 1.40 (27H, s, –O–C(CH_3)$_3$), 2.96 (3H, s, 4-N–CH_3), 3.39 (3H, s, 3-O–CH_3).

Found: C, 52.23; H, 8.02; N, 9.42.

Calcd. for $C_{32}H_{57}O_{12}N_5 \cdot \frac{1}{2}CO_2 \cdot H_2O$: C, 52.33; H, 8.24; N, 9.49.

1,2′,6′-Tri-*N*-*t*-butoxycarbonyldactimicin (**5**)

To an ice-cooled solution of **4** (300 mg) in dry ethanol 10 ml was added ethyl formimidate hydrochloride (300 mg) and stirred for 1 hour under ice-cooling. The reaction mixture was stirred for further 24 hours at room temperature to convert the excess imidate into ethyl orthoformate and ammonium chloride. Then, the mixture was concentrated under reduced pressure to give a colorless solid. To the solid was added ethyl acetate (50 ml) and insoluble material was removed by filtration. The ethyl acetate was evaporated under reduced pressure to give **5** as the mono hydrochloride (332 mg).

The salt was used in the following procedure without further purification.

Dactimicin (**1**)

To the crude salt of **5** (332 mg) was added 90% trifluoroacetic acid 10 ml and stirred for 20 minutes at room temperature. The excess trifluoroacetic acid was removed by evaporation under reduced pressure with addition of several portions of cold water. The residue was dissolved in ice-cooled 1.87 N sulfuric acid 2.0 ml. The solution was added to ice-cooled ethanol (50 ml) to give a precipitate, which was collected and washed with ice-cooled ethanol to give a colorless solid. To the solid was added cold water 3.0 ml and adjusted to pH 5.4 with Dowex 1×2 (OH^- form) resin. The resin was removed by filtration and the filtrate was lyophilized to give **1** as the disulfate trihydrate 209 mg (72% yield from **4**): mp 170~175°C (dec.); $[\alpha]_D^{25}$ +81° (*c* 1, H_2O).

Found: C, 31.77; H, 6.76; N, 12.05; S, 9.10.

Calcd. for $C_{18}H_{36}O_6N_6 \cdot 2H_2SO_4 \cdot 3H_2O$: C, 31.66; H, 6.79; N, 12.31; S, 9.39.

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