The Total Synthesis of Antibiotic Fortimicin B

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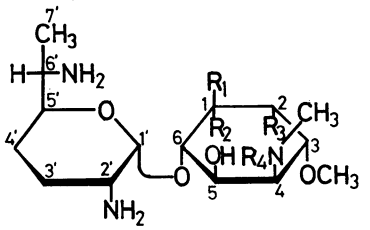
The total synthesis of antibiotic fortimicin B (**1b**) is described. The sugar component of **1b**: 6-*epi*-purpurosamine B was converted to the sugar chloride: 2,6-bis(2,4-dinitrophenylamino)-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranosyl chloride (**5**). The aminocyclitol component of **1b**: fortamine B was also converted to the partially protected aglycone (**9**). The condensation of aglycone **9** with the sugar chloride **5** gave the desired condensation product in 37% yield. The analogous condensation of the DL-form of the aglycone with **5** afforded the same condensation product in 19% yield, it was then converted to **1b** by step-by-step hydrolysis.

Fortimicin A (**1a**), B (**1b**),[1,2] and C,[3,4] SF-1854,[5] SF-2052,[6,7] sporaricin A, B,[8-10] KA-6606 III, and KA-6606 IV[11] are members of a family of structually similar aminocyclitol antibiotics which are found in fermentation broths of various strains of *Actinomycetes*. The general feature of the structures of the antibiotics is a pseudodisaccharide consisting of 6-*epi*-purpurosamine B and a novel aminocyclitol named fortamine. The structural diversity of the antibiotics is due to the configurational difference in the amino group on the 1-position, the existence or the absence of the hydroxyl group on the 2-position, and the variety of aminoacyl groups attached to the methylamino group on the 4-position.



	R_1	R_2	R_3	R_4
Fortimicin A (**1a**):	NH_2	H	OH	$COCH_2NH_2$
B (**1b**):	NH_2	H	OH	H
C:	NH_2	H	OH	$COCH_2NHCONH_2$
SF-1854:	NH_2	H	OH	$COCH_2NHCH{=}O$
SF-2052:	NH_2	H	OH	$COCH_2NHCH{=}NH$
Sporaricin A:	H	NH_2	H	$COCH_2NH_2$
B:	H	NH_2	H	H
KA-6606 III:	H	NH_2	H	$COCH_2NHCONH_2$
KA-6606 IV:	H	NH_2	H	$COCH_2NHCH{=}O$

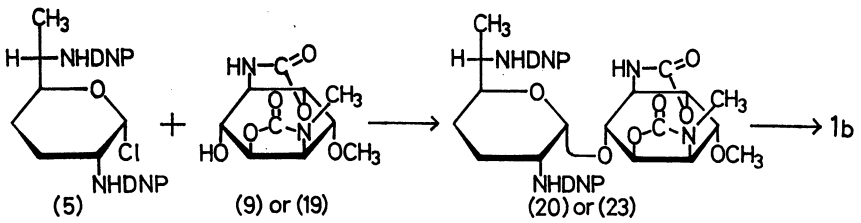
In preceding papers,[12-14] Suami and his coworkers have reported the synthesis of 6-*epi*-D-purpurosamine B and, in a communication letter,[15] a synthesis of the antibiotic fortimicin B in which natural fortamine B has been used as one of the starting materials. The synthesis of DL-fortamine B from *muco*-inosadiamine-3,6 has been described by a Kyowa Hakko research group.[16] We wish to report here the details of the total synthesis of fortimicin B, together with the synthesis of a DL-fortamine B derivative from 1,4-diazido-1,4-dideoxy-*muco*-inositol.

Fortimicin A, **1a**, the first members of this family to be discovered, exhibits high antimicrobial activities against Gram-positive and Gram-negative bacteria. The structures of **1a** and **1b** have been established by Egan and his coworkers.[17] The important biological activities and unique structures of these antibiotics make then attractive subjects of synthetic studies.

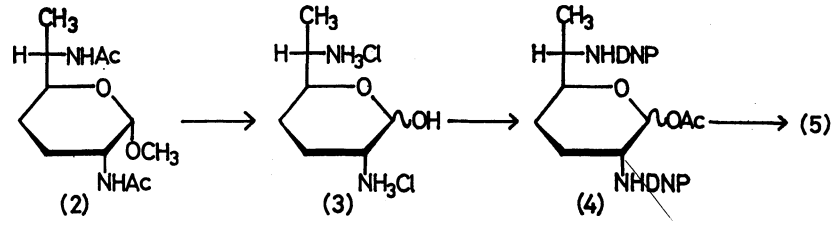
1a differs from **1b** in the existence of a glycyl group on the 1-methylamino group, and the former is converted into the latter by the loss of the glycyl group in an alkaline solution,[17] while it has been described in the literature that **1a** has been reconstructed from **1b** by a selective *N*-acylation.[18] Therefore, we have attempted to synthesize **1b** by condensing a glycosyl halide with a partially protected fortamine B derivative. Scheme 1 represents the reaction sequence leading to the total synthesis of **1b**.

The glycosyl chloride has been prepared by the reaction shown in Scheme 2. The acid hydrolysis of methyl 2,6-di-*N*-acetyl-2,6-diamino-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranoside (methyl 2,6-di-*N*-acetyl-6-*epi*-α-purpurosaminide B) (**2**)[13] in hydrochloric acid afforded the dihydrochloride (**3**). On treatment with acetic anhydride in the presence of diethyl ether-boron trifluoride, **3** gave the 1-*O*-acetyl derivative, which was then converted into the bis(*N*-2,4-dinitrophenyl) derivative (**4**) in 52% yield by reaction with 2,4-dinitrofluorobenzene in methanol containing triethylamine. The conversion of **4** into the crystalline glycosyl chloride (**5**) was accomplished in 82% yield by reaction with hydrogen chloride in the presence of acetyl chloride in dry diethyl ether.

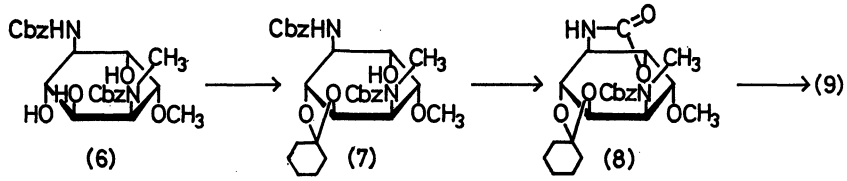
The aglycone was synthesized as follows. For the



Scheme 1.



Scheme 2.



Scheme 3.

conversion of optically active L-1,4-bis(*N*-benzyloxycarbonyl)-4-amino-1,4-dideoxy-6-*O*-methyl-1-methylamino-*chiro*-inositol (**6**)[19] into the partially protected aglycone (**9**), it was necessary to protect selectively the two amino groups on the 1- and 4-positions and the two hydroxyl groups on the 2- and 5-positions with an appropriate protective group, prior to the condensation with the glycosyl chloride **5**. This has been accomplished by the reaction route shown in Scheme 3.

The protection of the two vicinal *trans* hydroxyl groups on the 2- and 3-positions of **6** was achieved by treating **6** with 1,1-dimethoxycyclohexane in *N*,*N*-dimethylformamide (DMF) in the presence of an acid catalyst. The yield of the 2,3-*O*-cyclohexylidene derivative (**7**) from **6** was 67%. The protection of the 5-hydroxyl group of **7** was accomplished by the reaction of **7** with sodium hydride in DMF, forming a cyclic 4,5-*N*,*O*-carbamate (**8**) in 91% yield. The transformation of **8** into 1,2:4,5-di-*N*,*O*-carbonyl-fortamine B (**9**) was performed by the mild hydrolysis of **8** in aqueous acetic acid, followed by cyclic 1,2-*N*,*O*-carbonylation with sodium hydride in DMF; 83% yield.

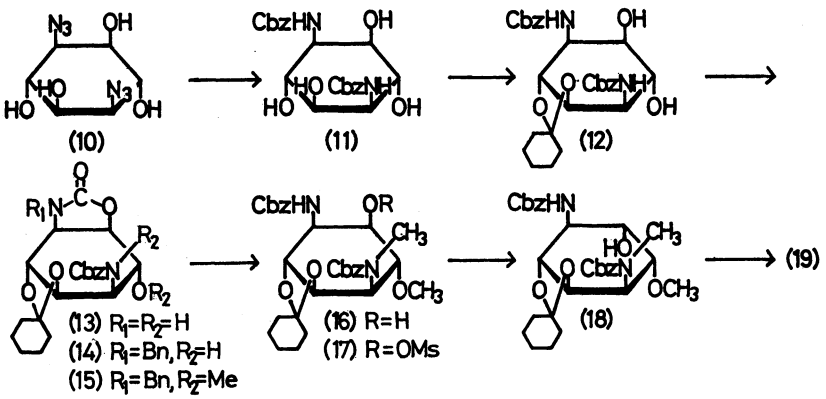
The reaction sequence shown in Scheme 4 represents a synthesis of the DL-form of the aglycone. As a starting material, 1,4-diazido-1,4-dideoxy-*muco*-inositol (**10**) was used; it was readily converted to *muco*-inosadiamine-1,4 by catalytic hydrogenation.[20] The stereochemistry of *muco*-inosadiamine-1,4 is similar to that of fortimicin B, except for the 5-hydroxyl group.

The catalytic hydrogenation of **10**, followed by benzyloxycarbonylation with benzyloxycarbonyl chloride in aqueous methanol containing sodium hydrogencarbonate, afforded 1,4-bis(*N*-benzyloxycarbonyl)-*muco*-inosadiamine-1,4 (**11**). The protection of one of the two sets of vicinal *trans* diols of **11** with 1,1-dimethoxycyclohexane in DMF furnished the 2,3-*O*-cyclohexylidene derivative (**12**) in 79% yield.

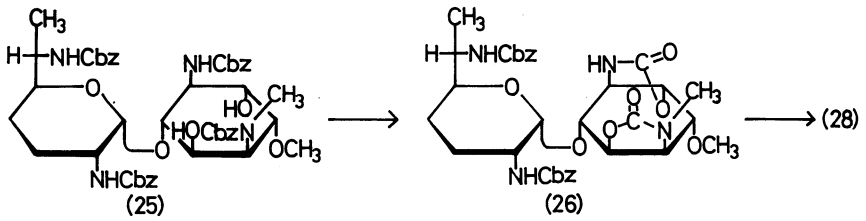
On treatment with sodium carbonate in aqueous dioxane, **12** was converted into the 4,5-*N*,*O*-carbamate (**13**) in 82% yield, since the formation of the cyclic carbamate occurs more readily between vicinal *cis*-arranged amino and hydroxyl groups than between those which are *trans*-related.

The *N*-benzylation of **13** in DMF with benzyl bromide and silver oxide afforded a mono-*N*-benzyl derivative (**14**) in 56% yield. The position of the benzyl group, shown in Scheme 4, has been deduced from the ^{1}H NMR spectroscopy and successive reaction products. The methylation of the 1-acylamino and the 6-hydroxyl groups of **14** with methyl iodide and silver oxide in DMF gave the 1-*N*-methyl-6-*O*-methyl derivative (**15**) in 82% yield.

The catalytic hydrogenolysis of **15** in the presence of palladium charcoal, followed by hydrolysis in a barium hydroxide solution, the catalytic hydrogenolysis of *N*-benzyl group in the presence of palladium black, and



Scheme 4.



Scheme 5.

N-acylation with benzyloxycarbonyl chloride afforded the 1,4-bis(*N*-benzyloxycarbonyl) derivative (**16**).

The mesylation of the 5-hydroxyl group of **16** with methanesulfonyl chloride in pyridine afforded the 5-*O*-sulfonate derivative (**17**) in 71% yield; this was, in turn, converted to the epimer by reaction with sodium acetate in DMF. The reaction mixture was hydrolyzed in a barium hydroxide solution, followed by *N*-acylation to give 1,4-bis(*N*-benzyloxycarbonyl)-2,3-*O*-cyclohexylidene-DL-fortamine B (**18**).

On treatment with sodium hydride, followed by hydrolysis and again treatment with sodium hydride, **18** was converted into the DL-form of aglycone (**19**) in 72% yield.

The attempted condensation of the optically active aglycone **9** with the glycosyl chloride **5** in dioxane in the presence of silver trifluoromethanesulfonate afforded two condensation products (**20** and **21**) in 37% and 9% yields respectively. The ^{1}H NMR spectrum of the main product **20** revealed a signal at δ 5.53 with a small coupling constant (3.3 Hz) which was due to the anomeric proton of the α-D-glycoside, while the spectrum of **21** showed a signal at δ 5.13 with a large coupling constant (8.1 Hz) which was attributed to the anomeric proton of the β-D-glycoside.

The analogous condensation of the DL-aglycone **19** with the glycosyl chloride **5** gave three component which were subsequently separated by column chromatography to give **22**, **23**, and **24** in 20, 19, and 23% yields respectively. The structural assigments of the condensation products were made by means of ^{1}H NMR spectroscopy. Compound **23** was identical with **20** in all respects, while **22** was identified as its diastereomer containing D-fortamine B. Compound **24** was a mixture of two products possessing β-D-glycosidic linkages. Compounds **20** and **23** were also identical with an authentic sample (**28**) prepared from natural fortimicin B by the reaction sequence shown in Scheme 5.

The removal of the protective groups of **23** by Amberlite IRA-400 (OH^-) resin, followed by hydrolysis in a barium hydroxide solution, afforded **1b** in 98% yield; it was then converted into tetra-*N*-acetylfortimicin B[17] (**25**). Compound **25** was identical with an authentic sample (**29**) prepared from natural fortimicin B by *N*-acetylation.

Experimental

General Procedures. The melting points were determined in capillary-tubes and are uncollected. The solutions were concentrated under reduced pressure below 40 °C. The optical rotations were meassured on a Japan Spectroscopic DIP-SL polarimeter. The infrared spectra were taken on a Hitachi HPL-225 spectrophotometer. ^{1}H NMR spectra were recorded on Varian EM-360A and 390 spectrometers at 60 and 90 MHz respectively. Deutriochloroform was used as a solvent, with tetramethylsilane as the internal standard. The peak position are given in δ values. TLC was preformed on Wakogel B-10 plates (Wako Pure Chemical Co., Ltd.), and silica-gel (Wakogel C-300) was employed for column chromatography.

*2,6-Diamino-2,3,4,6,7-pentadeoxy-*L*-lyxo-heptopyranose Dihydrochloride (6-*epi*-*D*-Purpurosamine B Dihydrochloride) (**3**).* A 1.0 g portion of methyl 2,6-diacetamido-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranoside (**2**)[13] was heated in 2 M (1 M=1 mol dm^{-3}) HCl (60 ml) under reflux. After 15 h, the solution was concentrated to give 0.89 g (99%) of crude **2** as a syrupy residue; it was recrystallized from ethanol to give 0.47 g (52%) of **3**; mp 183—184 °C (dec); $[\alpha]_D^{23}$ −15→−9.8° (*c* 1, methanol) $[\alpha]_D^{23}$ −5.4→+23.5° (*c* 1, water). Lit,[19] mp 186—191 °C (dec), $[\alpha]_D^{24}$ +6→+23° (*c* 0.6, water).

Found: C, 36.03; H, 7.50; N, 12.02; Cl, 30.20%. Calcd for $C_7H_{16}N_2O_2 \cdot 2HCl$: C, 36.06; H, 7.78; N, 12.02; Cl, 30.41%.

*1-O-Acetyl-2,6-bis(2,4-dinitrophenylamino)-2,3,4,6,7-pentadeoxy-*L*-lyxo-heptopyranose (**4**).* To a suspention of crude **3** (2.0 g) in acetic anhydride (20 ml), diethyl ether–boron trifluoride (3.3 ml) was added under ice cooling with agitation. After 12 h, the solution was concentrated. To a solution of the residue in 80% aq methanol, 2,4-dinitrofluorobenzene (4.0 g) was added, and a solution of triethylamine (4.8 ml) in methanol (20 ml) was dropped in over 1 h under ice cooling. After 16 h, the solution was diluted with chloroform, it washed with dil HCl and water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography using 1 : 15 (v/v) ethanol–toluene. The fractions corresponding to R_f 0.49 on TLC in 1 : 10 (v/v) ethanol–toluene were combined and concentrated to give 2.4 g (52%) of **4**: mp 120—122 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (2d, 3, J=7.2 Hz, J=6.3 Hz, H-7), 1.97·2.23 (2s, 3, intensity ratio 2 : 1, OAc), 5.65 (d, 2/3, $J_{1,2}$=8.7 Hz), 6.35 (d, 1/3, $J_{1,2}$=3.3 Hz).

Found: C, 47.42; H, 4.30; N, 15.45%. Calcd for $C_{21}H_{22}N_6O_{11}$: C, 47.19; H, 4.15; N, 15.73%.

*2,6-Bis(2,4-dinitrophenylamino)-2,3,4,6,7-pentadeoxy-β-*L*-lyxo-heptopyranosyl Chloride (**5**).* A suspension of **4** (1.0 g) in dry diethyl ether (200 ml) was staturated with dry HCl, and then, to the mixture, acetyl chloride (4 ml) was added. After 18 h, the crystals which was appeared were collected by filtration and washed with diethyl ether to give 0.78 g (82%) of **5**: mp 130—132 °C; $[\alpha]_D^{20}$ +130° (*c* 0.49, acetone); ^{1}H NMR (acetone-d_6) δ 1.45 (d, 3, $J_{6,7}$=6.6 Hz, H-7), 6.72 (d, 1, $J_{1,2}$=3.0 Hz, H-1), 8.69 (d, 1, J=9.0 Hz, NH), 8.80 (d, 1, J=9.9 Hz, NH); IR (KBr) 1610·1580 (Ar), 1510·1325 cm^{-1} (NO_2).

Found: C, 44.38; H, 3.81; N, 16.17; Cl, 7.18%. Calcd for $C_{19}H_{19}N_6ClO_9$: C, 44.67; H 3.75; N, 16.45; Cl, 6.94%.

1,4-Bis(N-benzyloxycarbonyl)-5,6-O-cyclohexylidenefortamine B (1L,4-Amino-1,4-bis(N-benzyloxycarbonyl)-2,3-O-cyclohexylidene-1,4-dideoxy-6-O-methyl-1-methylamino-chiro-*inositol) (**7**).* A

mixture of 1,4-bis(*N*-benzyloxycarbonyl)-fortamine B[19] (**6**, 2.0 g) and 1,1-dimethoxycyclohexane (15 ml) in DMF (60 ml) was heated at 70 °C under reduced pressure (30 mmHg)† in the presence of a catalytic amount of *p*-toluenesulfonic acid. After 3 h, triethylamine (1 ml) was added to the solution, and the mixture was concentrated. The residue was purified by column chromatography using 15 : 1 (v/v) benzene–ethanol. Fractions corresponding to R_f 0.52 on TLC in 10 : 1 (v/v) benzene–ethanol were collected and concentrated. The residue was recrystallized from ether–petroleum ether to give 1.46 g (63%) of **7**: mp 94—96 °C; $[\alpha]_D^{24}$ +25.1° (*c* 0.97, methanol) IR (KBr) 1700 (NC=O), 1615 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, C_6H_{10}), 3.07 (s, 3, NCH_3), 3.39 (s, 3, OCH_3), 4.52 (dd, 1, J=3.6 Hz, J=6.1 Hz, H-4), 5.10 (s, 2, CH_2 of benzyl), 5.24 (d, 1, J=7.5 Hz, NH-1), 7.37 (s, 10, $2\times C_6H_5$).

Found: C, 64.73; H, 6.76; N, 4.91%. Calcd for $C_{30}H_{38}N_2O_8$: C, 64.97; H, 6.91; N, 5.05%.

4-N-Benzyloxycarbonyl-1,2-N,O-carbonyl-5,6-O-cyclohexylidene-fortamine B (8). To a stirred solution of **7** (0.50 g) in DMF (10 ml), 50% sodium hydride (0.13 g) was added under ice cooling in a N_2 atmosphere. After 2 h, the solution was neutralized with acetic acid, diluted with water, and extracted with chloroform. The organic layer was concentrated, and the residue was purified by column chromatography, using 15:1 (v/v) benzene–ethanol. Fractions corresponding to R_f 0.67 on TLC in 10 : 1 (v/v) benzene–ethanol were collected and concentrated to give 0.34 g (84%) of crude **8** as an amorphous solid: mp 71 °C. It was recrystallized from chloroform–petroleum ether to give 0.23 g (57%) of **8**: mp 87—90 °C; $[\alpha]_D^{24}$ −9.1° (*c* 0.99, methanol); IR (KBr) 1780 (*trans* cyclic carbamate), 1695 cm^{-1} (NC=O); ^1H NMR ($CDCl_3$) δ 1.62 (m, 10, C_6H_{10}), 3.14 (s, 3, NCH_3), 3.43 (s, 3, OCH_3), 4.75 (m, 1, H-2), 5.17 (s, 2, CH_2 of benzyl), 5.52 (s, 1, NH-1), 7.39 (s, 5, C_6H_5).

Found: C, 62.10; H, 6.91; N, 6.17%. Calcd for $C_{23}H_{30}N_2O_7$: C, 61.87; H, 6.77; N, 6.28%.

1,2:4,5-Di-N,O-carbonylfortamine B (9). To a solution of crude **8** (0.35 g) in methanol (3 ml), 50% aq acetic acid (12 ml) was added, after which the mixture was warmed at 60 °C. After 2 h, the solution was concentrated. To a solution of the residue in DMF (10 ml), 50% sodium hydride (63 mg) was added. After 1 h at 0 °C in a N_2 atmosphere, the solution was neutralized with acetic acid and concentrated. The residue was purified by column chromatography, using 10 : 1 (v/v) benzene–ethanol. Fractions corresponding to R_f 0.42 on TLC in 5 : 1 (v/v) benzene–ethanol were collected and concentrated. The recrystallization of the residue from chloroform gave 168 mg (83%) of **9**: mp 225—228 °C; $[\alpha]_D^{20}$ −84.3° (*c* 1.06, methanol); IR (KBr) 1772 (*trans* cyclic carbamate), 1725 cm^{-1} (*cis* cyclic carbamate); ^1H NMR (CD_3OD) δ 3.01 (s, 3, NCH_3), 3.73 (s, 3, OCH_3), 4.63 (dd, 1, J=6.3 Hz, J=7.9 Hz, H-5).

Found: C, 46.41; H, 5.37; N, 10.59%. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85%.

1,4-Diamino-1,4-bis(N-benzyloxycalbonyl)-1,4-dideoxy-muco-inositol (11). A 1 g portion of 1,4-diazido-1,4-dideoxy-*muco*-inositol[20] (**10**) was hydrogenated in methanol (10 ml) in the presence of Raney nickel under a hydrogen atmosphere (3.4 kg/cm^2). After 14 h, the catalyst was removed by filtration and the filtrate was concentrated. To a solution of the residue in 50% aq methanol containing sodium hydrogencarbonate (1.4 g), a 30% solution of benzyloxycarbonyl chloride in toluene (7.4 ml) was added under ice cooling with agitation. After 12 h, the solution was neutralized with acetic acid and concentrated. The residue was purified by column chromatography using 1 : 5 (v/v) ethanol–toluene. Fractions corresponding to R_f 0.29 on TLC in the same solvent were collected and concentrated to give 1.70 g (88%) of **11**: mp 90—93 °C; IR (KBr) 1695 (NHCOO), 1520 cm^{-1} (NH); ^1H NMR (CD_3OD) δ 5.03 (s, 4, $2\times CH_2$ of benzyl), 7.29 (s, 10, $2\times C_6H_5$).

Found: C, 58.28; H, 5.90; N, 5.88%. Calcd for $C_{22}H_{26}N_2O_8\cdot 1/2\ H_2O$: C, 58.01; H, 5.98; N, 6.15%.

DL-*1,4-Diamino-1,4-bis(N-benzyloxycarbonyl)-2,3-O-cyclohexylidene-1,4-dideoxy*-muco-*inositol (12).* To a solution of **11** (1.0 g) in DMF (20 ml), 1,1-dimethoxycyclohexane (0.7 ml) and a catalytic amount of *p*-toluenesulfonic acid were added at 55—60 °C under reduced pressure (38—40 mmHg). After 2 h, triethylamine (1 ml) was added to the solution and the mixture was concentrated. The residue was purified by column chromatography using 1 : 7 (v/v) ethanol–toluene. Fractions corresponding to R_f 0.47 on TLC 1 : 5 (v/v) ethanol–toluene were collected and concentrated. The residue was recrystallized from ether to give 0.93 g (79%) of **12**: mp 98—100 °C; IR (KBr) 1705 (NHCOO), 1520 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, cyclohexylidene), 5.06 (s, 4, $2\times CH_2$ of benzyl), 5.06 (m, 1, NH), 5.77 (d, 1, J=9 Hz, NH), 7.33 (s, 10, $2\times C_6H_5$).

Found: C, 63.61; H, 6.62; N, 5.19%. Calcd for $C_{28}H_{34}N_2O_8$: C, 63.86; H, 6.51; N, 5.32%.

DL-*1,4-Diamino-1-N-benzyloxycarbonyl-4,5-N,O-carbonyl-2,3-O-cyclohexylidene-1,4-dideoxy*-muco-*inositol (13).* To a solution of **12** (1.0 g) in 60% aq dioxane (30 ml), sodium carbonate (0.2 g) was added. After 17 h at 65 °C the solution was diluted with water (50 ml) and repeatedly extracted with chloroform. The combined layer was concentrated, and the residue was purified by column chromatography, using 2: 3 (v/v) acetone–toluene. Fractions corresponding to R_f 0.26 on TLC in the same solvent were combined and concentrated to give 0.65 g (82%) of **13**: mp 105—107 °C; IR (KBr) 1765 (*cis* cyclic carbamate), 1710 cm^{-1} (NHCOO); ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, cyclohexylidene), 4.72 (t, 1, J=8.7 Hz, H-2), 5.09 (s, 2, CH_2), 5.84 (s, NH-1), 6.79 (s, 1, NH-4), 7.34 (s, 5, C_6H_5).

Found: C, 60.47; H, 6.43; N, 6.50%. Calcd for $C_{21}H_{26}N_2O_7$: C, 60.27; H, 6.26; N, 6.70%.

DL-*1,4-Diamino-4-N-benzyl-1-N-benzyloxycarbonyl-4,5-N,O-carbonyl-2,3-O-cyclohexylidene-1,4-dideoxy*-muco-*inositol (14).* To a stirred solution of **13** (0.10 g) in DMF (4 ml), benzyl bromide (0.06 ml) was added in the presence of silver oxide (0.07 g). After 18 h, the reaction mixture was filtered and the filtrate was diluted with water. The solution was extracted with chloroform and recrystallized from chloroform–ether to give 73 mg (56%) of **14**: mp 166 °C; IR (KBr) 1760 (carbamate); ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, C_6H_{10}), 4.18 (d, 1, J=14.5 Hz, CH_2 of *N*-benzyl), 4.73 (d, 1, J=14.5 Hz, CH_2 of *N*-benzyl), 4.56 (t, 1, J=8.2 Hz, H-5), 5.12 (s, 2, CH_2 of benzyloxycarbonyl), 7.41 (s, 10, $2\times C_6H_5$).

Found: C, 66.31; H, 6.40; N, 5.50%. Calcd for $C_{28}H_{32}N_2O_7$: C, 66.12; H, 6.34; N, 5.51%.

DL-*1,4-Diamino-4-N-benzyl-1-N-benzyloxycarbonyl-4,5-N,O-carbonyl-2,3-O-cyclohexylidene-1,4-dideoxy-1-N-methyl-6-O-methyl*-muco-*inositol (15).* To a stirred solution of **14** (0.56 g) in DMF (10 ml), methyl iodide (0.34 ml) and silver oxide (0.77 g) were added. After 18 h, The mixture was filtered and the filtrate was concentrated. The residue was purified by column chromatography, using 1 : 5 (v/v) acetone–toluene. Fractions corresponding to R_f 0.35 in the same solvent were collected to give 0.49 g (82%) of **15**: mp 107—110 °C; IR (KBr) 1763 (carbamate), 1696 cm^{-1} (NHCOO); ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, C_6H_{10}), 3.30 (s, 3, NCH_3), 3.49 (s, 3,

† 1 mmHg≈133.322 Pa.

OCH_3), 4.15 (d, 1, J=14.5 Hz, CH_2 of *N*-benzyl) 4.49 (t, 1, J=8.8 Hz, H-2), 4.77 (d, 1, J=14.5 Hz, CH_2 of *N*-benzyl), 5.14 (s, 2, CH_2 of benzyloxycarbonyl), 7.37 (s, 10, 2×C_6H_5).

Found: C, 67.36; H, 6.85; N, 5.19%. Calcd for $C_{30}H_{36}N_2O_7$: C, 67.14; H, 6.76; N, 5.22%.

DL-*1,4-Diamino-1,4-bis(*N-*benzyloxycarbonyl)-2,3-*O-*cyclohexylidene-1,4-dideoxy-1-*N-*methyl-6-*O-*methyl-*muco-*inositol (**16**)* Compound **15** (0.32 g) was hydrogenated in ethanol (10 ml) in the presence of 10% Pd on charcoal (30 mg) under a hydrogen atmosphere (3.4 kg/cm²). After 16 h, the catalyst was filtered off and the filtrate was concentrated. The residue was dissolved in dioxane (10 ml) and a 0.04 M $Ba(OH)_2$ solution (10 ml) was added. After 2 h at 100 °C, the solution was neutralized with CO_2 gas, and the resulting precipitate was removed by filtration. The filtrate was concentrated, and the residue was dissolved in aq. methanol (20 ml). To the solution, sodium hydrogencarbonate (0.2 g) and a 30% solution of benzyloxycarbonyl chloride in toluene (1 ml) were under ice cooling. After 4 h, the solution was concentrated, and residue was purified by column chromatography, using 1 : 5 (v/v) acetone–toluene. Fractions corresponding to R_f 0.26 on TLC in the same solvent were collected and concentrated to give 0.28 g (83%) of **16**: mp 77—82 °C; IR (KBr) 1695 (NHCOO), 1520 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 1.55 (m, 10, C_6H_{10}), 3.17 (s, 3, NCH_3), 3.32 (s, 3, OCH_3), 3.61 (s, 1, H-3), 4.63 (d, 1, J=5.2 Hz, H-4), 5.08 (s, 2, CH_2 of benzyl), 5.11 (s, 2, CH_2 of benzyl), 5.67 (d, 1, J=7.6 Hz, NH-1), 7.35 (s, 10, 2×C_6H_5).

Found: C, 63.72; H, 6.78; N, 4.85%. Calcd for $C_{30}H_{38}N_2O_8 \cdot 1/2\ H_2O$: C, 63.92; H, 6.97; N, 4.97%.

DL-*1,4-Diamino-1,4-bis(*N-*benzyloxycalbonyl)-2,3-*O-*cyclohexylidene-1,4-dideoxy-1-*N-*methyl-6-*O-*methyl-*chiro-*insoitol (**18**).* To a stirred solution of **16** (0.27 g) in pyridine (10 ml), methanesulfonyl chloride (2.7 ml) was added. After 27 h, the solution was concentrated and the residue was dissolved in chloroform. The solution was washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography, using 1 : 7 (v/v) acetone–toluene. Fractions corresponding to R_f 0.36 on TLC in the same solvent were collected and concentrated to give 0.22 g of the 5-*O*-mesyl derivative (**17**) as a residue. The residue (0.69 g) was dissolved in 90% aq DMF (60 ml) containing sodium acetate (4.4 g), after which the solution was heated under reflux. After 22 h, the solution was concentrated and the residue was dissolved in 50% aq dioxane (30 ml) containing barium hydroxide (1.2 g); the solution was then heated under reflux. After 5 h, the solution was neutralized with CO_2 gas and the resulting precipitate was removed by filtration. The filtrate was concentrated, and the residue was dissolved in aq methanol (40 ml). To the solution, sodium hydrogencarbonate (0.36 g) and a 30% solution of benzyloxycarbonyl chloride in toluene (1.85 ml) were added under ice cooling. After 4 h, the solution was concentrated and the residue was purified by column chromatography, using 1 : 5 (v/v) acetone–toluene. Fractions corresponding to R_f 0.40 on TLC in the same solvent were collected and concentrated to give 0.23 g (38%) of **18** as a syrup: ^1H NMR ($CDCl_3$) δ 1.5 (m, 10, C_6H_{10}), 3.08 (s, 3, NCH_3), 3.40 (s, 3, OCH_3), 4.51 (dd, 1, J=3.6 Hz, J=6.1 Hz, H-1), 5.09 (s, 2, CH_2 of benzyl), 5.13 (s, 2, CH_2 of benzyl), 5.20 (d, 1, J=7.5 Hz, NH-4), 7.37 (s, 10, 2×C_6H_5).

DL-*1,4-Diamino-1,2 : 4,5-di-*N,O-*carbonyl-1,4-dideoxy-1-*N-*methyl-6-*O-*methyl-*chiro-*inositol (**19**).* To a solution of **18** (192 mg) in DMF (10 ml), 50% sodium hydride (42 mg) was added under ice cooling. After 3 h, the solution was diluted with water and neutralized with acetic acid. The solution was extracted with chloroform, and the organic layer was concentrated. The residue was hydrolyzed in 50% aq acetic acid (10 ml) at 60 °C for 2 h and then concentrated. To a solution of the residue in DMF (10 ml), 50% sodium hydride (28 mg) was added, after which the mixture was worked up as has been described above. The residual product was purified on a silica-gel column, using 1 : 10 (v/v) methanol–toluene. Fractions corresponding to R_f 0.07 in the same solvent were collected and concentrated. The residue was recrystallized from chloroform to give 64 mg (72%) of **19**: mp 224—228 °C; IR (KBr) 1775 (*trans* cyclic carbamate), 1725 cm^{-1} (*cis* cyclic carbamate); ^1H NMR (CD_3OD) δ 3.02 (s, 3, NCH_3), 3.74 (s, 3, OCH_3), 4.64 (dd, 1, J=6.4 Hz, J=7.7 Hz, H-5).

Found: C, 46.24; H, 5.35; N, 10.57%. Calcd for $C_{10}H_{14}N_2O_6$; C, 46.51; H, 5.46; N, 10.85%.

*Condensation of **9** (L-Form) with **5**.* Compound **9** (0.10 g) was dissolved in freshly distilled dioxane (4 ml) at 100 °C. After the solution had cooled to the ambient temperature, **5** (0.58 g) and silver trifluoromethanesulfonate (0.29 g) were added, portion by portion to the solution (under N_2) with agitation in the dark. After 27 h, acetone was added to the reaction mixture, and it was filtered. The filtrate was concentrated, and the residue was purified by column chromatography, using 1 : 3 (v/v) acetone–toluene. Fractions corresponding to R_f 0.26 on TLC in the same solvent were collected and concentrated to give 0.11 g (37%) of **20**: mp 226—228 °C; $[\alpha]_D^{20}$ +43.2° (*c* 1.01, acetone); ^1H NMR (acetone-d_6) δ 1.44 (d, 3, $J_{6',7'}$=6.3 Hz, H-7'), 2.85 (s, 3, NCH_3), 3.61 (s, 3, OCH_3), 4.67 (dd, 1, J=6.0 Hz, J=7.5 Hz, H-5), 5.53 (d, 1, $J_{1',2'}$=3.3 Hz, H-1'), 7.20 (s, 1, NH-1), 7.3 (d, 1, J=9.0 Hz), 7.4 (d, 1, J=9.0 Hz), 8.24 (dd, 2, J=2.4 Hz, J=9.0 Hz), 8.92 (d, 1, J=2.4 Hz), 8.94 (d, 1, J=2.4 Hz) (total 6H, DNP), 8.82 (d, 2, J=8.1 Hz, NH-2',6').

Found: C, 47.50; H, 4.47; N, 14.99%. Calcd for $C_{29}H_{32}N_8O_{15}$: C, 47.54; H, 4.40; N, 15.30%.

Fractions corresponding to R_f 0.23 on TLC were collected to give 25 mg (9%) of the corresponding β-anomer **21**: mp 180—182 °C; $[\alpha]_D^{20}$ −12.5° (*c* 1.00, acetone); ^1H NMR (acetone-d_6) δ 1.47 (d, 3, $J_{6',7'}$=6.3 Hz, H-7'), 2.82 (s, 3, NCH_3), 3.44 (s, OCH_3), 4.48 (dd, 1, J=6.0 Hz, J=7.5 Hz), 5.13 (d, 1, $J_{1',2'}$=8.1 Hz), 6.33 (s, 1, NH-1), 7.33 (d, 1, J=9.3 Hz), 7.49 (d, 1, J=9.3 Hz), 8.29 (dd, 2, J=3.0 Hz, J=9.3 Hz), 8.94 (d, 1, J=3.0 Hz), 8.96 (d, 1, J=3.0 Hz) (total 6H, DNP), 8.64 (d, 1, J=8.4 Hz), 8.9 (d, 1, NH).

Found: C, 47.25; H, 4.45; N, 14.98%. Calcd for $C_{29}H_{32}N_8O_{15}$: C, 47.54; H, 4.40; N, 15.30%.

*Condensation of **19** (DL-Form) with **5**.* Compound **19** (49 mg) was dissolved in dioxane (5 ml). To the solution, **5** (288 mg) and silver trifluoromethanesulfonate (145 mg) were added, after which the reaction mixture was worked up as was described in the case of the previous condensation of **9** with **5**. Fractions corresponding to R_f 0.34 on TLC in 1 : 3 (v/v) acetone–toluene were collected and concentrated to give 28 mg (20%) of **22**: mp 306—307 °C; $[\alpha]_D^{20}$ +100.7° (*c* 1.12, acetone); ^1H NMR (DMSO-d_6) δ 1.36 (d, 3, $J_{6',7'}$= 5.1 Hz, H-7'), 2.80 (s, 3, NCH_3), 3.48 (s, OCH_3), 4.60 (dd, 1, J=6.0 Hz, J=7.5 Hz, H-5), 5.20 (d, 1, $J_{1',2'}$=3.0 Hz, H-1'), 8.16 (s, 1, NH-1), 7.23 (d, 2, J=10.8 Hz), 8.23 (m, 2), 8.65 (m, 2) (total 6H, DNP), 8.65 (d, 1, J=9 Hz, NH-2',6').

Found: C, 47.30; H, 4.37; N, 15.10%. Calcd for $C_{29}H_{32}N_8O_{15}$: C, 47.54; H, 4.40; N, 15.30%.

Fractions (R_f 0.26) were collected and concentrated to give 26 mg (19%) of **23**: mp 226—228 °C; $[\alpha]_D^{20}$ +41.9° (*c* 0.48, acetone); ^1H NMR (acetone-d_6) δ 1.44 (d, 3, $J_{6',7'}$= 6.3 Hz, H-7'), 2.87 (s, 3, NCH_3), 3.58 (s, 3, OCH_3), 4.66 (dd, 1, J=6.0 Hz, J=7.5 Hz, H-5), 5.54 (d, 1, J=3.3 Hz), 7.23

(s, 1, NH-1), 7.36 (d, 1, J=9.0 Hz), 7.46 (d, 1, J=9.0 Hz), 8.34 (dd, 2, J=2.4 Hz, J=9.0 Hz), 9.00 (d, 1, J=2.4 Hz), 9.02 (d, 1 J=2.4 Hz) (total 6H, DNP), 8.88 (d, 2, J=8.1 Hz, NH-2′,6′).

Fractions (R_f 0.23) were collected and concentrated to give 31 mg (23%) of a mixture of β-anomer **24**: ^{1}H NMR (acetone-d_6) δ 1.46 (d, 3, $J_{6',7'}$=6.3 Hz, H-7′), 2.77·2.99 (2s, 3, NCH_3), 3.37·3.40 (2s, 3, OCH_3), 4.91·5.05 (2d, 1, $J_{1',2'}$=8.1 Hz, H-1′), 6.30·6.33 (2s, 1, NH-1), 7.2—7.6 (m, 2), 8.2 (dd, 2, J=3.0 Hz, J=9.0 Hz), 8.86·8.89 (2d, 2, J=3.0 Hz) (total 6H, DNP), 8.56 (d, 1, J=8.1 Hz, NH), 8.8 (d, 1, NH).

*Fortimicin B (**1b**).* To a solution **23** (28 mg) in a mixture of acetone (2 ml) and methanol (6 ml), Amberlite IRA 400 (OH^-) resin (0.23 ml) and water (2 ml) were added. After 15 h at the ambient temperature with agitation, the resin was filtered off and the filtrate was concentrated to give 17 mg of a residue. The residue was dissolved in ethanol (4 ml), and to the solution, a 0.1 M barium hydroxide solution (10 ml) was added. The mixture was then heated at 85 °C overnight and neutralized with CO_2. The precipitate was filtered off, and the filtrate was concentrated. The residue was purified on a column of Amberlite CG-50 (NH_4^+) by the use of 1 M ammonia. Fractions showing ninhydrin-positive and R_f 0.38 on TLC in 1 : 1 : 4 (v/v) chloroform–concd ammonia–isopropyl alcohol were combined and concentrated to give 13 mg (98%) of **1b** as an amorphous residue, $[\alpha]_D^{16}$ +25.3° (*c* 0.65, water). Lit,[17] $[\alpha]_D^{25}$ +22.2° (*c* 0.1, water); ^{1}H NMR (D_2O) δ 1.07 (d, 3, $J_{6',7'}$=6.6 Hz, H-7′), 2.40 (s, 3, NCH_3), 3.46 (s, 3, OCH_3), 3.98 (dd, 1, J=4.5 Hz, J=9.2 Hz, H-5), 5.03 (d, 1, $J_{1',2'}$=3.0 Hz).

*Tetra-N-acetylfortimicin B (**25**).* To a solution of **1b** (13 mg) in methanol (4 ml), acetic anhydride (0.5 ml) was added. After 3 h, the solution was concentrated to give 18 mg (96%) of **25** as a monohydrate: mp 161—163 °C; $[\alpha]_D^{20}$ +91.6° (*c* 0.61, methanol). Lit,[22] $[\alpha]_D$ +92.72° (*c* 1.0, methanol). ^{1}H NMR (D_2O) δ 1.15 (d, 3, $J_{6',7'}$=6.0 Hz, H-7′), 2.00 (s, 3, NAc), 2.02 (s, 3, NAc), 2.05 (s, 3, NAc), 2.18 (s, 3, NAc), 3.10 (d, 3, J=10.2 Hz, NCH_3), 3.44 (s, 3, OCH_3).

*2′,6′-Bis(N-benzyloxycarbonyl)-1,2:4,5-di-N,O-carbonylfortimicin B (**27**).* To a solution of 1,2′,4,6′-tetrakis-*N*-(benzyloxycarbonyl)foltimicin B[19] (**26**, 0.50 g) in DMF (6 ml), 50% sodium hydride (70 mg) was added under ice cooling with agitation. After 6 h at the ambient temperature, the pH of the solution was adjusted to 4 by adding acetic acid. The solution was then diluted with cold water, and the precipitates were collected by filtration. Recrystallization of the product from chloroform–ether gave 0.31 g (81%) of **27**: mp 121—128 °C; $[\alpha]_D^{20}$ −1.6° (*c*, 1.3, methanol); ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 3, $J_{6',7'}$=6.3 Hz, H-7′), 2.83 (s, 3, NCH_3), 3.49 (s, 3, OCH_3), 6.75 (s, 1, NH-1), 7.36 (s, 10, 2×C_6H_5).

Found: C, 59.42; H, 6.12; N, 8.16%. Calcd for $C_{33}H_{40}N_4O_{11}$: C, 59.27; H, 6.03; N, 8.38%.

*1,2:4,5-Di-N,O-carbonyl-2′,6′-bis(N-2,4-dinitrophenyl)fortimicin B (**28**).* Compound **27** (150 mg) was hydrogenated in methanol (10 ml) in the presence of 10% Pd on charcoal (30 mg) under a hydrogen atmosphere (3.4 kg/cm^2). After 16h, the catalyst was filtered off and the filtrate was concentrated. To a solution of the residue in 67% aq ethanol (25 ml) containing sodium hydrogencarbonate (57 mg), 2,4-dinitrofluorobenzene (104 mg) was added under ice cooling. After 18 h, the solution was diluted with water and repeatedly extracted with chloroform. The combined organic layers were concentrated, and the residue was recrystallized from acetone–chloroform to give 139 mg (85%) of **28**: mp 226—228 °C; $[\alpha]_D^{20}$ +40.7° (*c* 0.87, acetone); IR (KBr) 1770 (cyclic carbamate), 1624·1594 (nitrophenyl), 1525·1535 cm^{-1} (NO_2); ^{1}H NMR (acetone-d_6) δ 1.45 (d, 3, $J_{6',7'}$=6.3 Hz, H-7′), 2.86 (s, 3, NCH_3), 3.61 (s, 3, OCH_3), 4.66 (dd, 1, J=6.0 Hz, J=7.5 Hz, H-5), 5.53 (d, 1 $J_{1',2'}$=3.3 Hz, H-1′), 7.20 (s, 1, NH-1), 7.32·7.42 (2d, 2, J=9.0 Hz), 8.26 (dd, 2, J=2.4 Hz, J=9.0 Hz), 8.94·8.96 (2d, 2, J=2.4 Hz), 8.83 (d, 2, NH-2′ and NH-6′).

Found: C, 47.23; H, 4.47; N, 14.91%. Calcd for $C_{29}H_{32}N_8O_{15}$: C, 47.54; H, 4.40; N, 15.30%.

*Tetra-N-acetylfortimicin B (**29**).* To a solution of natural fortimicin B (58.8 mg) in methanol (10 ml), acetic anhydride (4 ml) was added. After 3 h, the solution was concentrated to give 82.2 mg (94%) of **29** as a monohydrate: mp 161—163 °C; $[\alpha]_D^{20}$ +92.5° (*c* 0.72, methanol); ^{1}H NMR (D_2O) δ 1.17 (d, 1, $J_{6',7'}$=7.2 Hz, H-7′), 2.00 (s, 3, NAc), 2.02 (s, 3, NAc), 2.05 (s, 3, NAc), 2.18 (s, 3, NAc), 3.09 (d, 3, J=10.6 Hz, NCH_3), 3.43 (s, 3, OCH_3).

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References

1) T. Nara, M. Yamamoto, I. Kawamoto, K. Takayama, R. Okachi, S. Takasawa, T. Sato, and S. Sato, *J. Antibiot.*, **30**, 533 (1977).

2) R. Okachi, S. Takasawa, T. Sato, S. Sato, M. Yamamoto, I. Kawamoto, and T. Nara, *J. Antibiot.* **30**, 541 (1977).

3) M. Sugimoto, S. Ishii, R. Okachi, and T. Nara, *J. Antibiot.*, **32**, 868 (1979).

4) T. Iida, M. Sato, I. Matsubara, Y. Mori, and K. Shirahata, *J. Antibiot.*, **32**, 1273 (1979).

5) S. Inouye, T. Shomura, and K. Ohba, *J. Antibiot.*, **33**, 510 (1980).

6) S. Inouye, K. Ohba, T. Shomura, M. Kojima, T. Tsuruoka, J. Yoshida, N. Kato, M. Ito, S. Amano, S. Omoto, N. Ezaki, and T. Niida, *J. Antibiot.*, **32**, 1354 (1979).

7) T. Shomura, M. Kojima, J. Yoshida, M. Ito, S. Amano, K. Totsugawa, T. Niwa, S. Inouye, T. Ito, and T. Niida, *J. Anitibiot.* **33**, 924 (1980).

8) T. Deushi, A. Iwasaki, K. Kamiya, T. Kunieda, T. Mizuguchi, M. Nakayama, H. Itoh, T. Mori, and T. Oda, *J. Antibiot.*, **32**, 173 (1979).

9) A. Iwasaki, H. Itoh, and T. Mori, *J. Antibiot.*, **32**, 180 (1979).

10) T. Deushi, M. Nakayama, I. Watanabe, and T. Mori, *J. Antibiot.*, **32**, 187 (1979).

11) T. Mori, T. Deushi, A. Iwasaki, T. Kunieda, T. Mizoguchi, K. Kamiya, M. Nakayama, H. Itoh, and T. Oda, Ger. Offen, 2813021, Oct. 5, 1978; Japan Kokai, 79-66603, May 29, 1979.

12) T. Suami, Y. Honda, and T. Kato, *Chem. Lett.*, **1978**, 1125.

13) T. Suami, Y. Honda, T. Kato, M. Masu, and K. Matsuzawa, *Bull. Chem. Soc. Jpn.*, **53**, 1372 (1980).

14) Y. Honda and T. Suami, *Bull. Chem. Soc. Jpn.*, **54**, 2825 (1981).

15) T. Suami and Y. Honda, *Chem. Lett.*, **1980**, 641.

16) H. Sano, K. Shirahata, and Y. Mori, Abstract paper of the Annual Meeting of the Chemical Society of Japan, 3S01, p, 1107 (1980), delivered on April 3, 1980, Osaka, Japan.

17) R. S. Egan, R. S. Stanaszek ,M. Cirovic, S. L. Mueller, J. Tadanier, J. R. Martin, P. Collum, A. W. Goldstein, R. L. Devault, A. C. Sinclair, E. E. Fager, and L. A. Mitscher, *J.*

Antibiot., **30**, 552 (1977).

18) J. Tadanier, J. R. Martin, P. Kurath, A. W. Goldstein, and P. Johnson, *Carbohydr. Res.*, **79**, 91 (1980).

19) H. Sano, T. Sakaguchi, and Y. Mori, *Bull. Chem. Soc. Jpn.*, **52**, 2727 (1979).

20) T. Suami, F. W. Lichtenthaler, and Ogawa, *Bull. Chem. Soc. Jpn.*, **40**, 1488 (1967); a mixture of 2,4,5,6-tetra-*O*-acetyl-1,3-di-*O*-tosyl-*myo*-inositol[21] (3.0 g) and sodium azide (3.0 g) in 90% aq 2-methoxyethanol (54 ml) was heated under reflux. After 45 h, the mixture was filtered and the filtrate was concentrated. The residue was extracted with hot ethyl acetate, and the organic layer was concentrated. The residual product was recrystallized from ethyl acetate to give 0.56 g (53%) of 1,4-diazido-1,4-dideoxy-*muco*-inositol; mp 139—140 °C. Found: C, 31.28; H, 4.38; N, 36.36%. Calcd for $C_6H_{10}N_6O_4$: C, 31.31; H, 4.38; N, 36.51%.

21) S. J. Angyal, P. T. Gilham, and G. J. H. Melrose, *J. Chem. Soc.*, **1965**, 5252.

22) R. L. Giralami and J. M. Stamm, *J. Antibiot.*, **30**, 564 (1977).