SYNTHESES OF SPORARICIN ANALOGUES, 2-DEOXY-4-N-GLYCYL-6-O-(α-NEBROSAMINYL)FORTAMINE AND ITS 3-DE-O-METHYL COMPOUND

TAKASHI YAMAGUCHI, YOSHINORI KYOTANI, ISAMU WATANABE, SEIICHI SATO, YOSHIO TAKAHASHI, MASAHIKO NAGAKURA and TOSHIHITO MORI

Tokyo Research Laboratories, Kowa Co., Ltd.,
Higashimurayama, Tokyo 189, Japan

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2-Deoxy-4-N-glycyl-6-O-(α-nebrosaminyl)fortamine (**21**) and 3-de-O-methyl-2-deoxy-4-N-glycyl-6-O-(α-nebrosaminyl)fortamine (**27**) were prepared starting from lividamine. The syntheses include four key steps, that is, transformation of 2-deoxystreptamine moiety of lividamine to 4-N,3-O-didemethyl-2-deoxyfortamine, selective 4-N-methylation of the new aminocyclitol moiety, selective attachment of a glycyl residue to the methylamino group at C-4 and selective amination at C-6′.

Recently we reported[1~3] a new aminoglycoside antibiotic sporaricin A (**1**) which was highly active against Gram-positive and Gram-negative bacteria including aminoglycoside-resistant strains. The compound was found[3] to be structurally related to fortimicin A (**2**)[4~6].

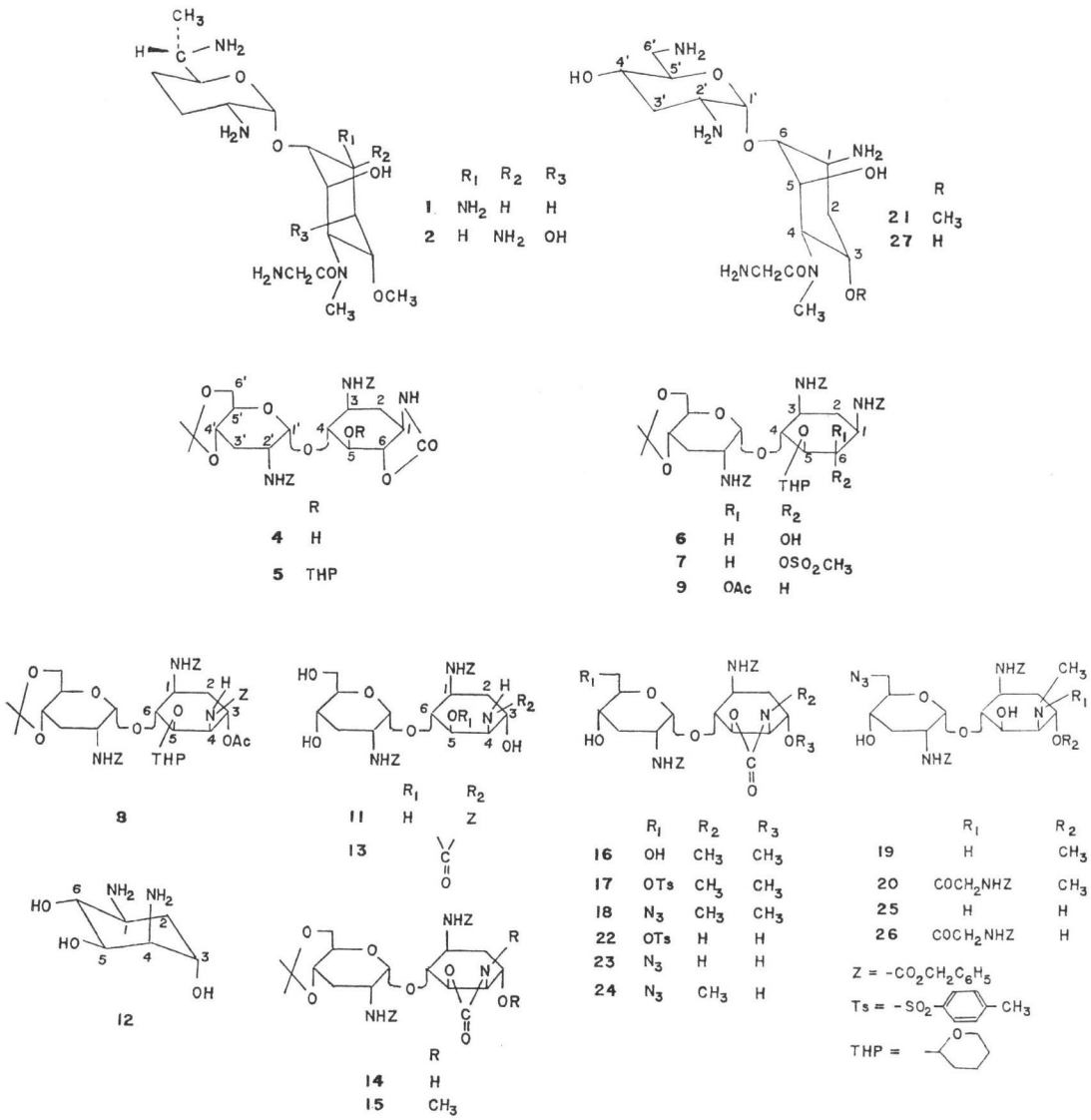
Fortimicin A and sporaricin A are similar but unique in structure among aminoglycoside antibiotics since they have a glycyl residue attached to their respective 1,4-diaminocyclitol moieties. The contributing factor for the antibacterial activity of the compounds towards resistant bacteria, however, is thought to be the lack of the hydroxyl groups at C-3′ and 4′ in their molecules. On the other hand, in ribostamycin, removal of only the 3′-hydroxyl group gave rise to a compound more active than the ribostamycin derivative lacking the hydroxyl groups at C-3′ and 4′[7]. Judging from the above result, we thought it meaningful to prepare sporaricin analogues which bore the 4′-hydroxyl group. In this paper we describe the syntheses of 2-deoxy-4-N-glycyl-6-O-(α-nebrosaminyl)fortamine(**21**) and its 3-de-O-methyl analogue(**27**). As the starting material, we chose lividamine[8], which was easily obtained by hydrolysis of lividomycins.

The problems we encountered in these syntheses were the transformation of the 2-deoxystreptamine moiety of lividamine to 4-N,3-O-didemethyl-2-deoxyfortamine moiety, selective 4-N-methylation of the new-born aminocyclitol moiety and selective attachment of a glycyl residue to the methylamino group at C-4 of the moiety.

At first we describe the transformation of the aminocyclitol portion. 3,2′-Bis(N-benzyloxycarbonyl)-1-N:6-O-carbonyllividamine[9] (**3**), which was prepared from lividamine in two steps, was treated with 2,2-dimethoxypropane in the presence of acidic catalyst to give the 4′,6′-O-isopropylidene derivative (**4**). Treatment of **4** with 3,4-dihydro-2*H*-pyran in the presence of acidic catalyst gave diastereoisomeric 5-O-tetrahydropyranyl derivatives (**5** and **5′**) which were formed in almost equal amounts. Alcoholysis of the mixture of **5** and **5′** with sodium benzylate in benzyl alcohol cleaved the 1,6-carbamate to give the 1-N-benzyloxycarbonyl-6-hydroxyl derivatives (**6** and **6′**). One of them (**6**) showing lower

Rf value on tlc was treated with mesyl chloride in pyridine to give the 6-O-mesyl derivative (**7**). Mesylation of the other diastereomer showing higher Rf value was, however, unsuccessful recovering the starting material (**6'**). Treatment of **7** with sodium acetate in N,N-dimethylformamide (DMF) gave two products. The major one (**8**) was proved to have the 3-O-acetyl-4-N-benzyloxycarbonyl-4-N,3-O-didemethyl-2-deoxyfortamine structure as the aminocyclitol portion. The conversion of the 2-deoxystreptamine moiety of **7** to the 4-N,3-O-didemethyl-2-deoxyfortamine structure was confirmed by the fact that **8** was led to 4-N,3-O-didemethyl-2-deoxyfortamine (**12**) by successive O-deblocking (to give **11**), acidic hydrolysis and catalytic hydrogenolysis. The structure of **12** was positively determined by its ^{1}H-NMR spectrum (Fig. 2). By the spin decoupling method, the *trans*-diaxial relationship of the 3-hydroxyl and 4-amino groups was shown. The formation of **8** was explained by *trans*-diaxial opening of an intermediate N-benzyloxycarbonylaziridine, which, in turn, was formed by displace-

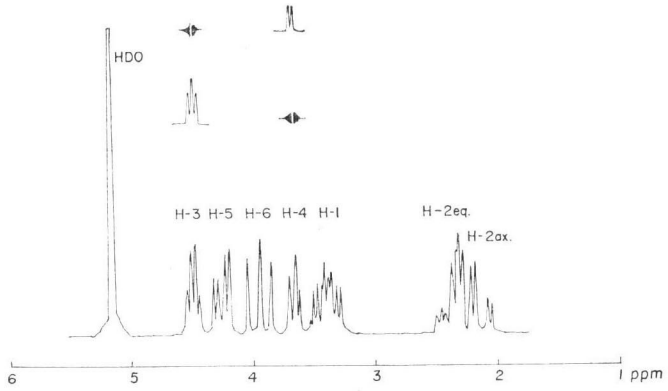
Fig. 1.



ment of the mesyloxy group in **7**.

As for the minor product (**9**), it was assumed to be a direct substituted product, that is, a product having a 6-*epi*-2-deoxystreptamine moiety. The reason for the assignment was that the tris(N-benzyloxycarbonyl) derivative (**10**) formed by de-O-protection of **9** consumed 1 mole of periodate after 24 hours, but was not identical with tris(N-benzyloxycarbonyl)lividamine.

Fig. 2. 100 MHz ^{1}H-NMR spectrum of **12** in D_2O.



In the next place, introduction of methyl groups to the 3-hydroxyl and 4-amino groups of the 4-N,3-O-didemethyl-2-deoxyfortamine portion is described. Treatment of **11** with sodium hydride in N,N-dimethylformamide gave a 4,5-cyclic carbamate derivative (**13**). This was indicated by the presence of an absorption peak at 1765 cm^{-1} in its IR spectrum. Since the relationship between the benzyloxycarbonylamino group at C-4 and the hydroxyl group at C-3 was *trans*-diaxial, the carbamate of **13** was assumed to be the 4,5- and not the 3,4-cyclic carbamate. Treatment of **13** with 2,2-dimethoxypropane in the presence of acidic catalyst gave a 4′,6′-O-isopropylidene product (**14**). The compound **14** was then treated with a mixture of methyl iodide, barium oxide and barium hydroxide in N,N-dimethylformamide, when 4-N,3-O-dimethyl derivative (**15**) was produced in 67% yield. Selective introduction of a methyl group at the C-4 amino group is described later (see the description of compound **19**).

In the next place, introduction of an amino group at C-6′ is described. Acidic solvolysis of **15** gave the diol (**16**). The compound **16** was treated with tosyl chloride to give the 6′-tosyl derivative (**17**). The tosyloxy group was then displaced with azido group. Selective hydrolysis of the cyclic carbamate of the azido derivative (**18**) was performed by use of a limited amount of barium hydroxide in dioxane to give the methylamino alcohol (**19**). In the ^{1}H-NMR spectrum of **19**, an up-field shift ($\approx$0.4 ppm) of the N-methyl resonance at C-4 was observed in comparison to the corresponding N-methyl resonances of compounds **15**$\sim$**18**. This indicates that the N-methyl groups of **15**$\sim$**18** are attached to the position bearing carbamate groups, respectively.

The compound **19** was then condensed with N-benzyloxycarbonylglycine by the active ester method using N-hydroxysuccinimide to give the 4-N-(N-benzyloxycarbonylglycyl) derivative (**20**) in 83% yield. Finally, catalytic reduction of the azido group and hydrogenolysis of the protecting groups with palladium black gave the target product (**21**).

The synthesis of another target compound (**27**) will be described next.

Selective tosylation of **13** followed by substitution with sodium azide gave the 6′-azido compound (**23**). Treatment of **23** with limited amounts of methyl iodide, barium oxide and barium hydroxide gave the 4-N-methyl derivative (**24**) in 90% yield. Under these conditions O-methylation was substantially avoided. Selective hydrolysis of the cyclic carbamate of **24** by a similar manner as described in the preparation of **19** gave the methylamino alcohol (**25**). Introduction of a N-benzyloxycarbonyl-

glycyl group to the methylamino group at C-4 of **25** gave the condensation product (**26**) in 57% yield. Catalytic reduction and hydrogenolysis of **26** gave the deblocked title compound (**27**).

In the ^{1}H-NMR spectrum of **21** (Fig. 3), the resonance of H-4 (5.12 ppm) exhibited one large coupling ($J_{3,4}=11.6$ Hz). This indicates that the relationship between H-3 and H-4 is *trans*-diaxial. Judging from the above and the other ^{1}H-NMR data, the cyclitol ring of **21** is in the $^1C_4$ conformation in spite of the $^4C_1$ conformation of **12**. This conformational inversion was also observed in the case of **27**. These phenomena accord with the relationship between fortimicins A and B[6].

Fig. 3. 100 MHz ^{1}H-NMR spectrum of **21** in D_2O.

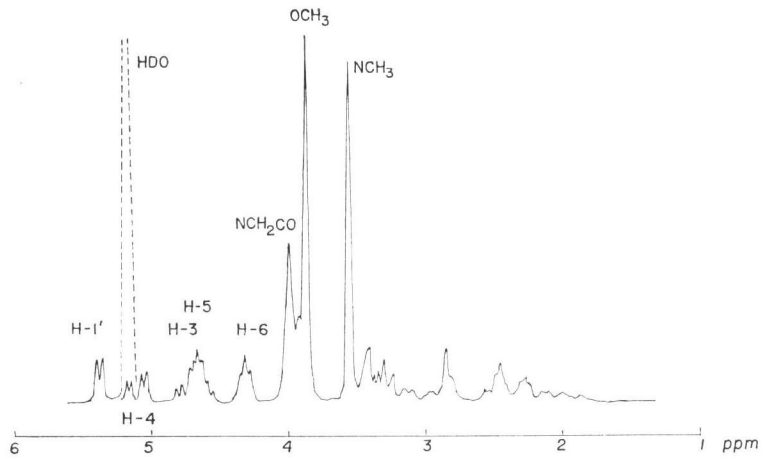


Table 1. Antimicrobial spectra of **21**, **27** and sporaricin A.

Test organisms	M.I.C. (mcg/ml)		
	21	**27**	Sporaricin A
Staphylococcus aureus FDA 209P	0.78	0.78	0.20
Bacillus anthracis	0.78	1.56	0.20
Bacillus cereus	3.13	1.56	0.78
Bacillus subtilis ATCC 6633	0.20	0.39	0.20
Streptococcus faecalis	50	50	12.5
Escherichia coli NIHJ	6.25	6.25	1.56
Escherichia coli K-12 ML1410	6.25	12.5	1.56
Escherichia coli K-12 ML1410 R-81[a]	6.25	6.25	1.56
Escherichia coli K-12 ML1410 R-83[b]	12.5	12.5	1.56
Escherichia coli K-12 ML1410 R-101[c]	6.25	12.5	1.56
Proteus vulgaris OX-19	3.13	3.13	0.78
Proteus inconstans[d]	1.56	3.13	0.78
Klebsiella pneumoniae PCI 602	3.13	6.25	0.78
Pseudomonas aeruginosa No. 99[e]	>100	>100	>100
Pseudomonas aeruginosa TI-13[a]	6.25	3.13	3.13
Pseudomonas aeruginosa A_3	6.25	3.13	3.13
Pseudomonas aeruginosa GN 157[b]	6.25	3.13	6.25
Pseudomonas aeruginosa GN 315[f]	>100	>100	6.25
Serratia sp.	1.56	3.13	0.78

Medium: nutrient agar (Eiken Chemical Co., Ltd., Japan)
a) APH(3′)-I b) APH(3′)-II c) AAD(2″) d) AAC(2′) e) AAC(3)-I f) AAC(6′)

The minimal inhibitory concentrations of **21** and **27** are compared with those of sporaricin A in Table 1. Antimicrobial activities of **21** and **27** were weaker than the activity of sporaricin A. The compound **27** showed similar activity to that of sporaricin A against *Pseudomonas*.

We obtained 2-deoxyfortimicin B, the aminocyclitol of which was identical with those of **21** and **27** in N,O-situation and configuration, from the culture broth of *Saccharopolyspora hirsuta* subsp. *kobensis* KC 6606, together with sporaricins A and B. On this subject we will report elsewhere.

Experimental

Thin-layer chromatography (tlc) was carried out on E. Merck DC-Alufolien $60F_{254}$ with anisaldehyde-sulfuric acid spray for detection unless otherwise stated. For column chromatography, silica gel (Wakogel C-200) was used. Optical rotations were measured on a Digital polarimeter DIP-4 of Japan Spectroscopic Co., Ltd. Infrared and ^{1}H-NMR spectra were recorded by instruments made by Japan Spectroscopic Co., Ltd. (Model IRA-1) and Japan Electron Optics Lab. (Model JNM-MH-100), respectively.

3,2′-Bis(N-benzyloxycarbonyl)-1-N:6-O-carbonyl-4′,6′-O-isopropylidenelividamine (**4**)

To a solution of 3,2′-bis(N-benzyloxycarbonyl)-1-N:6-O-carbonyllividamine[9] (**3**) (843 mg) and anhydrous *p*-toluenesulfonic acid (20 mg) in dry DMF (10 ml), 2,2-dimethoxypropane (14 ml) was added and the solution was heated at 70°C for 2 hours. Aqueous sodium hydrogen carbonate was added and the mixture was concentrated to a solid. Addition of water (50 ml) gave precipitates which were filtered, washed with water, and dried to give **4** as a solid, 860 mg (96%); $[\alpha]_D^{23}$ +41° (*c* 1, DMF); IR ν_{max}^{KBr} cm^{-1}: 1775 (C=O of 1,6-carbamate), 1715 (C=O of N-benzyloxycarbonyl); ^{1}H-NMR (CD_3OD) δ: 1.34 and 1.50 (each 3H, s, $C(CH_3)_2$).

Found: C, 59.71; H, 6.35; N, 6.42%.
Calcd for $C_{32}H_{39}N_3O_{11}$: C, 59.90; H, 6.13; N, 6.55%.

3,2′-Bis(N-benzyloxycarbonyl)-1-N:6-O-carbonyl-4′,6′-O-isopropylidene-5-O-tetrahydropyranyllividamine (**5** and **5′**)

To a solution of **4** (2.1 g) and anhydrous *p*-toluenesulfonic acid (50 mg) in dry DMF (24 ml), 3,4-dihydro-2*H*-pyran (12 ml) was added and the solution was kept at room temperature for 3 hours. Usual work up gave the diastereoisomeric mixture **5** and **5′** as a solid, 2.3 g (97%), which as used without purification.

1,3,2′-Tris(N-benzyloxycarbonyl)-4′,6′-O-isopropylidene-5-O-tetrahydropyranyllividamine(**6** and **6′**)

A solution of **5** (200 mg) in dry benzyl alcohol (4 ml) containing sodium benzylate (300 mg) was kept at room temperature for 20 hours under nitrogen. The reaction mixture showed, on tlc with chloroform - methanol (15: 1), two marked spots at Rf 0.58 (**6**) and 0.69 (**6′**, the diastereoisomer of **6**). The solution was poured into water (200 ml) and resulting precipitates were dissolved in chloroform. The solution was washed with aqueous sodium chloride, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **6** as a solid, 67.0 mg (29%) and **6′** as a solid, 69.2 mg (30%).

Compound **6**: $[\alpha]_D^{23}$ +40° (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1710 (C=O), 1695 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.33 and 1.46 (each 3H, s, $C(CH_3)_2$).

Found: C, 63.44; H, 6.36; N, 5.34%.
Calcd for $C_{44}H_{55}N_3O_{13}$: C, 63.37; H, 6.65; N, 5.04%.

Compound **6′**: $[\alpha]_D^{23}$ +26° (*c* 1, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1720 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.35 and 1.47 (each 3H, s, $C(CH_3)_2$).

Found: C, 63.56; H, 6.41; N, 5.28%.
Calcd for $C_{44}H_{55}N_3O_{13}$: C, 63.37; H, 6.65; N, 5.04%.

1,3,2′-Tris(N-benzyloxycarbonyl)-4′,6′-O-isopropylidene-6-O-mesyl-5-O-tetrahydropyranyllividamine (**7**)

To an ice cold solution of **6** (680 mg) in dry pyridine (10 ml), methanesulfonyl chloride (300 mg) was added and the mixture was kept at room temperature for 1 hour. Aqueous sodium hydrogen carbonate was added and the mixture was extracted with chloroform. The solution was washed with water and aqueous sodium chloride, dried (Na_2SO_4), and concentrated to give a syrup, which was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **7** as a solid, 580 mg (78%); $[\alpha]_D^{23}$ +35° (*c* 1, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (C=O), 1710 (C=O), 1185 (SO_3); ^{1}H-NMR ($CDCl_3$) δ: 1.37 and 1.45 (each 3H, s, $C(CH_3)_2$), 2.73 (3H, s, CH_3SO_2).

Found: C, 59.50; H, 6.43; N, 4.89; S, 3.34%.
Calcd for $C_{45}H_{57}N_3O_{15}S$: C, 59.26; H, 6.30; N, 4.61; S, 3.52%.

3-O-Acetyl-1,4-bis(N-benzyloxycarbonyl)-6-O-(2-N-benzyloxycarbonyl-4,6-O-isopropylidene-α-lividosaminyl)-4-N,3-O-didemethyl-2-deoxy-5-O-tetrahydropyranylfortamine (**8**) and 6-O-acetyl-1,3,2′-tris(N-benzyloxycarbonyl)-6-*epi*-4′,6′-O-isopropylidene-5-O-tetrahydropyranyllividamine (**9**)

To a solution of **7** (1.01 g) in dry DMF (27 ml), sodium acetate (2.7 g) was added and the mixture was stirred at 100°C for 1 hour, then at 120°C for 2 hours. On tlc with chloroform - ethyl acetate (2: 1), the reaction mixture showed two marked spots at Rf 0.55 (**8**) and 0.46 (**9**) and some minor ones. The mixture was concentrated to a syrup. After addition of water, the mixture was extracted with chloroform. The solution was washed with aqueous sodium chloride, dried (Na_2SO_4), and concentrated to give a syrup. The syrup was chromatographed on a column of silica gel with chloroform - ethyl acetate (5: 1). Compounds **8** and **9** were eluted in this order to give a solid 520 mg (54%) and a solid 185 mg (19%), respectively.

Compound **8**: $[\alpha]_D^{23}$ +35° (*c* 1, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (C=O), ^{1}H-NMR ($CDCl_3$) δ: 1.40 and 1.47 (each 3H, s, $C(CH_3)_2$), 1.94 (3H, s, CH_3CO).

Found: C, 62.80; H, 6.54; N, 4.72%.
Calcd for $C_{46}H_{57}N_3O_{14}$: C, 63.07; H, 6.56; N, 4.80%.

Compound **9**: $[\alpha]_D^{23}$ +22° (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.37 and 1.46 (each 3H, s, $C(CH_3)_2$), 2.05 (3H, s, CH_3CO).

Found: C, 62.86; H, 6.47; N, 4.74%.
Calcd for $C_{46}H_{57}N_3O_{14}$: C, 63.07; H, 6.56; N, 4.80%.

1,4-Bis(N-benzyloxycarbonyl)-6-O-(2-N-benzyloxycarbonyl-α-lividosaminyl)-4-N,3-O-didemethyl-2-deoxyfortamine (**11**)

A solution of **8** (520 mg) in methanol containing 5% ammonia (10 ml) was kept at room temperature for 2 hours and then concentrated. The residue was dissolved in a mixture of acetone - acetic acid - water (2: 9: 1, 24 ml). The solution was kept at 70°C for 3 hours and then concentrated. The residue was chromatographed on a column of silica gel with chloroform - methanol (20: 1) to give **11** as a solid, 322 mg (76%); $[\alpha]_D^{23}$ +30° (*c* 0.5, DMF).

Found: C, 60.69; H, 6.02; N, 5.68%.
Calcd for $C_{36}H_{43}N_3O_{12}$: C, 60.92; H, 6.11; N, 5.92%.

4-N,3-O-Didemethyl-2-deoxyfortamine (**12**)

A solution of **11** (120 mg) in 5.5 N methanolic HCl (6 ml) was heated in a sealed tube at 80°C for 8 hours. Concentration followed by co-evaporation with water gave a residue. The solution of the residue in aqueous acetic acid (1: 2, 3 ml) was hydrogenated with palladium black. The reaction mixture was filtered and the filtrate was evaporated to give a solid, which was chromatographed on a column (1 × 25 cm) of CM-Sephadex C-25 (NH_4 form) with 0.05 ~ 0.30 N ammonia with gradient increase in concentration to give **12**, 25 mg (91%); $[\alpha]_D^{23}$ +32° (*c* 1, H_2O); ^{1}H-NMR (D_2O, TMS as the external reference) δ: 2.20 (1H, double t, $J_{1,2ax.} = J_{2ax.,2eq.} = 12.0$, $J_{2ax.,3} = 3.6$ Hz. H-2ax.), 2.41 (1H, m, $J_{1,2eq.} = 4.5$, $J_{2eq.,3} = 3.6$ Hz. H-2eq.), 3.43 (1H, m, $J_{1,6} = 9.4$ Hz. H-1), 3.70 (1H, t, $J_{3,4} = J_{4,5} = 3.6$ Hz. H-4), 3.94 (1H, t, $J_{5,6} = 9.4$ Hz. H-6), 4.32 (1H, double d, J = 3.6 and 9.4 Hz. H-5), 4.51 (1H, q, J = 3.6 Hz. H-3). Irradiation of the H-3 signal turned the H-4 triplet to a doublet. Irradiation of the H-4 signal collapsed the H-3 quartet to a triplet.

Found: C, 39.67; H, 8.71; N, 15.78%.
Calcd for $C_6H_{14}N_2O_3 \cdot H_2O$: C, 39.99; H, 8.95; N, 15.54%.

1-N-Benzyloxycarbonyl-6-O-(2-N-benzyloxycarbonyl-4,6-O-isopropylidene-α-lividosaminyl)-4-N: 5-O-carbonyl-4-N,3-O-didemethyl-2-deoxyfortamine (**14**)

To a solution of **11** (780 mg) in dry DMF (15 ml), 50% oily sodium hydride (160 mg) was added and the mixture was vigorously stirred at room temperature for 1 hour. After addition of acetic acid (1 ml), the mixture was concentrated and the residue was extracted with dioxane. Concentration of the solution gave crude **13** as a solid (760 mg) which was derived to the 4′,6′-O-isopropylidene derivative in a manner as described for the preparation of **4**. The product was purified by chromatography on a column of silica gel with chloroform - methanol (15: 1) to give **14** as a solid, 525 mg (75%); $[\alpha]_D^{23}$ +32° (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O of 4,5-carbamate), 1715 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.30 and 1.46 (each 3H, s, $C(CH_3)_2$).

Found:	C, 59.69; H, 6.05; N, 6.53%.
Calcd for $C_{32}H_{39}N_3O_{11}$:	C, 59.90; H, 6.13; N, 6.55%.

1-N-Benzyloxycarbonyl-6-O-(2-N-benzyloxycarbonyl-4,6-O-isopropylidene-α-lividosaminyl)-4-N: 5-O-carbonyl-2-deoxyfortamine (**15**)

To an ice cold solution of **14** (100 mg) in dry DMF (2 ml), methyl iodide (2 ml), barium oxide (200 mg) and barium hydroxide monohydrate (250 mg) were added and the mixture was stirred in the dark in the cold for 5 minutes and then at room temperature for 1 hour. Additional aliquot (50 mg) of barium hydroxide monohydrate was added and the mixture was stirred for further 1 hour. Chloroform was added, the mixture was filtered, and the filtrate was evaporated to give a syrup, which was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **15** as a solid, 70 mg (67%); $[\alpha]_D^{23}$ +6.7° (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (C=O), 1705 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.35 and 1.46 (each 3H, s, $C(CH_3)_2$), 2.78 (3H, s, NCH_3), 3.36 (3H, s, OCH_3).

Found:	C, 60.67; H, 6.73; N, 6.47%.
Calcd for $C_{34}H_{43}N_3O_{11}$:	C, 60.97; H, 6.48; N, 6.27%.

1-N-Benzyloxycarbonyl-6-O-(2-N-benzyloxycarbonyl-α-lividosaminyl)-4-N: 5-O-carbonyl-2-deoxyfortamine (**16**)

Compound **15** (74 mg) was treated with acetic acid in a manner as described for the preparation of **11** to give **16** as a solid, 58 mg (84%); $[\alpha]_D^{25}$ +12° (*c* 0.5, MeOH); IR ν_{max}^{KBr} cm^{-1}: 1750 (C=O), 1685 (C=O); ^{1}H-NMR (CD_3OD) δ: 2.72 (3H, s, NCH_3), 3.32 (3H, s, OCH_3).

Found:	C, 59.18; H, 6.23; N, 6.41%.
Calcd for $C_{31}H_{39}N_3O_{11}$:	C, 59.13; H, 6.24; N, 6.67%.

1-N-Benzyloxycarbonyl-6-O-(2-N-benzyloxycarbonyl-6-O-tosyl-α-lividosaminyl)-4-N: 5-O-carbonyl-2-deoxyfortamine (**17**)

To a cold solution (−15°C) of **16** (56 mg) in dry pyridine (1 ml), *p*-toluenesulfonyl chloride (60 mg) was added and the solution was allowed to stand at the temperature for 20 hours. Work up in the usual manner gave a syrup, which was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **17** as a solid, 47 mg (67%); $[\alpha]_D^{25}$ +10° (*c* 1, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O), 1715 (C=O), 1705 (C=O), 1170 (SO_3); ^{1}H-NMR ($CDCl_3$) δ: 2.40 (3H, s, $SO_2C_6H_4CH_3$), 2.79 (3H, s, NCH_3), 3.34 (3H, s, OCH_3).

Found:	C, 58.55; H, 5.51; N, 5.63; S, 4.23%.
Calcd for $C_{38}H_{45}N_3O_{13}S$:	C, 58.23; H, 5.79; N, 5.36; S, 4.09%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-4-N: 5-O-carbonyl-2-deoxyfortamine (**18**)

To a solution of **17** (220 mg) in dry DMF (5 ml), sodium azide (220 mg) was added and the mixture was stirred at 60°C for 10 hours. The mixture was concentrated, and the residue was dissolved in chloroform. The solution was washed with aqueous sodium chloride, dried (Na_2SO_4), and concentrated to give a syrup. It was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **18** as a solid, 149 mg (81%); $[\alpha]_D^{25}$ +12° (*c* 1, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2090 (N_3), 1765 (C=O), 1710 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.78 (3H, s, NCH_3), 3.36 (3H, s, OCH_3).

Found: C, 56.66; H, 5.84; N, 12.95%.
Calcd for $C_{31}H_{38}N_6O_{10}$: C, 56.87; H, 5.85; N, 12.84%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-2-deoxyfortamine (**19**)

To a solution of **18** (140 mg) in dioxane (5.6 ml), 0.1 M barium hydroxide solution (5.6 ml) was added and the mixture was stirred at 60°C for 18 hours. Introduction of carbon dioxide followed by concentration gave a residue, which was extracted with chloroform. Concentration of the solution gave a solid, which was chromatographed on a column of silica gel with chloroform - methanol (20: 1) to give **19** as a solid, 78 mg (58%); $[\alpha]_D^{25}$ +25° (*c* 0.5, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2085 (N_3), 1685 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s, NCH_3), 3.32 (3H, s, OCH_3).

Found: C, 57.02; H, 6.24; N, 13.11%.
Calcd for $C_{30}H_{40}N_6O_9$: C, 57.31; H, 6.41; N, 13.37%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-4-N-(N-benzyloxycarbonylglycyl)-2-deoxyfortamine (**20**)

To a solution of **19** (139 mg) in dry dioxane (4 ml), N-hydroxysuccinimide ester (165 mg) of N-benzyloxycarbonylglycine and triethylamine (100 mg) were added and the solution was kept at 90°C overnight. The mixture was concentrated and the residue was dissolved in chloroform. The solution was washed with water, dried (Na_2SO_4), and concentrated to give a syrup. The syrup was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **20** as a solid, 150 mg (83%); $[\alpha]_D^{25}$ +40° (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2100 (N_3), 1710 (C=O), 1640 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.90 (3H, s, NCH_3), 3.30 (3H, s, OCH_3).

Found: C, 58.41; H, 5.77; N, 11.69%.
Calcd for $C_{40}H_{49}N_7O_{12}$: C, 58.60; H, 6.02; N, 11.96%.

2-Deoxy-4-N-glycyl-6-O-(α-nebrosaminyl)fortamine (**21**)

A solution of **20** (70 mg) in acetic acid (1.5 ml) was hydrogenated with palladium black. Chromatography of the product in a manner as described for the preparation of **12** gave **21** as a solid, 21 mg (63%); $[\alpha]_D^{23}$ +169° (*c* 1, H_2O); IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O); ^{1}H-NMR (D_2O, TMS as the external reference) δ: 3.56 (3H, s, NCH_3), 3.88 (3H, s, OCH_3), 4.32 (1H, t, J=3.0 Hz. H-6), 4.66 (1H, t, J=3.0 Hz. H-5), 4.72 (1H, double t, $J_{2eq.,3}$=4.0, $J_{2ax.,3}=J_{3,4}$=11.6 Hz. H-3), 5.12 (1H, double d, H-4), 5.36 (1H, d, J=3.6 Hz. H-1′).

Found: C, 46.65; H, 8.48; N, 16.75%.
Calcd for $C_{16}H_{33}N_5O_6\cdot H_2O$: C, 46.93; H, 8.62; N, 17.10%.

1-N-Benzyloxycarbonyl-6-O-(2-N-benzyloxycarbonyl-6-O-tosyl-α-lividosaminyl)-4-N:5-O-carbonyl-4-N,3-O-didemethyl-2-deoxyfortamine (**22**)

Compound **13** (186 mg) was tosylated in a manner as described for the preparation of **17** to give **22** as a solid, 138 mg (59%); $[\alpha]_D^{23}$ +14° (*c* 1, DMF); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O), 1700 (C=O), 1175 (SO_3); ^{1}H-NMR ($CDCl_3$) δ: 2.43 (3H, s, $SO_2C_6H_4CH_3$).

Found: C, 57.03; H, 5.61; N, 5.68; S, 4.01%.
Calcd for $C_{36}H_{41}N_3O_{13}S$: C, 57.21; H, 5.47; N, 5.56; S, 4.24%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-4-N:5-O-carbonyl-4-N,3-O-didemethyl-2-deoxyfortamine (**23**)

Compound **22** (131 mg) was treated with sodium azide in a manner as described for the preparation of **18** to give **23** as a solid, 98 mg (90%); $[\alpha]_D^{23}$ +10° (*c* 0.87, MeOH); IR ν_{max}^{KBr} cm^{-1}: 2085 (N_3), 1750 (C=O), 1690 (C=O).

Found: C, 55.62; H, 5.51; N, 13.69%.
Calcd for $C_{29}H_{34}N_6O_{10}$: C, 55.59; H, 5.47; N, 13.41%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-4-N:5-O-carbonyl-3-de-O-methyl-2-deoxyfortamine (**24**)

To an ice cold solution of **23** (67.7 mg) in dry DMF (1.6 ml), methyl iodide (1 ml), barium oxide (102 mg) and barium hydroxide monohydrate (34 mg) were added and the mixture was stirred for 2

hours in the dark. Additional aliquots (5 mg, and 5 mg after 2 hours) of barium hydroxide monohydrate were added and the mixture was stirred for further 2.5 hours. After addition of ethanol (10 ml), the mixture was filtered, and the filtrate was concentrated to give a syrup. The syrup was chromatographed on a column of silica gel with chloroform - methanol (100: 1) to give **24** as a solid, 62.3 mg (90%); $[\alpha]_D^{23}$ +11° (*c* 0.7, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2085 (N_3), 1740 (C=O), 1690 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.78 (3H, s, NCH_3).

Found: C, 56.51; H, 5.43; N, 12.98%.
Calcd for $C_{30}H_{36}N_6O_{10}$: C, 56.24; H, 5.66; N, 13.12%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-3-de-O-methyl-2-deoxyfortamine (**25**)

To a solution of **24** (55.0 mg) in dioxane (2.5 ml), 0.1 M barium hydroxide solution (2.5 ml) was added and the mixture was stirred at 50°C for 22 hours. Work up in a manner as described for the preparation of **19** gave **25** as a solid, 35.6 mg (67%); $[\alpha]_D^{25}$ +28° (*c* 0.5, MeOH); IR ν_{max}^{KBr} cm^{-1}: 2100 (N_3), 1700 (C=O); ^{1}H-NMR (CD_3OD) δ: 2.35 (3H, s, NCH_3).

Found: C, 56.41; H, 6.44; N, 13.38%.
Calcd for $C_{29}H_{38}N_6O_9$: C, 56.67; H, 6.23; N, 13.67%.

6-O-(6-Azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-4-N-(N-benzyloxycarbonylglycyl)-3-de-O-methyl-2-deoxyfortamine (**26**)

Compound **25** (52 mg) was treated as described for the preparation of **20** to give **26** as a solid, 39 mg (57%); $[\alpha]_D^{23}$ +42° (*c* 1, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2100 (N_3), 1736 (C=O), 1710 (C=O), ^{1}H-NMR ($CDCl_3$) δ: 3.10 (3H, s, NCH_3).

Found: C, 58.41; H, 5.82; N, 11.89%.
Calcd for $C_{39}H_{47}N_7O_{12}$: C, 58.13; H, 5.88; N, 12.17%.

3-De-O-methyl-2-deoxy-4-N-glycyl-6-O-(α-nebrosaminyl)fortamine (**27**)

Compound **26** (39 mg) was treated as described for the preparation of **21** to give **27** as a solid, 13 mg (71%); $[\alpha]_D^{23}$ +178° (*c* 0.5, H_2O); IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O); ^{1}H-NMR (D_2O, TMS as the external reference) δ: 3.59 (3H, s, NCH_3), 4.32 (1H, t, J=3.0 Hz. H-6), 4.65 (1H, t, J=3.0 Hz. H-5), 5.03 (1H, double t, $J_{2eq.,3}$=4.0, $J_{2ax.,3}=J_{3,4}$=11.6 Hz. H-3), 5.05 (1H, double d, H-4), 5.37 (1H, d, J=3.6 Hz. H-1′).

Found: C, 45.23; H, 8.50; N, 17.44%.
Calcd for $C_{15}H_{31}N_5O_6\cdot H_2O$: C, 45.56; H, 8.41; N, 17.71%.

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