FORTIMICIN ANALOGS *VIA* GLYCOSIDE FORMATION

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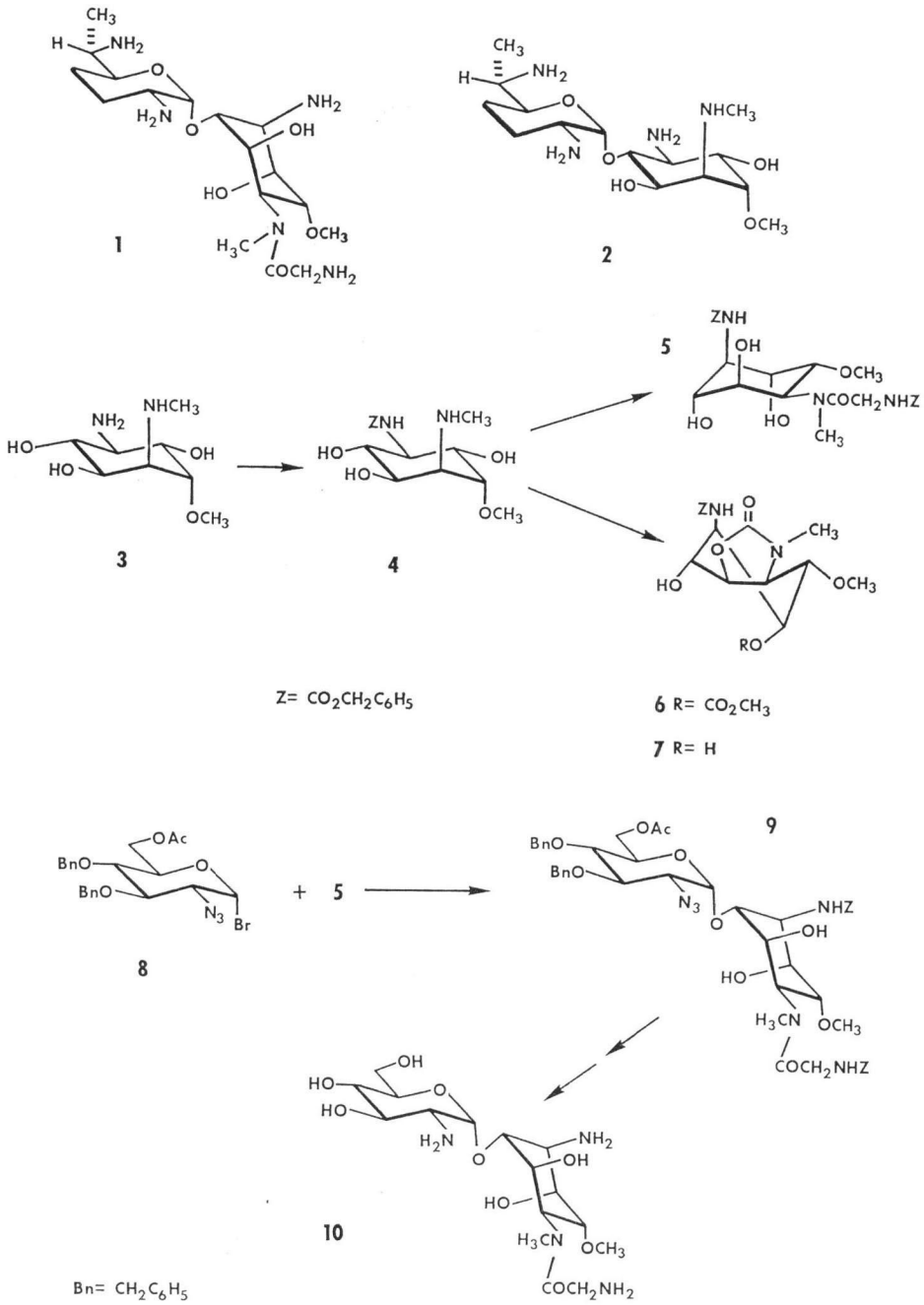
Fortimicin analogs have been prepared *via* glycosylation of suitably protected derivatives of the aminocyclitol fortamine. The analogs contain changes in the sugar portion of the molecule and/or in the manner of its attachment to the cyclitol.

Fortimicins A (**1**) and B (**2**) are aminoglycosidic aminocyclitol antibiotics discovered in fermentations of *Micromonospora olivoasterospora*[1]. These antibiotics are pseudodisaccharides in which 6-*epi*-purpurosamine B (**19**) is attached through an α-glycosidic linkage to O-6 of a novel 1,4-diaminocyclitol (**3**), fortamine[2]. Unlike fortimicin B, fortimicin A possesses a 4-*N*-glycyl substituent and exhibits potent, broad-spectrum antibacterial activity. Also noteworthy is the $^1C_4$ conformation of the cyclitol portion of fortimicin A in which the 4-$NCH_3COCH_2NH_2$ group is equatorial. In fortimicin B, at and above physiological pH, the cyclitol assumes the $^4C_1$ conformation in which the 4-$NHCH_3$ group is axial. Several fortimicin analogs incorporating changes in the sugar portion of the molecule and/or in the manner of its attachment to the cyclitol have been prepared by glycosylation of suitably blocked fortamines. These analogs are not readily attainable through direct chemical modification and provide some insight into fortimicin structure activity relationships.

Fortamine[3] (**3**), derived from fortimicin A or B by acid hydrolysis, was protected for glycosylation in two ways. The intermediate 1-*N*-benzyloxycarbonylfortamine (**4**) was prepared directly from **3** by use of *N*-benzyloxycarbonyloxysuccinimide. Treatment of the latter (**4**) with the *N*-hydroxysuccinimide activated ester derivative of *N*-benzyloxycarbonylglycine provided the desired 1-*N*-benzyloxycarbonyl-4, *N*-(*N*-benzyloxycarbonylglycyl)fortamine (**5**). Glycosylation of **5** has the advantage of providing 4-*N*-glycyl or fortimicin A analogs directly provided that the relative reactivity of the various hydroxyl groups directs glycosylation to the C-6 hydroxyl. The second blocked fortamine was prepared by reaction of **4** with N,N′-carbonyldiimidazole in tetrahydrofuran and subsequent treatment with methanol and hydrochloric acid to provide 1-*N*-benzyloxycarbonyl-4,5-carbamoyl-2-*O*-methoxycarbonylfortamine (**6**). This cyclic carbamate has only the C-6 hydroxyl group free for glycosylation and a flattened $^4C_1$ conformation as determined by analysis of its pmr spectrum and that of the 2-*O*-demethoxycarbonyl analog (**7**) using spin-decoupling experiments. The free hydroxyl group is in a pseudoequatorial orientation in this conformation.

Condensation of 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide[4] (**8**) with **5** under modified KOENIGS-KNORR conditions with mercuric cyanide in dichloroethane gave, as the only isolable product, 6-*O*-(6′-*O*-acetyl-2′-azido-3′,4′-di-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4-*N*-(*N*-benzyloxycarbonylglycyl)fortamine (**9**) in 8% yield. The presence of a non-participating azido group at C-2 in **8** led to the expected α-glycoside (**9**) as indicated by a doublet ($J_{1',2'} \cong$ 3 Hz) at δ 5.84 ppm in the pmr spectrum due to the anomeric Cl′H. The failure of **9** to undergo oxidation with lead tetraacetate in acetic acid indicates that the glycoside bond is not at C-2 of fortamine.



O-Deacylation of **9**, followed by catalytic hydrogenation, provided 6-*O*-(2′-amino-2′-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**10**), whose structure was established by analysis of pmr, cmr (Table 1), and mass spectra.

When 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride[5] (**11**) was condensed with **5** under similar conditions glycosylation occurred at the 5-position to give, as the only isolable product, 5-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4-*N*-(*N*-benzyl-

Table 1. Cmr chemical shifts in D_2O solution.*

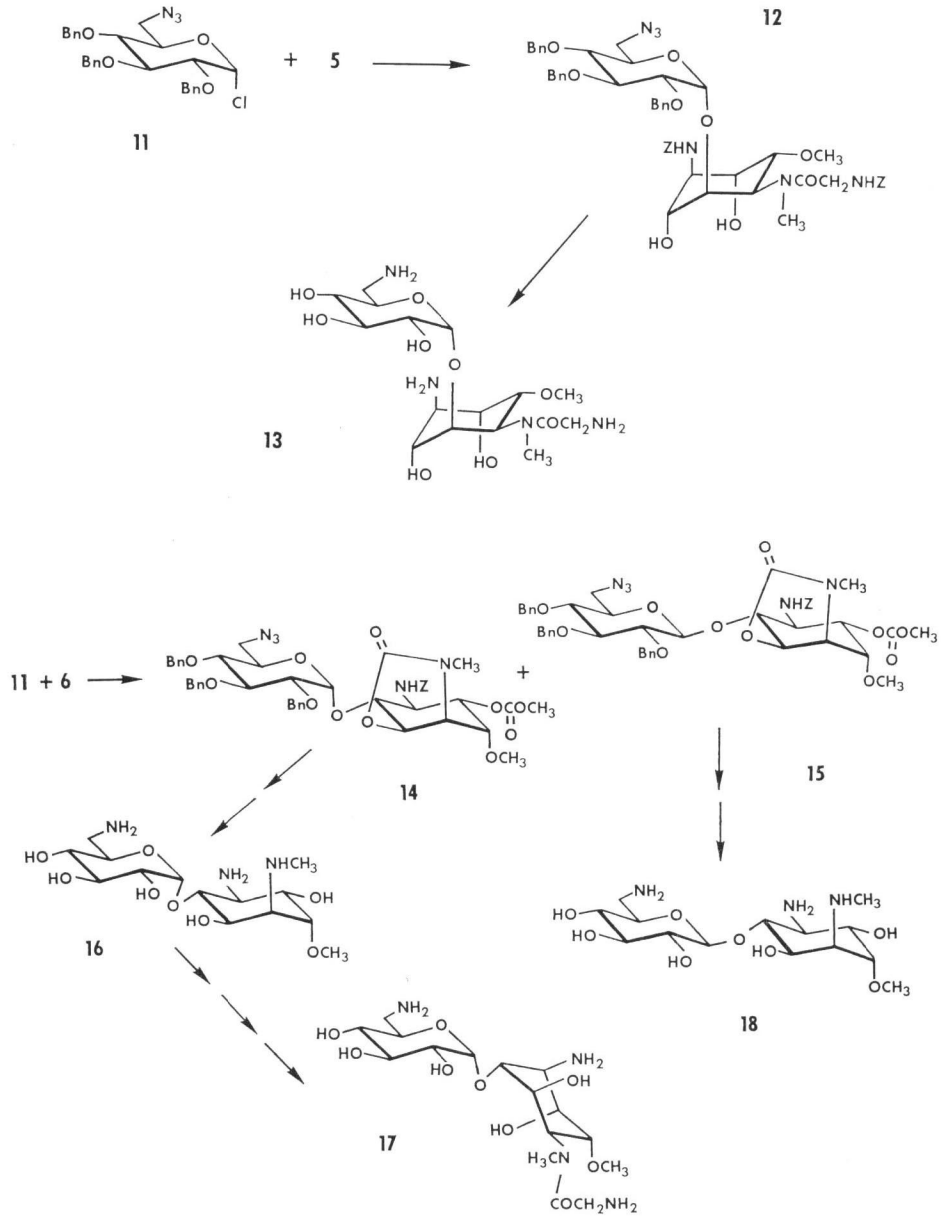
Compound	1[2,9]	2[2]	3	10	13	16	17	18	22	23
pD	10.8	11.2	10.6	9.0	9.4	10.5	9.3	11.2	10.0	10.0
Carbons										
CO	176.2	—	—	176.0	175.3	—	176.0	—	176.0	—
1′	100.1	102.5	—	100.1	98.0	100.9	98.7	104.4	106.9	106.7
6	78.4	84.2	73.7	78.9	71.4	84.5	79.0	85.3	81.4	83.6
5′	74.9	75.1	—	73.6	73.6	73.7	73.4	74.5	80.9	82.1
3	73.7	79.9	80.2	73.6	77.9	79.9	73.7	79.8	73.6	79.5
5	72.9	71.3	71.2	73.3	76.4	71.1	72.8	71.8	74.6	70.9
2	71.1	71.2	71.2	70.7	71.0	71.1	70.5	71.0	70.9	69.4
OCH_3	56.4	59.3	59.3	56.4	57.2	59.3	56.8	59.3	56.4	59.2
4	55.4	60.9	60.9	55.5	54.9	60.5	56.6	60.3	54.1	60.1
1	52.5	53.8	55.1	52.4	53.0	53.7	52.7	54.4	52.7	54.1
6′	50.6	50.4	—	61.6	41.7	42.4	43.6	42.4	51.6	50.2
2′	50.2	50.6	—	55.5	71.8	73.7	72.2	76.5	51.7	52.1
Gly-CH_2	43.4	—	—	42.8	41.7	—	43.6	—	43.0	—
4-NCH_3	32.2	35.4	35.4	32.3	33.4	35.4	32.1	35.3	32.4	35.2
4′	27.4	27.4	—	70.7	70.6	71.8	71.9	69.7	29.8	26.9
3′	26.9	27.1	—	74.6	72.4	72.7	73.0	74.5	27.3	30.3
6′-CH_3	18.5	18.6	—	—	—	—	—	—	17.4	18.3

* Assignments were confirmed by means of pD-titrations.

oxycarbonylglycyl)fortamine (**12**) in 7% yield. A doublet ($J_{1',2'} \cong 3$ Hz) at 5.85 ppm in the pmr spectrum of **12** and the failure of **12** to undergo lead tetraacetate oxidation confirm a 5- or 6-*O*-α-glycoside. Catalytic hydrogenation of **12** provided 5-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**13**) whose structure was again established by analysis of pmr, cmr (Table 1), and mass spectra.

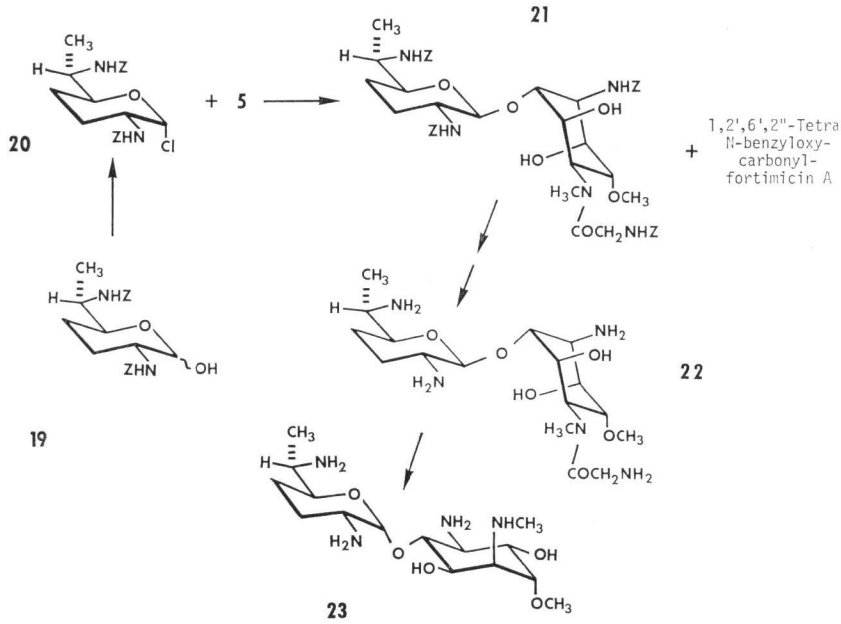
The desired 6-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4,5-carbamyl-2-*O*-methoxycarbonylfortamine (**14**) and its β-analog (**15**) were obtained by condensation of **11** with **6** in 34% and 16% yield, respectively. The intermediates **14** and **15** were deblocked by hydrogenation followed by alkaline hydrolysis to provide the fortimicin B analogs, 6-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)fortamine (**16**) and its β-analog (**18**), respectively. The various structures were confirmed by analysis of pmr, cmr (Table 1), and mass spectra. Treatment of **16** with benzyloxycarbonyl active ester gave the 1,6′-di-*N*-benzyloxycarbonyl derivative which was then reacted with benzyloxycarbonylglycine by the mixed-anhydride procedure to provide the 1,6′,2″-tri-*N*-benzyloxycarbonylfortimicin A analog of **16**. The latter was deblocked in the usual way to give 6-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**17**), whose structure was established by analysis of pmr, cmr (Table 1), and mass spectra.

The tetra-*N*-benzyloxycarbonyl derivative of 1′-*epi*-fortimicin A (**21**) was obtained in 14% yield by condensation of 2,6-di-*N*-benzyloxycarbonyl-α-6-*epi*-purpurosaminyl B chloride (**20**) with **5** under the modified KOENIGS-KNORR conditions. The sugar chloride (**20**) was derived from 2,6-di-*N*-benzyloxycarbonyl-6-*epi*-purpurosamine B[3] (**19**) by the acetyl chloride method[6]. The corresponding α-glycoside, 1,2′,6′,2″-tetra-*N*-benzyloxycarbonylfortimicin A, was also obtained (10% yield) from the reaction mixture, suggesting that the C-6 hydroxyl group is more reactive, under these conditions, than the C-2 and



C-5 hydroxyls to both α- and β-glycosylation. The failure of **21** to undergo lead tetraacetate oxidation further confirms a 5- or 6-*O*-β-glycoside. Removal of the blocking groups from **21** provided 1′-*epi*-fortimicin A (**22**). Mild, alkaline conditions[2] afforded a ready conversion of **22** to 1′-*epi*-fortimicin B (**23**). Analysis of the pmr, cmr (Table 1), and mass spectra of **22** and **23** establish their respective structures as 1′-*epi*-fortimicin A and 1′-*epi*-fortimicin B.

The fact that the latter glycosylation reaction gave a 1.4: 1 mixture of β- and α-glycosides [(**21**) and tetra-*N*-benzyloxycarbonylfortimicin A respectively] despite the presence of a reputed participating blocking group (benzyloxycarbonylamido) at the 2-position of the sugar chloride is most interesting. The presence of a participating group at C-2 was anticipated to provide β-glycosides exclusively during



the glycosylation. The presence of an α-glycoside in the reaction mixture can be rationalized by invoking neighboring group participation with the benzyl carbamate at the 6-position in competition with the benzyl carbamate at the 2-position. Substituents at positions other than C-2 have been observed to influence the steric course of glycosylation reactions. The production of α-glycosides has been associated with sugar halides possessing functional groups at C-4 and/or C-6 with the potential for participation[7,8].

The cyclitol portion of each of the reported fortimicin A analogs (**10**, **13**, **17** and **22**) is in the preferred $^1C_4$ or fortimicin A conformation as evidenced by the large and small couplings of the C-4 proton and by the chemical shift of the cyclitol carbons in their respective pmr and cmr spectra. Both 6-amino-6-deoxyglucose, present in analogs **13** and **17**, and 2-amino-2-deoxyglucose, present in **10**, are constituents of a number of aminoglycoside antibiotics. However, only compound **17** exhibited weak antibacterial activity (1~2% that of fortimicin A) against a variety of Gram-positive and Gram-negative microorganisms.

Experimental

Pmr spectra were recorded on a Varian Associated HA-100 spectrometer or on a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system in deuterated solvents. Chemical shifts are reported in ppm down-field from internal TMS and coupling constants are reported in Hz. CMR spectra were measured on a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system and on a JEOL FX-900. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. Mass spectra were obtained on an AEI MS-902 spectrometer at 50 eV using the direct insertion probe. Optical rotations were measured with a Hilger and Watts polarimeter. Reported pD values are uncorrected pH meter readings of deuterated solutions.

Microanalytical results are reported for those products which could be prepared free of solvent and carbonates formed by atmospheric exposure.

Fortamine (3)

A solution of fortimicin B (**2**) (100 g, 0.29 mole) in 6 M hydrochloric acid (1.5 liters) was refluxed for 16 hours. The hydrolysate was evaporated under reduced pressure to a dark syrup, reconstituted in 1 liter of distilled water, and passed over a 3-liter column of AG 1X-8 ion exchange resin (OH^- form). Fractions containing fortamine were combined, evaporated to a syrup under reduced pressure and treated with absolute ethanol (200 ml) to provide crystalline fortamine (free base) (37 g, 63%); m.p. 193~197°C (dec.); $[\alpha]_D^{25}$ −88° (*c* 1.0, H_2O); Mass Spec.: M^+ meas. 206.1268; $C_8H_{18}N_2O_4$ requires 206.1249; *Anal*: Calcd. for $C_8H_{18}N_2O_4$, C, 46.59; H, 8.80; N, 13.59; O, 31.03; Found: C, 46.58; H, 9.16; N, 13.19; O, 31.41; PMR (D_2O): 2.88 s NCH_3 (3H); 3.29 t C1H (1H); 3.58 q C4H (1H); 3.73 t C6H (1H); 3.95 s OCH_3 (3H); 4.10 q C2H (1H); 4.15 q C3H (1H); 4.27 q C5H (1H). $J_{1,2}$=10 Hz, $J_{2,3}$=3 Hz, $J_{3,4}$=2 Hz, $J_{4,5}$=5 Hz, $J_{5,6}$=10 Hz, $J_{1,6}$=10 Hz.

1-*N*-Benzyloxycarbonylfortamine (4)

To a cooled solution of fortamine (**3**) (18 g; 0.087 mole) in a mixture of water (260 ml) and methanol (530 ml) was added sodium bicarbonate (7.8 g; 1 equiv.) and *N*-(benzyloxycarbonyloxy)succinimide (22 g; 1 equiv.) and the mixture stirred overnight at room temperature. The reaction mixture was evaporated to a residue under reduced pressure. Pure product (**4**) was isolated by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 75: 25: 2) and crystallized from chloroform (100 ml) (18 g, 61%); mp 156~160°C; $[\alpha]_D^{25}$ −45° (*c* 0.98, MeOH); *Anal*: Calcd. for $C_{16}H_{24}N_2O_6$, C, 56.45; H, 7.11; N, 8.23; O, 28.20; Found: C, 56.15; H, 7.31; N, 8.03; O, 27.96; PMR (D_2O): δ 2.92 s NCH_3 (3H); 3.71 t C1H (1H); 3.89 s OCH_3 (3H); 5.57 s OCH_2Ph (2H), 7.87 s C_6H_5 (5H).

1-*N*-Benzyloxycarbonyl-4-*N*-(2′-*N*-benzyloxycarbonylglycyl)fortamine (5)

To a solution of **4** (6.6 g; 20 mmole) in chloroform (150 ml) was added *N*-benzyloxycarbonylglycine-*N*-hydroxysuccinimide active ester (10 g; 1.6 equiv.) and sodium bicarbonate (1.6 g; 1 equiv.) and the mixture stirred overnight at room temperature. The reaction mixture was washed with water, dried, filtered and evaporated under reduced pressure to a syrup. Pure product (**5**) was isolated by silica gel column chromatography (chloroform - methanol, 10: 1) as a white foam (5.7 g, 55%); mp 82~84°C; $[\alpha]_D^{23}$ +47° (*c* 1.0, MeOH); *Anal*: Calcd. for $C_{26}H_{33}N_3O_9$, C, 58.75; H, 6.26; N, 7.91; O, 27.09; Found: C, 58.54; H, 6.34; N, 7.72; O, 26.91; PMR ($CDCl_3$): 2.98 s NCH_3 (3H); 3.30 s OCH_3 (3H); 5.09 s OCH_2-Ph (4H); 7.33 s C_6H_5 (10H).

1-*N*-Benzyloxycarbonyl-4,5-carbamyl-2-*O*-methoxycarbonylfortamine (6)

To a solution of **4** (17.7 g; 0.052 mole) in tetrahydrofuran (400 ml) was added *N*,*N*′-carbonyldiimidazole (16.7 g; 2 equiv.) and the mixture stirred overnight at room temperature. Methanol (250 ml) was added and, after stirring for 1 hour, 10 drops of conc. hydrochloric acid was added and stirring was continued for an additional hour. Solvent was removed from the reaction mixture by evaporation under reduced pressure; the residue taken up in methylene chloride; the organic phase washed with water, dried, filtered and evaporated to a syrup under reduced pressure. Pure product (**6**) was isolated by silica gel column chromatography (methylene chloride - methanol, 98: 2) as a colorless foam (9.3 g, 42%); mp 94~97°C; $[\alpha]_D^{25}$ −37° (*c* 1.0, MeOH); *Anal*: Calcd. for $C_{19}H_{24}N_2O_9$, C, 53.77; H, 5.70; N, 6.60; O, 33.93; Found: C, 53.89; H, 5.85; N, 6.47; O, 33.98; IR ($CDCl_3$): 3580, 3425, 1754, 1730, 1520, 1450, 1405, 1330, 1270, 1105, 1055, 975 cm^{-1}; PMR (pyridine-d_5, 110°C): δ 2.72 s NCH_3 (3H); 3.26 s OCH_3 (3H); 3.54 s $COOCH_3$ (3H); 3.81 q C4H (1H); 3.95 q C3H (1H); 4.03 q C1H (1H); 4.18 t C6H (1H); 4.56 t C5H (1H); 5.08 s OCH_2Ph (2H); 5.35 q C2H (1H). $J_{1,2}$=5.0 Hz, $J_{2,3}$=2.5 Hz, $J_{3,4}$=7.0 Hz, $J_{4,5}$=8.0 Hz, $J_{5,6}$=8.0 Hz, $J_{1,6}$=8.0 Hz.

2,6-Di-*N*-benzyloxycarbonyl-α-6-*epi*-purpurosaminyl B chloride (20)

A solution of 2,6-di-*N*-benzyloxycarbonyl-6-*epi*-purpurosamine B[3] (**19**) (1.3 g; 3.0 mmole) in acetyl chloride (26 ml) was stirred for 20 hours at room temperature[6]. The reaction mixture was taken up in methylene chloride (100 ml) and the solution slowly added to vigorously stirred ice-water (100 ml). The mixture was transferred to a separatory funnel and the organic layer immediately drawn off into a cold saturated solution of sodium bicarbonate (100 ml). The methylene chloride layer was again separated and run into a flask containing anhydrous magnesium sulfate. The mixture was filtered and evaporated

under reduced pressure to provide **20** as a pale yellow syrup to be used immediately in the glycosylation reaction (1.3 g, 96%); PMR ($CDCl_3$): δ 1.16 d C6CH_3 (3H, J=6.0 Hz); 5.09 s OCH_2Ph (4H); 6.21 d C1H (1H, J=3.0 Hz); 7.32 s C_6H_5 (10H).

6-*O*-(6′-*O*-Acetyl-2′-azido-2′,3′-di-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4-*N*-(*N*-benzyloxycarbonylglycyl)fortamine (**9**)

To a solution of **5** (1.0 g; 1.9 mmole) in dry dichloroethane (50 ml), freshly prepared powdered Drierite (5.0 g), dried mercuric cyanide (2.0 g) and freshly prepared 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide[4] (**8**) (1.16 g; 2.3 mmole) were added and the mixture refluxed for 20 hours with vigorous stirring. An additional portion of the sugar bromide (**8**) (2.3 mmole) was added and the reaction was continued for 24 hours. The reaction mixture was filtered, the filtrate washed with 5% sodium bicarbonate, dried and evaporated to a solid (3.1 g). The major product (**9**) was isolated by silica gel column chromatography (methylene chloride - methanol; 0~1%) as a colorless foam (150 mg, 8%); PMR ($CDCl_3$): δ 2.01 s 6′-OCOCH_3 (3H); 3.04 s NCH_3 (3H); 3.35 s OCH_3 (3H); 4.5~5.1 OCH_2Ph (8H); 5.84 d C1′H (1H, $J_{1',2'}$ 3 Hz); ~7.3 s C_6H_5 (20H).

6-*O*-(2′-Amino-2′-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**10**)

A solution of **9** (110 mg; 0.12 mmole) in methanol (2 ml) was treated with sodium methoxide (7 mg; 0.012 mmole) for 4 hours at room temperature. Methylene chloride (50 ml) was added to the reaction mixture, and the organic phase washed with water, dried and evaporated to a colorless foam (120 mg); PMR ($CDCl_3$): δ 3.03 NCH_3 (3H); 3.35 s OCH_3 (3H); 4.6~5.1 OCH_2PH (8H); 5.78 d C1′H (1H, $J_{1',2'}\cong$ 3 Hz); ~7.3 s C_6H_5 (20H).

A solution of the latter in a mixture of methanol (36 ml) and 0.2 M methanolic hydrochloric acid (3.6 ml) was treated with hydrogen (3 atm) over 5% palladium on carbon (110 mg) for 4 hours at room temperature. The mixture was filtered and the filtrate evaporated under reduced pressure to give **10** as the hydrochloride salt. Pure product (**10**) in the form of the free base was isolated by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 1 : 2 : 1) as a colorless foam (32 mg, 63%); $[\alpha]_D^{25}$ +108° (*c* 0.1, H_2O); Mass Spec. M^+ meas. 424.2161; $C_{16}H_{32}N_4O_9$ requires 424.2169; PMR (D_2O, pD 9.02): δ 3.28 s NCH_3 (3H); 3.95 s OCH_3 (3H); 3.99 s Gly-CH_2 (2H); 5.26 q C4H (1H; $J_{3,4}\cong$ 10 Hz; $J_{4,5}\cong$3 Hz).

5-*O*-(6′-Azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4-*N*-(*N*-benzyloxycarbonylglycyl)fortamine (**12**)

To a solution of **5** (3.5 g; 6.6 mmole) in dry dichloroethane (50 ml), freshly prepared powdered Drierite (14.8 g), dried mercuric cyanide (7.4 g) and freshly prepared 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride[5] (**11**) (3.3 g; 6.6 mmole) were added and the mixture refluxed for 20 hours with vigorous stirring. The reaction mixture was filtered, the filtrate washed with 5% sodium bicarbonate, dried and evaporated to a solid (7.6 g). The major product (**12**) was isolated by silica gel column chromatography (toluene - ethanol, 0~3.5%) as a colorless foam (426 mg, 7%); IR ($CHCl_3$); 3410, 3330, 2110, 1710, 1650, 1510, 1450, 1400, 1360, 1315, 1280, 1155, 1070, 1025, 1000 cm^{-1}; PMR ($CDCl_3$): δ 2.96 s NCH_3 (3H); 3.40 s OCH_3 (3H); 4.5~5.1 OCH_2Ph (10H); 5.85 d C1′H (1H, $J_{1',2'}\simeq$3 Hz); ~7.3 s C_6H_5 (25 H).

5-*O*-(6′-Amino-6′-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**13**)

A solution of **12** (74 mg; 0.075 mmole) in a mixture of methanol (20.5 ml) and 0.2 M methanolic hydrochloric acid (4.5 ml) was treated with hydrogen (3 atm) over 5% palladium on carbon (75 mg) for 4 hours at room temperature. The mixture was filtered and the filtrate evaporated at reduced pressure to give pure product (**13**) as the hydrochloride salt (40 mg, 99%); Mass Spec.: M^+ meas. 424.2164; $C_{16}H_{32}N_4O_9$ requires 424.2169; PMR (D_2O, pD 2.87): 3.60 s NCH_3 (3H); 3.99 s OCH_3 (3H); 4.61 s Gly-CH_2 (2H); 5.49 q C4H (1H, $J_{3,4}$=7.0 Hz, $J_{4,5}$=3.5 Hz); 5.73 d C1′H (1H, $J_{1',2'}$=2.3 Hz).

6-*O*-(6′-Azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucopyranosyl)-1-*N*-benzyloxycarbonyl-4,5-carbamyl-2-*O*-methoxycarbonylfortamine (**14**) and the β-analog (**15**)

To a solution of **6** (1.5 g; 3.6 mmole) in dry dichloroethane (50 ml), freshly prepared powdered Drierite (9.5 g), dried mercuric cyanide (3.8 g) and freshly prepared **11** (1.8 g; 3.4 mmole) were added

and the mixture refluxed for 20 hours with vigorous stirring. The reaction mixture was filtered, the filtrate washed with 5% sodium bicarbonate, dried and evaporated under reduced pressure to a syrup (3.2 g). The two products were isolated by silica gel column chromatography (methylene chloride - methanol, 0~1%) as colorless foams; **14** (1.09 g, 34%); IR ($CHCl_3$): 2114, 1710, 1655 cm^{-1}; PMR ($CDCl_3$): δ 2.93 s NCH_3 (3H); 3.44 s OCH_3 (3H); 3.79 s $COOCH_3$ (3H); 4.4~5.1 OCH_2Ph (8H); 5.21 q C2H (1H, $J_{1,2}$=4.0 Hz, $J_{2,3}$=2.0 Hz); 5.47 d C1'H α (1H, $J_{1',2'}$=3.5 Hz); ~7.3 s C_6H_5 (20H). **15** (510 mg, 16%); IR ($CHCl_3$): 2114, 1755, 1720, 1655 cm^{-1}; PMR ($CDCl_3$): δ 2.88 s NCH_3 (3H); 3.41 s OCH_3 (3H); 3.73 s $COOCH_3$ (3H); 4.5~5.0 OCH_2Ph (8H); 5.16 q C2H (1H, $J_{1,2}$=4.0 Hz, $J_{2,3}$=2.0 Hz); 5.30 d C1'H β (1H, $J_{1',2'}$=7.5 Hz); 7.3 s C_6H_5 (20H).

6-*O*-(6'-Amino-6'-deoxy-α-D-glucopyranosyl)fortamine (**16**)

A solution of **14** (1.09 g; 1.24 mmole) in a mixture of methanol (13 ml) and 0.2 M methanolic hydrochloric acid (37 ml) was treated with hydrogen (3 atm) over 5% palladium on carbon (1.1 g) for 4 hours at room temperature. The mixture was filtered and the filtrate evaporated under reduced pressure to give 6-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-4,5-carbamyl-2-*O*-methoxycarbonylfortamine as the hydrochloride salt (600 mg, 99%); IR (nujol): 1715, 1660 cm^{-1}; PMR (D_2O, pD 2.16); δ 3.39 s NCH_3 (3H); 4.00 s OCH_3 (3H); 4.36 s $COOCH_3$ (3H); 5.67 q C2H (1H, $J_{1,2}$=8.2 Hz, $J_{2,3}$=2.5 Hz); 5.93 d C1'H (1H, $J_{1',2'}$=3.0 Hz). The latter (600 mg; 1.22 mmole) was refluxed under nitrogen in a mixture of methanol (75 ml) and 4 M sodium hydroxide (25 ml) for 5 hours. The reaction mixture was cooled, adjusted to pH 8 with hydrochloric acid and evaporated under reduced pressure to a residue. Pure product (**16**) was isolated by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 4: 4: 1) as a white foam (442 mg, 96%); Mass Spec.: M^+ meas. 367.1968; $C_{14}H_{29}N_3O_8$ requires 367.1955; PMR (D_2O, pD 10.52): δ 2.89 s NCH_3 (3H); 3.96 s OCH_3 (3H); 4.52 q C5H (1H; $J_{4,5}$= 4.5 Hz, $J_{5,6}$=9.0 Hz); 5.69 d C1'H (1H; $J_{1',2'}$=3.5 Hz); (pD 0.97): δ 3.32 s NCH_3 (3H); 4.00 s OCH_3 (3H); 4.53 q C3H (1H; $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.0 Hz); 4.77 t C6H (1H, $J_{5,6}\cong$3 Hz, $J_{1,6}\cong$4 Hz); 5.08 t C5H (1H; $J_{4,5}\cong$3 Hz); 5.71 d C1'H (1H; $J_{1',2'}$=3.0 Hz).

6-*O*-(6'-Amino-6'-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine (**17**)

To a solution of **16** (340 mg; 0.91 mmole) in a mixture of acetonitrile (40 ml), water (15 ml) and triethylamine (0.25 ml; 1.8 mmole) was added benzyloxycarbonyl-*N*-hydroxysuccinimide active ester (567 mg; 2.28 mmole) and the mixture stirred at room temperature for 20 hours. The reaction mixture was evaporated under reduced pressure to a syrup and 6-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-1,6'-di-*N*-benzyloxycarbonylfortamine was isolated by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 90: 10: 1) as a colorless foam (297 mg, 51%); PMR (D_2O; pD 4.43): δ 3.26 s NCH_3 (3H); 3.86 s OCH_3 (3H); 5.42 s CH_2Ph (4H); 5.65 d C1'H (1H; $J_{1',2'}$=3.0 Hz); 7.69 s C_6H_5 (10H).

A solution of *N*-benzyloxycarbonylglycine (50 mg, 0.24 mmole) and triethylamine (0.033 ml; 0.24 mmole) in dry toluene (3 ml) was cooled in an ice-acetone bath and *iso*-butylchloroformate (0.031 ml; 0.24 mmole) was added. After 30 minutes, the above blocked pseudodisaccharide (100 mg; 0.16 mmole) in a mixture of dry toluene (3 ml) and dry dimethylformamide (1 ml) was added and the reaction stirred for 1 hour in the cold and for 16 hours at room temperature. The reaction mixture was evaporated under reduced pressure to a residue and the product, 1,6',2''-tri-*N*-benzyloxycarbonyl-6-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-4-*N*-glycylfortamine was isolated by silica gel column chromatography (methylene chloride - methanol, 19: 1) as a colorless foam (66 mg, 50%). This material was then dissolved in a mixture of methanol (27 ml) and 0.2 M methanolic hydrochloric acid (3 ml) and treated with hydrogen (3 atm) over 5% palladium on carbon (70 mg) for 4 hours at room temperature. The mixture was filtered and the filtrate evaporated at reduced pressure to give the product (**17**) as the hydrochloride salt (42 mg, 98%); Mass Spec.: $(M-H_2O)^+$ meas. 406.2064; $C_{16}H_{30}N_4O_8$ requires 406.2064; PMR (D_2O, pD 1.96); 3.62 s NCH_3 (3H); 3.97 s OCH_3 (3H); 4.57 s Gly CH_3 (2H), 5.40 q C4H (1H, $J_{3,4}$=11.0 Hz, $J_{4,5}$=2.5 Hz); 5.65 d C1'H (1H, $J_{1',2'}$=3.5 Hz).

6-*O*-(6'-Amino-6'-deoxy-β-D-glucopyranosyl)fortamine (**18**)

A solution of **15** (510 mg; 0.58 mmole) in 0.2 M methanolic hydrochloric acid (18 ml) was treated with hydrogen (3 atm) over 5% palladium on carbon (500 mg) for 4 hours at room temperature. The

mixture was filtered and the filtrate evaporated at reduced pressure to give 6-*O*-(6′-amino-6′-deoxy-β-D-glucopyranosyl)-4,5-carbamyl-2-*O*-methoxycarbonylfortamine as the hydrochloride salt (290 mg, 102%). The latter was refluxed under nitrogen in a mixture of methanol (45 ml) and 4 M sodium hydroxide (15 ml) for 8 hours. The reaction mixture was cooled, adjusted to pH 8 with hydrochloric acid and evaporated under reduced pressure to a residue. Pure product (**18**) was isolated by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 2: 3: 1) as a colorless foam (207 mg, 95%); Mass Spec.: M^+ meas. 367.1966; $C_{14}H_{29}N_3O_8$ required 367.1955; PMR (D_2O; pD 11.21): 2.82 s NCH_3 (3H); 3.90 s OCH_3 (3H); 4.32 q C5H (1H; $J_{4,5}$=4.5 Hz, $J_{5,6}$=9.5 Hz); 5.01 d C1′H (1H, $J_{1',2'}$=7.2 Hz).

1,2,6′,2″-Tetra-*N*-(benzyloxycarbonyl)-1′-*epi*-fortimicin A (**21**)

To a solution of **5** (1.6 g; 3.0 mmole) in dry dichloroethane (50 ml), freshly prepared powdered Drierite (8.0 g), dried mercuric cyanide (3.5 g) and freshly prepared **20** (1.3 g; 2.9 mmole) were added and the mixture refluxed for 27 hours with vigorous stirring. The reaction mixture was filtered, the filtrate washed with 5% sodium bicarbonate, dried and evaporated under reduced pressure to a syrup (3.0 g). Two products were isolated by silica gel column chromatography (methylene chloride - methanol, 0~1.5%) in addition to unreacted **5** (450 mg, 28%). The second product was identified as 1,2,6′,2″-tetra-*N*-(benzyloxycarbonyl)fortimicin A (290 mg, 10%) by comparison with an authentic sample, while the first was the desired 1′-*epi*-analog (**21**) (410 mg, 14%); PMR ($CDCl_3$): 3.49 s NCH_3 (3H); 3.81 s OCH_3 (3H); 5.08~5.12 OCH_2Ph (8H); 7.32 s C_6H_5 (20H).

1′-*epi*-Fortimicin A hydrochloride (**22**)

A solution of **21** (109 mg; 0.116 mmole) in a mixture of methanol (15 ml) and 0.2 M methanolic hydrochloric acid (10 ml) was treated with hydrogen (3 atm) over 5% palladium on carbon (100 mg) for 4 hours at room temperature. The mixture was filtered and the filtrate evaporated at reduced pressure to give **22** (61 mg, 95%); Mass Spec.: M^+ meas. 405.2587; $C_{17}H_{35}N_5O_6$ requires 405.2576; PMR (D_2O, pD 2.63): δ 1.81 d 6′-CH_3 (3H, $J_{6',CH}$=6.7 Hz); 3.61 s NCH_3 (3H); 3.95 s OCH_3 (3H); 4.57 s Gly-CH_2 (2H); 5.39 q C4H (1H; $J_{3,4}$=9.0 Hz, $J_{4,5}$=4.0 Hz); 5.36 d C1′H (1H; $J_{1',2'}\cong$9 Hz).

1′-*epi*-Fortimicin B sulfate (**23**)

A solution of **22** (50 mg; 0.12 mmole) in 1 M ammonium hydroxide (10 ml) was allowed to stand at room temperature for 2 weeks. Pure product (**23**) was isolated from the reaction mixture by silica gel column chromatography (methylene chloride - methanol - conc. ammonia, 1: 1: 1 lower phase) as a colorless foam (20 mg, 48%); Mass Spec.: $(M+1)^+$ meas. 349.2428; $C_{15}H_{33}N_4O_5$ requires 349.2451; PMR (D_2O; pD 9.98): 1.51 d 6′-CH_3 (3H, $J_{6',CH}$=6.5 Hz); 2.83 s NCH_3 (3H); 3.90 d OCH_3 (3H); 4.35 q C5H (1H, $J_{4,5}$=4.5 Hz, $J_{5,6}$=9.5 Hz); 4.83 d C1′H (1H, $J_{1',2'}$=8.0 Hz).

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