Synthesis of a Lividomycin B Analogue, 5-*O*-[3-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-3′-deoxyparomamine

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5-*O*-[3-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-3′-deoxyparomamine was prepared by two different routes. The first route involves glycosylation of a protected 1-*N* : 6-*O*-carbonyl-3′-deoxyparomamine with a protected 3-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)ribofuranosyl bromide and subsequent removal of the protecting groups. In the second route, 1-*N* : 6-*O*-carbnyl-3′-deoxyparomamine was glycosylated with a protected ribosyl bromide having 3-*O*-benzylthiocarbonyl group to give a pseudotrisaccharide and, after de-3-*O*-benzylthiocarbonylation, the product was further condensed with a protected 1-bromide of 2-amino-2-deoxy-D-glucose. This synthesized lividomycin B analogue showed only very weak antibacterial activity and the role of the 6‴-amino group of lividomycin in the action was suggested.

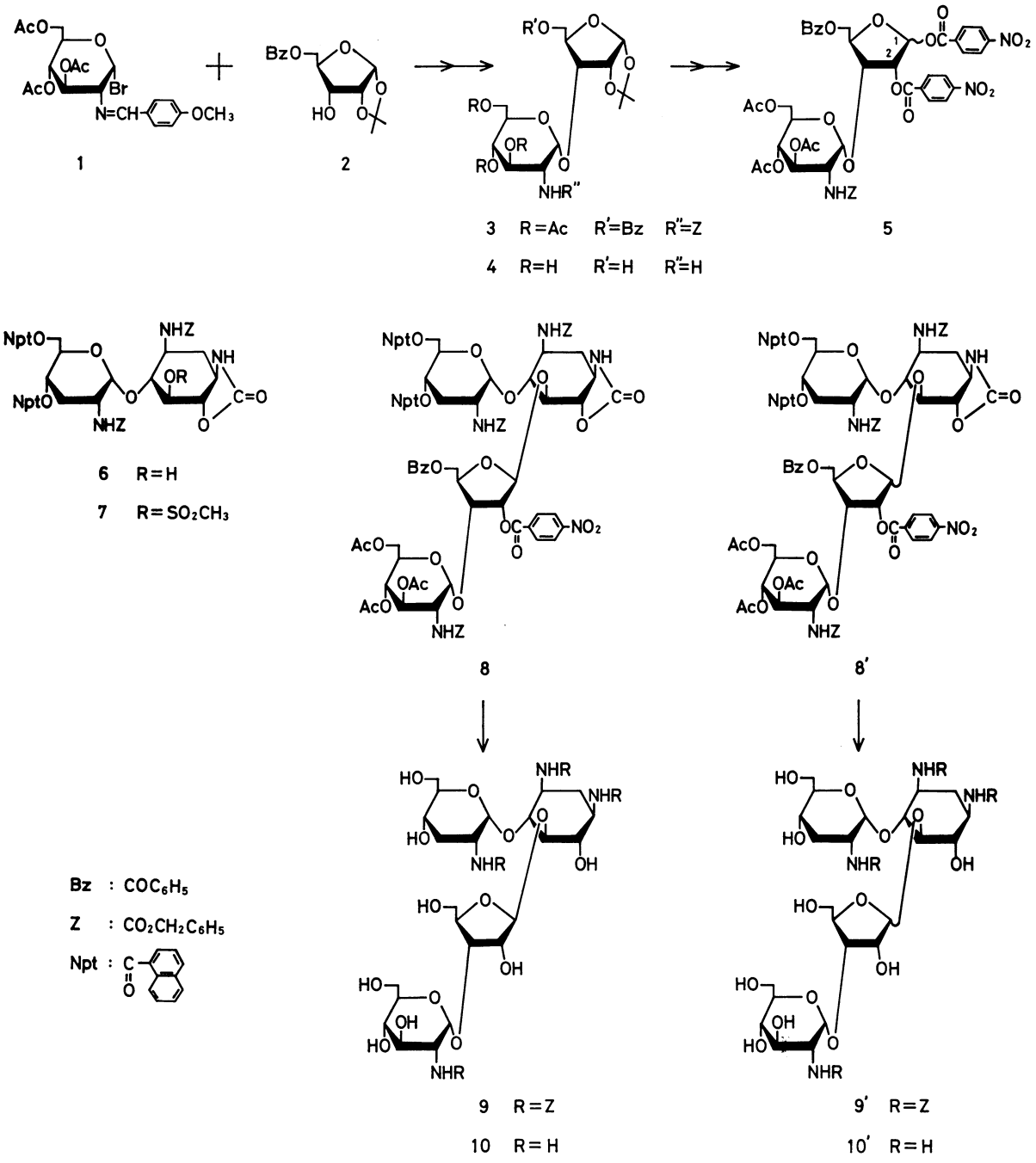
Lividomycin B[1] is an aminoglycoside antibiotic which belongs to a pseudotetrasaccharide. Since its pseudotrisaccharide[2] portion which lacks the 2,6-diamino-2,6-dideoxy-L-idopyranose moiety has only very weak antibacterial activity, this diaminohexose moiety has been suggested to enhance the antibacterial activity in a great extent. However, it has not yet been certain, which amino group in this diaminohexose moiety has the predominant role to increase the activity. Therefore, we attempted to replace this diaminohexose moiety of lividomycin B with 2-amino-2-deoxy-D-glucose.

Among two synthetic routes considered, we at first prepared this lividomycin B analogue by condensation of a disaccharide with a pseudodisaccharide. The disaccharide was prepared by condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)amino-α-D-glucopyranosyl bromide[3] (**1**) with 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose[4] (**2**) in benzene in the presence of mercury(II) cyanide as catalyst. Acid hydrolysis of the *N*-(*p*-methoxybenzylidene) group of the condensation product followed by *N*-benzyloxycarbonylation gave the protected disaccharide (**3**) in 54% yield (based on **2**). The high yield formation of the α-D-glucoside may be ascribed in part to the presence of the 2-*N*-(*p*-methoxybenzylidene) group.[5] To confirm the α-D-glucoside linkage of **3**, the *O*-acyl and *N*-benzyloxycarbonyl groups of **3** were removed. The PMR spectrum and the optical rotation of the deacylated product (**4**) substantiated the α-D-glucoside linkage. Then, the isopropylidene group of **3** was removed by 95% formic acid treatment without affecting the glucosyl bond and the *O*-acyl groups, and the resulting free hydroxyl groups at C-1 and 2 were *p*-nitrobenzoylated to give **5**. The presence of the 2-*O*-(*p*-nitrobenzoyl) group prevents undesirable orthoester[6] formation in the subsequent glycosylation. Treatment of **5** with hydrogen bromide in dichloromethane gave the corresponding 1-bromide.

The protected pseudodisaccharide, namely 3,2′-bis(*N*-benzyloxycarbonyl)-1-*N* : 6-*O*-carbonyl-3′-deoxy-4′,6′-di-*O*-(α-naphthoyl)paromamine (**6**), which is moderately soluble in organic solvents, was prepared by treatment of tris(*N*-benzyloxycarbonyl)-3′-deoxyparomamine[7] with sodium hydride in *N*,*N*-dimethylformamide (DMF)[8] followed by regioselective acylation with α-naphthoyl chloride. The positions of the α-naphthoyl groups in **6** were confirmed by the hydrolysis of mono-*O*-mesyl derivative (**7**) of **6**. Paper chromatography of the acidic hydrolyzate of **7** gave no detectable 2-deoxystreptamine, whereas the same treatment of **6** gave 2-deoxystreptamine, indicating that the 5-hydroxyl group of **6** was mesylated. Condensation of **6** with the abovementioned 1-bromide of **5** was carried out in dichloromethane in the presence of mercury(II) cyanide, and, the condensation products having β-D (**8**) and α-D (**8′**) glycoside linkages were separated by silica gel chromatography in yields of 33 and 47%, respectively. Treatment of **8** and **8′** with sodium benzylate in benzyl alcohol removed all the *O*-acyl groups and, simultaneously cleaved the 1,6-carbamate into 1-benzyloxycarbonylamino and 6-hydroxyl groups to give **9** and **9′**, respectively. Hydrogenolysis of **9** and **9′** with palladium black removed the benzyloxycarbonyl groups and gave the desired pseudotetrasaccharide (**10**) and its α-D-anomer (**10′**), respectively.

The anomeric configurations of **10** and **10′** were identified by their PMR spectra, and their pseudotetrasaccharide structures were supported by paper chromatography of their methanolyzates.

In another route, the pseudodisaccharide derivative (**6**) was successively glycosylated with a protected ribofuranosyl bromide and then with the glucosamine derivative (**1**). The aforementioned protected ribose **2** was led to 3-*O*-benzylthiocarbonyl derivative (**11**) by treatment with benzylthiocarbonyl chloride in pyridine. This protecting group[9] was chosen because of its easy elimination by oxidation with hydrogen peroxide to liberate the free hydroxyl group at C-3 of the ribose moiety. Acidic solvolysis of **11** to remove the isopropylidene group followed by *p*-nitrobenzoylation gave methyl 3-*O*-benzylthiocarbonyl-2-*O*-(*p*-nitrobenzoyl)-β-D- and α-D-riboside (**12** and **12′**). The structures of **12** and **12′** were confirmed by their PMR spectra as well as by those of the debenzylthiocarbonylated product (**13** and **13′**) of **12** and **12′**. In the spectrum of **12′** a virtual coupling[10] between H-1 and 3 was observed and it was further observed that 2-*O*→3-*O*-(*p*-nitrobenzoyl) migration gradually occurred when the solu-

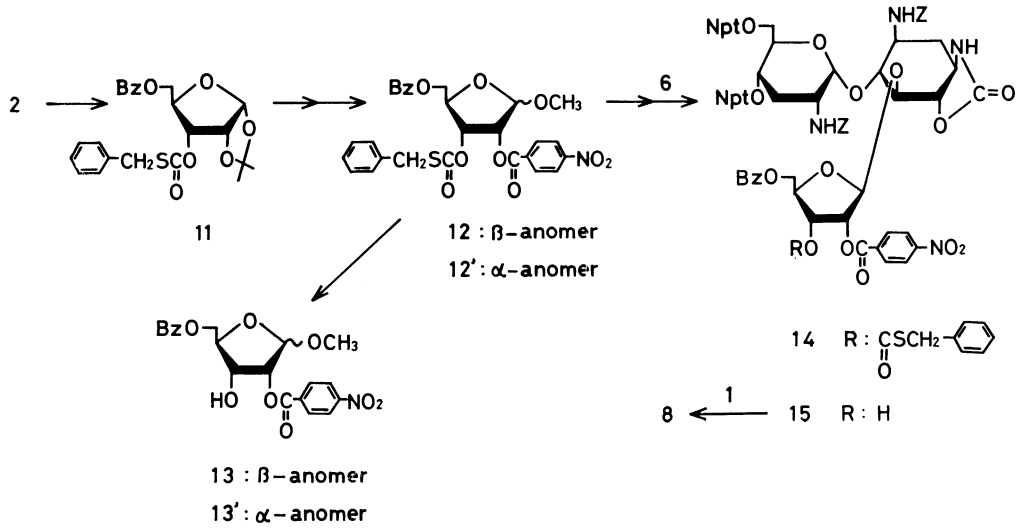


tion (in pyridine-d_5 containing deuterium oxide) of **13** and **13'** were allowed to stand (see Experimental).

Treatment of **12** with hydrogen bromide in acetic acid gave the 1-bromide, which was used without purification, for coupling with **6** in a manner described above to give the condensation product (**14**) in 66% yield. The β-anomeric configuration (at C-1'') of **14** could not be determined at this stage, but was made clear at the last step which gave **10** identical with that prepared by the alternative route aforementioned. Removal of the benzylthiocarbonyl group with hydrogen peroxide in acetic acid gave the product (**15**) having a free hydroxyl group at C-3''. In this reaction, a slight amount of 3''-*O*-(*p*-nitrobenzoyl) derivative was formed by migration. The structure of **15** was confirmed by recovery of **14** from **15** with the benzylthiocarbonyl chloride treatment. The condensation of **15** with **1** was achieved in benzene in the presence of silver reagents to give **8**, in 30% yield, which was led to the desired product (**10**).

The overall yield of **10** by the second route was lower than that by the first route. However, it should be noted that the first route gave a larger amount of the α-riboside (**8'**) than the desired β-riboside (**8**). In the second route no α-riboside was obtained.

The synthesized pseudotetrasaccharide (**10**) showed much weaker antibacterial activities than lividomycin B and this result suggested that the 6'''-amino group



of lividomycin B has a predominant role in increasing the antibacterial activity. The corresponding α-anomer (**10′**) showed no antibacterial activity.

Experimental

Thin-layer chromatography (TLC) was carried out on Wakogel B-5 with sulfuric acid spray for detection unless otherwise stated. For column-chromatography, silica gel (Wakogel C-200) was used. Descending paper chromatography was performed on Toyo-Roshi Paper No. 50 with 1-butanol–pyridine–water–acetic acid (6 : 4 : 3 : 1), and spots were visualized by spraying 0.5% ninhydrin in pyridine. PMR spectra were recorded at 60 and 90 MHz with Hitachi R-24A and Varian EM-390 spectrometers, respectively.

*5-O-Benzoyl-1,2-O-isopropylidene-α-D-ribofuranose (**2**).* The compound was prepared in a different way from the reported one.[4] 5-*O*-Benzoyl-1,2-*O*-isopropylidene-D-*erythro*-pentofuranos-3-urose[11] (2 g) dissolved in methanol was treated with sodium borohydride in a usual manner to give **2** as needles (from ethyl acetate–diisopropyl ether, 1.7 g, 86%), mp 81—82 °C (lit,[4] 78—79 °C), $[\alpha]_D^{26}$ +32° (*c* 1, $CHCl_3$) (lit,[4] +20.05° in $CHCl_3$).

*3-O-(3,4,6-Tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-5-O-benzoyl-1,2-O-isopropylidene-α-D-ribofuranose (**3**).* To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)amino-α-D-glucopyranosyl bromide[3] (**1**, 1.34 g) in dry benzene (18 ml), 5-*O*-benzoyl-1,2-*O*-isopropylideneribofuranose[2] (**2**, 0.58 g,) mercury(II) cyanide (1.3 g), and calcium sulfate (Drierite 2.7 g) were added and the mixture was stirred at room temperature overnight. After addition of chloroform (100 ml), the mixture was centrifuged. The organic layer separated was washed with aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. The resulting syrup was dissolved in a mixture of methanol (36 ml) and 50% aqueous acetic acid (16 ml) and the solution was kept at room temperature for 4 h. After addition of powdered sodium carbonate (7.1 g), the solution was concentrated. The residue was extracted with acetone and the extract was concentrated to dryness. To an ice cold solution of the residue in aqueous acetone (3 : 10, 50 ml), sodium carbonate (900 mg) and benzyl chloroformate (1.8 g) were added and the mixture was stirred for 1 h in the cold. Evaporation followed by extraction with chloroform and evaporation of the solvent gave a syrup. Since **2** which remained unreacted had the same mobility with that of **3** on column chromatography, **3** was separated after acetylation of the remaining **2**. A mixture of the syrup and acetic anhydride (2 ml) in pyridine (40 ml) was allowed to stand at 37 °C overnight. Evaporation, extraction of the residue with chloroform, washing of the solution (aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate, and water), drying (Na_2SO_4), and evaporation of the solvent gave a syrup. The syrup was chromatographed over silica gel with benzene–ethyl acetate (8 : 1) to give **3** (syrup), 748 mg (54%), $[\alpha]_D^{20}$ +102° (*c* 1, $CHCl_3$); IR (KBr): 1740, 1720 cm^{-1}; PMR ($CDCl_3$) δ: 1.22 and 1.53 (each 3H s, $C(CH_3)_2$), 1.94, 2.00, and 2.02 (each 3H s, Ac), 5.07 (1H d, J=3.5 Hz, H-1′), 5.91 (1H d, J=3.5 Hz, H-1).

Found: C, 58.67; H, 5.78; N, 1.78%. Calcd for $C_{35}H_{41}NO_{15}$: C, 58.74; H, 5.77; N, 1.96%.

*3-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-1,2-O-isopropylidene-α-D-ribofuranose (**4**).* A solution of **3** (100 mg) in methanol containing 5% ammonia (5 ml) was kept at room temperature overnight and the solution was concentrated. To a solution of the residue in dioxane (2 ml), water (1 ml) and two drops of acetic acid were added and the mixture was hydrogenated with palladium black in an atmospheric pressure of hydrogen. After filtration, the filtrate was concentrated to give a syrup, which was chromatographed over CM-Sephadex C-25 (NH_4 form) with 0.05 M aqueous ammonia to give a ninhydrin-positive solid of **4** as hemihydrate, 20 mg (40%), $[\alpha]_D^{18}$ +165° (*c* 0.5, H_2O); PMR (D_2O) δ: 1.43 and 1.61 (each 3H s, $C(CH_3)_2$), 2.78 (1H q, $J_{1',2'}$=3.7 Hz, $J_{2',3'}$=9.5 Hz, H-2′), 4.95 (1H m, H-2), 5.12 (1H d, J=3.7 Hz, H-1′), 5.96 (1H d, J=3.8 Hz, H-1).

Irradiation of H-2′ collapsed the doublet of H-1′ to a singlet and irradiation of H-1′ collapsed the quartet of H-2′ to a doublet (J=9.5 Hz). Irradiation of H-1 (δ 5.96) collapsed the multiplet of H-2 to an incomplete triplet ($J\approx$1.9 Hz).

Found: C, 46.75; H, 6.96; N, 3.70%. Calcd for $C_{14}H_{25}NO_{19}\cdot 1/2H_2O$: C, 46.66; H, 7.27; N, 3.89%.

*3-O-(3,4,6-Tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-5-O-benzoyl-1,2-bis-O-(p-nitrobenzoyl)-D-ribofuranose (**5**).* A solution of **3** (621 mg) in 95% aqueous formic acid (30 ml) was kept at room temperature for 3.5 h. Concentration *in vacuo* at room temperature gave a syrup. The chloroform solution of the syrup was washed with aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. To a solution of the resulting syrup in dry pyridine (8 ml), *p*-nitrobenzoyl chloride (626 mg) was added and the solution was kept at room temperature overnight.

After addition of water (0.1 ml), the solution was concentrated. The chloroform solution (50 ml) of the residue was washed with aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The resulting syrup was chromatographed over silica gel with benzene–ethyl acetate (10 : 1) to give a syrup of **5**, 572 mg (68%), $[\alpha]_D^{22}$ +72° (*c* 1, $CHCl_3$); IR (KBr): 1725, 1520 cm^{-1}.

Found: C, 56.82; H, 4.65; N, 4.02%. Calcd for $C_{46}H_{43}N_3O_{21}$: C, 56.73; H, 4.45; N, 4.31%.

*3,2′-Bis(*N*-benzyloxycarbonyl)-1-*N*:6-O-carbonyl-3′-deoxy-4′,6′-di-O-(α-naphthoyl)paromamine (**6**).* To an ice-cold solution of 1,3,2′-tris(*N*-benzyloxycarbonyl)-3′-deoxyparomamine[7] (3.0 g) in DMF (60 ml), 50% oily suspension (600 mg) of sodium hydride was added under nitrogen atmosphere and the mixture was stirred vigorously for 2.5 h. After addition of acetic acid (0.8 ml), the mixture was poured into ice-water. The precipitates of 1,6-carbamate were filtered and dried (2.35 g). To a cold (−20 °C) solution of the solid in pyridine (45 ml), α-naphthoyl chloride (610 mg × 3) was added and the solution was kept at −10 °C for 9 h (3 h × 3). After concentration, the residue was dissolved in chloroform. The solution was washed (with aq $KHSO_4$, aq $NaHCO_3$, and H_2O) as described for **3** to give a solid, which was chromatographed over silica gel with chloroform–ethanol (50 : 1) to give a solid of **6**, 2.78 g (76%), $[\alpha]_D^{22}$ +69° (*c* 1, $CHCl_3$); IR (KBr): 1775 cm^{-1} (shoulder, cyclic carbamate).

Found: C, 67.31; H, 5.26; N, 4.64%. Calcd for $C_{51}H_{47}N_3O_{13}$: C, 67.32; H, 5.21; N, 4.62%.

*3,2′-Bis(*N*-benzyloxycarbonyl)-1-*N*:6-O-carbonyl-3′-deoxy-5-O-mesyl-4′,6′-di-O-(α-naphthoyl)paromamine (**7**).* To a solution of **6** (180 mg) in dry pyridine (4 ml), methanesulfonyl chloride (200 mg) was added and the solution was kept at room temperature overnight. The solution showed, on TLC with chloroform–methanol (30 : 1), a single spot at R_f 0.33. Work up in a usual manner gave a solid of **7**, 172 mg (88%), $[\alpha]_D^{24}$ +49° (*c* 1, $CHCl_3$); IR (KBr): 1180, 1350 (Ms) cm^{-1}; PMR: δ of SO_2CH_3: 3.07 (in $CDCl_3$), 3.18 (in $CDCl_3$–CD_3OD=1 : 1), 3.43 (in C_5D_5N).

Found: C, 63.00; H, 5.16; N, 4.16; S, 3.47%. Calcd for $C_{52}H_{49}N_3O_{15}S$: C, 63.21; H, 5.00; N, 4.25; S, 3.25%.

*Acidic Methanolysis of **7**.* A solution of **7** in 2 M methanolic hydrogen chloride was heated at 80 °C for 40 h. As a control experiment, **6** was similarly treated. Paper chromatography of the methanolyzate of **7** gave no 2-deoxystreptamine, whereas that of **6** did.

*5-O-[3-O-(3,4,6-Tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-5-O-benzoyl-2-O-(*p*-nitrobenzoyl)-β-* and *α-D-ribofuranosyl]-3,2′-bis(*N*-benzyloxycarbonyl)-1-*N*:6-O-carbonyl-3′-deoxy-4′,6′-di-O-(α-naphthoyl)paromamine (**8** and **8′**).* *A. From **5** and **6**:* To a cold (−10 °C) solution of **5** (1.75 g) in dry dichloromethane (50 ml), hydrogen bromide was introduced until saturation and the solution was kept at −10 °C for 30 min. Colorless needles of *p*-nitrobenzoic acid was deposited. Filtration followed by concentration of the filtrate at room temperature gave a syrup, which was dissolved in dichloromethane (11 ml). To the solution, **6** (516 mg), mercury(II) cyanide (1.85 g), and calcium sulfate (Drierite 3.75 g) were added and the mixture was vigorously stirred at room temperature overnight. On TLC with chloroform–ethanol (30 : 1), the reaction mixture showed two marked spots at R_f 0.37 (**8**), 0.29 (**8′**). The mixture was filtered with aid of chloroform. The filtrate was concentrated to give a syrup, which was chromatographed over silica gel with chloroform–ethanol (100 : 1) to give a solid of **8**, 316 mg (33% from **6**) and a solid of **8′**, 456 mg (47% from **6**).

Compound **8**: $[\alpha]_D^{20}$ +51° (*c* 1, $CHCl_3$): IR(KBr): 1780 (s), 1725, 1530 cm^{-1}.

Found: C, 62.59; H, 5.05; N, 4.05%. Calcd for $C_{90}H_{85}N_5O_{30}$: C, 62.97; H, 4.99; N, 4.08%.

Compound **8′**: $[\alpha]_D^{20}$ +69° (*c* 0.5, $CHCl_3$): IR(KBr): 1780 (s), 1725, 1530 cm^{-1}.

Found: C, 63.04; H, 5.05; N, 4.04%.

*B. From **1** and **15**:* To a solution of **1** (222 mg) in dry benzene (4 ml), **15** (194 mg), freshly prepared silver carbonate (230 mg), silver perchlorate (23 mg), and calcium sulfate (Drierite 550 mg) were added and the mixture was stirred at room temperature overnight in a dark place. The reaction mixture was filtered with aid of chloroform (20 ml) and the filtrate was concentrated. The residue was dissolved in a mixture of chloroform (2 ml), methanol (4 ml), acetic acid (1 ml) and water (1 ml), and the solution was kept at room temperature overnight. After addition of powdered sodium carbonate (930 mg), the mixture was concentrated *in vacuo*. The residue was extracted with chloroform and the extract was washed with water, dried (Na_2SO_4), and concentrated to give a solid. To a cold (0 °C) solution of the solid in aqueous acetone (1 : 2, 6 ml), benzyl chloroformate (150 mg) and sodium carbonate (600 mg) were added and the mixture was stirred at 0 °C for 1.5 h. After evaporation of the mixture was extracted with chloroform. The solution was washed with water, dried (Na_2SO_4), and evaporated to give a solid, which was chromatographed over silica gel with chloroform–ethanol (60 : 1) to give a solid of **8**, 77 mg (30% based on **14**), $[\alpha]_D^{23}$ +52° (*c* 1, $CHCl_3$). Recovered **15** by the chromatography was 80 mg (41%).

Found: C, 62.61; H, 5.11; N, 3.87%. Calcd for $C_{90}H_{85}N_5O_{30}$: C, 62.97; H, 4.99; N, 4.08%.

*1,3,2′-Tris(*N*-benzyloxycarbonyl)-5-O-[3-O-(2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-β- and α-D-ribofuranosyl]-3′-deoxyparomamines (**9** and **9′**).* To a solution of **8** (42 mg) in dry dioxane (0.17 ml), 0.27 M sodium benzylate in benzyl alcohol (0.63 ml, benzyl alcohol was dried over molecular sieves 4A) were added and the solution was kept at 37 °C for 2 h. After addition of chloroform (27 ml), the organic solution was washed thoroughly with water, dried (Na_2SO_4), and concentrated to give a syrup. The syrup was chromatographed over silica gel with chloroform (to elute the alcohol remained) and then with chloroform–ethanol (4 : 1) to give **9**, 23 mg of solid (83%), $[\alpha]_D^{19}$ +58° (*c* 0.4, MeOH).

Found: C, 56.65; H, 5.89; N, 4.94%. Calcd for $C_{55}H_{68}N_4O_{22}\cdot H_2O$: C, 57.19; H, 6.11; N, 4.85%.

Compound **9′** was obtained from **8′** in a similar manner as described above in a yield of 83%; $[\alpha]_D^{19}$ +113° (*c* 0.5, MeOH).

Found: C, 56.90; H, 5.98; N, 4.88%.

*5-O-[3-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-β- and α-D-ribofuranosyl]-3′-deoxyparomamines (**10** and **10′**).* A mixture of **9** (80 mg) in aqueous dioxane (1 : 3, 4 ml) containing two drops of acetic acid was hydrogenated with palladium black in an atmospheric pressure of hydrogen. On TLC (E. Merck, silica gel 60 F_{254}) with chloroform–methanol–17% aqueous ammonia (1 : 4 : 3), the solution showed a major spot at R_f 0.51 (in the case of **10′**, R_f 0.59). Concentration of the solution gave a solid, which was chromatographed over CM-Sephadex C-25 (NH_4 form) with 0.05 M aqueous ammonia to give **10** as a solid of monocarbonate, 34 mg (73%); PPC, $R_{f\ \text{lividomycin B}}$ 1.49, $[\alpha]_D^{23}$ +77° (*c* 0.7, H_2O); PMR (D_2O+DCl, pH ≈3) δ: 5.34 (1H d, $J\approx4$ Hz, H-1′ or 1‴), 5.36 (1H s, H-1″), 5.53 (1H d, $J=3.0$ Hz, H-1′ or 1‴).

Found: C, 43.27; H, 7.28; N, 8.26%. Calcd for $C_{23}H_{44}N_4O_{14}\cdot H_2CO_3$: C, 43.50; H, 6.99; N, 8.45%.

Compound **10′** was obtained from **9′** in a similar manner

as described above in a yield of 77.5% as monocarbonate; PPC, R_f lividomycin B 1.63, $[\alpha]_D^{23}$ +119° (*c* 0.7, H_2O); PMR (D_2O)δ: 4.95 (1H d, $J\approx4$ Hz), 5.13 (1H d, $J\approx2.7$ Hz), 5.23 (1H d, $J\approx3$ Hz) (each anomeric proton); in D_2O+DCl (pH≈3): δ: 5.34 (1H d, $J\approx3$ Hz), 5.35 (1H d, $J\approx4$ Hz), and 5.48 (1H d, $J\approx2.5$ Hz) (each anomeric proton).

Found: C, 43.63; H, 7.22; N, 8.29%.

Acidic Methanolysis of ***10*** *and* ***10′***. A solution of **10**, **10′**, or lividomycin B in 0.4 M methanolic hydrogen chloride was heated at 65 °C for 25 h, and the solution was examined by TLC (E. Merck, silica gel 60 F_{254}) with chloroform–methanol–17% aqueous ammonia (2 : 2 : 1). The methanolyzates of **10** and **10′** gave the same TLC pattern. From the comparison of the R_f values of the spots on the chromatograms with those of the hydrolyzate of lividomycin B and other reference substances, assignments of the spots were made: R_f 0.12 (2-deoxystreptamine), 0.25 (3′-deoxyparomamine), 0.52 [methyl 3-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)riboside], 0.58 (methyl 2-amino-2-deoxy-D-glucoside), 0.62 (methyl 2-amino-2,3-dideoxy-D-glucoside).

*5-O-Benzoyl-3-O-benzylthiocarbonyl-1,2-O-isopropylidene-α-D-ribofuranose (****11****).* To a solution of **2** (3.0 g) in pyridine (50 ml), benzylthiocarbonyl chloride (3 ml) was added and the solution was kept at room temperature overnight. After addition of water (0.5 ml), the solution was concentrated and the chloroform solution of the residue was washed (with aq $KHSO_4$, aq $NaHCO_3$, and H_2O), dried (Na_2SO_4), and concentrated. The residue was recrystallized from methanol to give colorless needles, 4.2 g (93%), mp 68—69 °C, $[\alpha]_D^{23}$ +141° (*c* 1, $CHCl_3$); PMR ($CDCl_3$) δ: 1.38 and 1.60 (each 3H s, $C(CH_3)_2$), 4.17 (2H s, CH_2S), 5.94 (1H m, J=5 Hz, H-1).

Found: C, 62.27; H, 5.47; S, 7.12%. Calcd for $C_{23}H_{24}O_7S$: C, 62.15; H, 5.44; S, 7.12%.

*Methyl 5-O-Benzoyl-3-O-benzylthiocarbonyl-2-O-(p-nitrobenzoyl)-β- and α-ribofuranosides (****12*** *and* ***12′****).* To a solution of **11** (3.5 g) in dry dichloromethane (21 ml), 0.4 M methanolic hydrogen chloride (50 ml) was added and the solution was kept at room temperature for 40 h. Pyridine (3 ml) was added and the solution was concentrated with intermittent additions of pyridine to give a syrup. To a solution of the syrup in pyridine (30 ml), a solution of *p*-nitrobenzoyl chloride (3.3 g) in dichloromethane (8 ml) was added and the solution was kept at room temperature for 3 h. On TLC with benzene–ethyl acetate (30 : 1), the solution showed two spots at R_f 0.54 (major **12**) and 0.50 (minor, **12′**). Work up as described for **11** gave a syrup, which was chromatographed over silica gel with benzene–ethyl acetate (100 : 1) to give a pale-yellow syrup of **12**, 2.96 g (66%), and pale-yellow needles (recrystallized from methanol) of **12′**, 1.05 g (24%).

Compound **12**: $[\alpha]_D^{24}$ +90° (*c* 1, $CHCl_3$).

Found: C, 59.52; H, 4.50; N, 2.41; S, 5.41%. Calcd for $C_{28}H_{25}NO_{10}S$: C, 59.25; H, 4.44; N, 2.47; S, 5.65%.

PMR ($CDCl_3$) δ: 3.43 (3H s, OCH_3), 4.07 (2H s, CH_2S), 5.15 (1H s, H-1), 5.6—5.85 (2H m, H-2,3). In pyridine-d_5: δ 5.38 (1H s, which sharpend on irradiation at δ 6.0, H-1), 6.0 [1H d, $J_{2,3}$=5 Hz, with small splittings ($J_{1,2}\approx1$ Hz), which disappeared, on irradiation at δ 5.38, to give a sharp d, H-2], 6.08 (1H t, $J_{2,3}=J_{3,4}$=5 Hz, H-3). On irradiation at δ 4.8, the triplet of H-3 collapsed to a doublet.

Compound **12′**: mp 110—110.5 °C, $[\alpha]_D^{20}$ +147° (*c* 1, $CHCl_3$).

Found: C, 58.95; H, 4.46; N, 2.39; S, 5.45%. Calcd for $C_{28}H_{25}NO_{10}S$: C, 59.25; H, 4.44; N, 2.47; S, 5.65%.

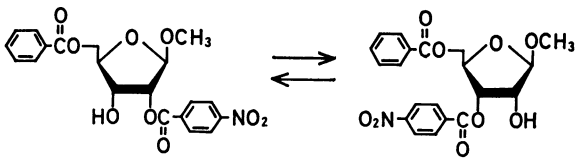
PMR (in $CDCl_3$ at 90 MHz) δ: 3.49 (3H s, OCH_3), 4.13 (2H AB q, J_{gem}=13 Hz, CH_2S), 5.2—5.45 (2H m, H-1,2), 5.55—5.75 (1H m, H-3). Since any signal distance of H-1,2 multiplet and that of H-3 multiplet did not accord each other, the occurrence of virtual coupling[10] between H-1 and H-3 was suggested. To clarify this, the solvent was changed: among the solvents tested, pyridine-d_5 was found most suitable and the signals of **12′** assignable to H-1,2, and 3 were separated each other without virtual coupling as follows: δ (at 90 MHz) 3.46 (3H s), 4.27 (2H s, CH_2S), 4.7—5.0 (3H, H-4,5,5′), 5.60 (1H d, $J_{1,2}$=4.7 Hz, H-1), 5.78 (1H q, J=4.7 and 7.2 Hz (=$J_{2,3}$), H-2), 6.12 (1H double q, J=7, ≈2, and ≈1 Hz, H-3). Irradiation at δ 4.8, the multiplet of H-3 collapsed to a doublet (J=7 Hz).

*Methyl 5-O-Benzoyl-2-O-(p-nitrobenzoyl)-β- and α-D-ribofuranosides (****13*** *and* ***13′****).* To a solution of **12** (or **12′**) (100 mg) in chloroform (1 ml), acetic acid (3 ml) and 30% aqueous hydrogen peroxide (1 ml) was added and the solution was kept at room temperature overnight. The solution was pound, with stirring, into an ice–water (150 ml) containing sodium hydrogencarbonate (4.5 g). The mixture was extracted with chloroform and the solution was washed with water, dried (Na_2SO_4), and concentrated to give a solid of **13** (or **13′**).

Compound **13**: Yield 67 mg (91%), colorless needles (from benzene–hexane), mp 134—135 °C, $[\alpha]_D^{23}$ +6° (*c* 1, $CHCl_3$).

Found: C, 57.46; H, 4.63; N, 3.23%. Calcd for $C_{20}H_{19}NO_9$: C, 57.55; H, 4.59; N, 3.36%.

PMR (pyridine-d_5) δ: 3.45 (3H s, OCH_3), 5.34 (1H s, H-1), 5.80 (1H d, J=4.4 Hz, H-2). On addition of D_2O followed by keeping the solution at room temperature for 1 h, the doublet of H-2 (δ 5.79) was gradually weakened with concomitant increase in intensity of a quartet at 5.91 (J=4.0 and 7.0 Hz, H-3 of **13m**) although the singlet of H-1 (δ 5.40) remained unchanged. This shows that an acyl migration occurred as shown below.



13 13m

Compound **13′**: Yield 58 mg (79%), pale-yellow needles (from benzene–hexane); mp 73.5—74.5 °C, $[\alpha]_D^{23}$ +80° (*c* 0.8, $CHCl_3$).

Found: C, 57.73; H, 4.63; N, 3.38%. Calcd for $C_{20}H_{19}NO_9$: C, 57.55; H, 4.59; N, 3.36%.

PMR (pyridine-d_5) δ: 3.55 (3H s, OCH_3), 5.53 (1H d, $J_{1,2}$=4.5 Hz, H-1), 5.70 (1H q, J=4.5 and 5.9 Hz(=$J_{2,3}$), H-2). On addition of water, the signal pattern was gradually changed possibly by the similar migration described above, but the migration was much slower than that for **13**.

*5-O-[5-O-Benzoyl-3-O-(benzylthiocarbonyl)-2-O-(p-nitrobenzoyl)-β-D-ribofuranosyl]-3,2′-bis(N-benzyloxycarbonyl)-1-N : 6-O-carbonyl-3′-deoxy-4′,6′-di-O-(α-naphthoyl)paromamine (****14****).* To a solution of **12** (1.9 g) in dry dichloromethane (5 ml), hydrogen bromide saturated in acetic acid (6 ml) was added and the solution was kept at room temperature for 1 h. The solution was concentrated *in vacuo* and the resulting syrup was dissolved in dichloromethane (40 ml). The solution was washed as fast as possible with aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated to give a syrup. Immediately, to a solution of the syrup in dichloromethane (9 ml), **6** (1.0 g), mercury (II) cyanide (1.9 g) and calcium sulfate (Drierite, 3.8 g), were added and the mixture was vigorously stirred at room temperature overnight. The reaction mixture was filtered with aid of chloroform and the filtrate was concentrated. The resulting syrup was chromato-

graphed over silica gel with chloroform–ethyl acetate (5 : 1) to give a solid of **14**, 1.05 g (66% based on **6**), $[\alpha]_D^{23}$ +33° (*c* 0.5, $CHCl_3$); IR (KBr): 1780, 1725, 1530 cm^{-1}.

Found: C, 64.50; H, 4.89; N, 3.79; S, 2.38%. Calcd for $C_{78}H_{68}N_4O_{22}S$: C, 64.81; H, 4.74; N, 3.88; S, 2.22%.

5-O-[*5*-O-*Benzoyl-2*-O-(p-*nitrobenzoyl*)-β-D-*ribofuranosyl*]-*3,2'*-*bis*(N-*benzyloxycarbonyl*)-*1*-N : *6*-O-*carbonyl-3'-deoxy-4',6'-di*-O-(α-*naphthoyl*)*paromamine* (***15***). To a solution of **14** (600 mg) in acetic acid (18 ml), 30% aqueous hydrogen peroxide (2.1 ml) was added and the solution was kept at room temperature overnight. On TLC with chloroform–ethanol–acetic acid (150 : 5 : 1), the solution showed two spots at R_f 0.49 (major, **15**) and 0.41 (trace, 3″-*p*-nitrobenzoate isomer?). The solution was concentrated at room temperature and the residue was dissolved in chloroform. The solution was washed with water, dried (Na_2SO_4), and concentrated. The resulting solid was chromatographed over silica gel with chloroform–ethanol–acetic acid (500 : 10 : 1) to give a solid of **15**, 424 mg (79%), $[\alpha]_D^{23}$ +18° (*c* 0.5, $CHCl_3$).

Found: C, 65.03; H, 4.96; N, 4.17%. Calcd for $C_{70}H_{62}N_4O_{21}$: C, 64.91; H, 4.82; N, 4.33%.

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