SYNTHESES OF 5-*O*-[2-*O*- AND 3-*O*-(6-AMINO-6-DEOXY-β-L-IDOPYRANOSYL)-β-D-RIBOFURANOSYL]-1-*N*-[(*S*)-4-AMINO-2-HYDROXYBUTANOYL]-3′-DEOXYPAROMAMINE

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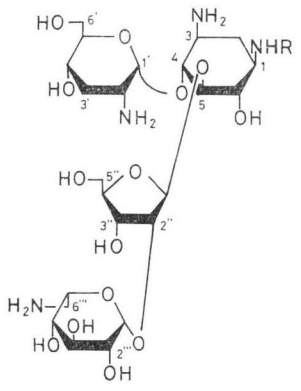
Synthesis and antibacterial activities of the title compounds are described.

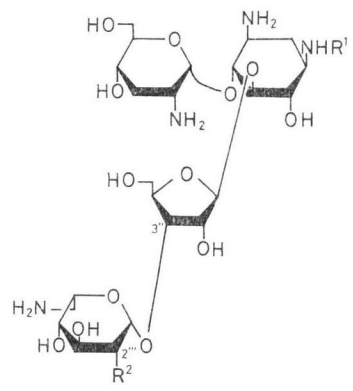
Recently we have prepared[1] two lividomycin B analogues which contain 6-amino-6-deoxy-β-L-idopyranose instead of 2,6-diamino-2,6-dideoxy-β-L-idopyranose of lividomycin B (**1**), and, it was observed that the 3′′-*O*-glycoside analog (**3**) had weaker antibacterial activity than lividomycin B while the position isomer, 2′′-*O*-glycoside analog (**2**), was less active than **3** (see Table 1). It was further observed that the presence of the 2′′′-amino group on lividomycin B has a potent influence on its antibacterial activity. This paper describes the syntheses of 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] derivatives (**10**, **11**) of **2** and **3** in order to see the effect of the aminoacyl residue for the antibacterial activity of **2** and **3**. The side chain residue is well-known for enhancing the activities of parent aminoglycoside antibiotics as exemplified by butirosins[2] and amikacin[3].

As the key intermediates for the syntheses of **10** and **11**, we utilized 5-*O*-[2-*O*-[1] (**4**) and 3-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-L-idopyranosyl)-5-*O*-benzoyl-β-D-ribofuranosyl]-4′,6′-di-*O*-benzoyl-3,2′-bis(*N*-benzyloxycarbonyl)-1-*N*: 6-*O*-carbonyl-3′-deoxyparomamine[1] (**5**). Treatment of **4** and **5** in a basic medium selectively cleaved the benzoyl and the 1,6-carbamate groups to give ninhydrin-positive derivatives (**6** and **7**), respectively, having 1-amino groups free. Coupling of the active ester of (*S*)-4-

Fig. 1.

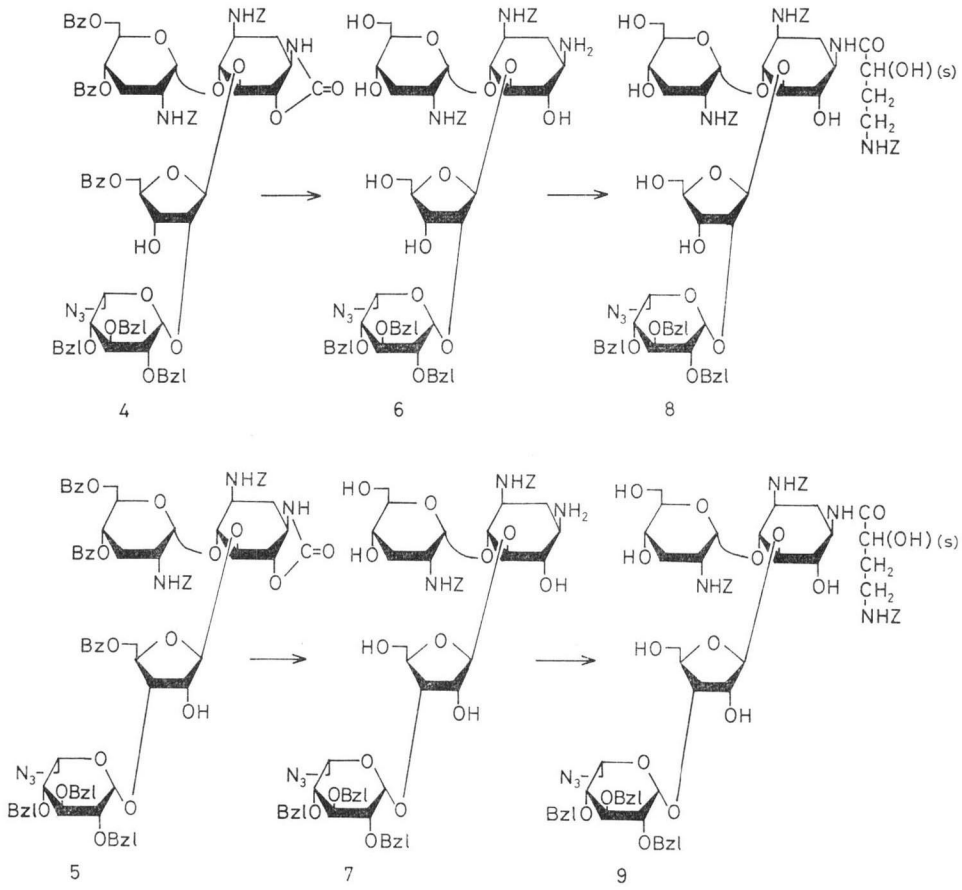


	R
2	H
10	$COCH(OH)CH_2CH_2NH_2$ (s)



	R^1	R^2
1	H	NH_2
3	H	OH
11	$COCH(OH)CH_2CH_2NH_2$ (s)	OH

Fig. 2.



benzyloxycarbonylamino-2-hydroxybutanoic acid[3] to the 1-amino groups of **6** and **7** was successfully carried out in tetrahydrofuran to give the corresponding 1-*N*-acyl derivatives (**8** and **9**). Finally, treatment of the derivatives with sodium metal in liquid ammonia (−50°C) simultaneously cleaved the benzyl and the benzyloxycarbonyl groups and reduced the azido into an amino group to give the desired 1-*N*-acyl derivatives (**10** and **11**, respectively).

Antibacterial spectra of **10** and **11** are shown in Table 1 with those of **2**, **3**, and lividomycin B (**1**). The results show that the introduction of (*S*)-4-amino-2-hydroxybutanoyl residue to the 1-amino group of **3** improves the activity of the parent antibiotic (**3**). It is noteworthy that **11** possesses activity against resistant strains having 5″-*O*-phosphorylating enzyme, whereas the isomer (**10**) having the aminoacyl residue attached at the 1-amino group of the 2″-*O*-glycosyl isomer (**2**) shows marked deterioration in the activity.

Experimental

^{1}H NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer. Thin-layer chromatography (TLC) was carried out on DC-Fertigplatten Kieselgel 60 F_{254} (E. Merck). For column chromatography, silica gel (Wakogel C-200) was used.

Table 1. Antibacterial spectra of lividomycin B (**1**), **2**, **3**, **10**, and **11**.

Test organisms[a]	MIC (mcg/ml)				
	1	**2**	**3**	**10**	**11**
Staphylococcus aureus FDA 209P	0.39	6.25	3.12	100	3.12
Micrococcus luteus PCI 1001	12.5	>100	50	>100	100
Bacillus subtilis NRRL B558	<0.2	0.78	<0.2	3.12	<0.2
Klebsiella pneumoniae PCI 602	0.78	6.25	1.56	50	1.56
Salmonella typhi T-63	3.12	12.5	6.25	100	3.12
Escherichia coli NIHJ	1.56	12.5	3.12	100	3.12
E. coli K-12	3.12	50	12.5	>100	6.25
E. coli K-12 R5	3.12	50	12.5	>100	12.5
E. coli K-12 ML1629[c]	>100	>100	>100	>100	6.25
E. coli K-12 ML1410 R81[c]	>100	>100	>100	>100	12.5
E. coli LA290 R55	3.12	25	6.25	>100	12.5
E. coli C600 R135	3.12	25	6.25	100	6.25
E. coli W677	1.56	25	3.12	100	6.25
E. coli JR66/W677	6.25	50	25	100	12.5
Pseudomonas aeruginosa A3	12.5	100	50	>100	100
Ps. aeruginosa No. 12	12.5	100	50	>100	50
Ps. aeruginosa TI13	12.5	100	50	>100	25
Ps. aeruginosa 99	25	100	100	>100	>100
Ps. aeruginosa GN315	25	>100	>100	>100	25
Mycobacterium smegmatis 607[b]	0.39	3.12	6.25	100	1.56

a) Agar dilution streak method (nutrient agar, 10^8 CFU/ml, 37°C, 18 hours).
b) 48 hours.
c) Resistant strain having the enzyme phosphorylating 3′- and 5″-hydroxyl groups.

5-*O*-[2-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-3,2′-bis(*N*-benzyloxycarbonyl)-3′-deoxyparomamine (**6**)

To a solution of **4** (349 mg) in dioxane (20 ml) was added 0.025 M barium hydroxide solution (5 ml) at hourly intervals (totally 20 ml) and the solution was kept at 60°C for 4 hours in total. After introduction of carbon dioxide, followed by filtration, the solution was concentrated. The residue was chromatographed on a silica gel column with chloroform - methanol - triethylamine (6: 1: 0.01) to give a ninhydrin-positive solid of **6**, 185 mg (67% as hemicarbonate), $[\alpha]_D^{26}+48°$ (*c* 1, chloroform); Rf 0.52 (TLC with chloroform - methanol - triethylamine=6: 1: 0.01); IR(KBr): 1530, 1700, 2100 cm^{-1} (N_3): 1760 cm^{-1} (cyclic carbamate) observed in **4** had disappeared.

Anal. Calcd. for $C_{66}H_{72}N_6O_{18}\cdot\frac{1}{2}H_2CO_3$: C 60.75, H 6.11, N 7.03%.
Found: C 61.02, H 6.00, N 7.22%.

5-*O*-[3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-3,2′-bis(*N*-benzyloxycarbonyl)-3′-deoxyparomamine (**7**)

Compound **5** (205 mg) was treated similarly as described for **6** to give **7**, 101 mg (62% as hemicarbonate), $[\alpha]_D^{26}+44°$ (*c* 1, chloroform); Rf 0.50 (TLC with the same solvent system described for **6**); IR (KBr): 1530, 1700, 2100 cm^{-1}.

Anal. Calcd. for $C_{66}H_{72}N_6O_{18}\cdot\frac{1}{2}H_2CO_3$: C 60.75, H 6.11, N 7.03%.
Found: C 60.87, H 6.26, N 6.92%.

5-*O*-[2-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-3,2′-bis(*N*-benzyloxycarbonyl)-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyl]-3′-deoxyparomamine (**8**)

A mixture of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoic acid[8] (75 mg), *N*-hydroxysuccinimide (35 mg) and dicyclohexylcarbodiimide (65 mg) in tetrahydrofuran (3 ml) was stirred for 1 hour in

an ice-bath. To the mixture, a solution of **6** (120 mg) in tetrahydrofuran (3 ml) containing triethylamine (0.2 ml) was added and the mixture was stirred for further 2 hours at ice-bath temperature. After filteration, the solution was concentrated and the residue was chromatographed on a silica gel column (20 g) with chloroform (50 ml), then with chloroform - methanol (20: 1) as developer. Since the product (**8**) obtained (130 mg) still contained slight amounts of impurities having similar mobilities, it was subjected to preparative TLC with chloroform - methanol - triethylamine (6: 1: 0.01) to give pure solid of **8**, 104 mg (74%), $[\alpha]_D^{26}+28°$ (*c* 1, chloroform): Rf 0.63 (TLC with the solvent system described for **6**).

Anal. Calcd. for $C_{72}H_{85}N_7O_{22}$: C 61.76, H 6.08, N 7.01%.
Found: C 61.70, H 6.12, N 6.82%.

5-*O*-[3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-3,2′-bis(*N*-benzyloxycarbonyl)-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoyl]-3′-deoxyparomamine (**9**)

Compound **7** (90 mg) was treated similarly as described for **8** to give **9**, 72 mg (68%), $[\alpha]_D^{26}+26°$ (*c* 1, chloroform); Rf 0.61 (TLC with the same solvent system described for **6**).

Anal. Calcd. for $C_{72}H_{85}N_7O_{22}$: C 61.76, H 6.08, N 7.01%.
Found: C 61.83, H 6.13, N 6.96%.

5-*O*-[2-*O*-[6-Amino-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3′-deoxyparomamine (**10**)

A solution of **8** (86 mg) in tetrahydrofuran (1.5 ml) was added to a solution of sodium metal (*ca.* 200 mg) in liquid ammonia (*ca.* 5 ml, −50°C) and the deep-blue solution was kept at the temperature for 1 hour. Addition of water until the solution became colorless, followed by gradual warming to room temperature, and evaporation under diminished pressure, gave a residue. An aqueous solution of the residue was poured onto a column of Dowex 50W×2 (NH_4^+) resin, and the column was washed with water, then eluted with 2 M aqueous ammonia. Ninhydrin-positive fractions were collected and evaporated. The residue was chromatographed on a column of CM-Sephadex C-25 (NH_4^+) with aqueous ammonia (0→0.5 M, gradually changed) to give a solid of **10**, 26 mg (50% as dicarbonate monohydrate), $[\alpha]_D^{26}+44°$ (*c* 1, water); $Rf_{\text{lividomycin B}}$ 0.38 (*n*-butanol - pyridine - water - acetic acid=6: 4: 3: 1); ^1H NMR (D_2O): δ 5.20 (1H d, $J_{1''',2'''}\simeq 1.5$ Hz, H-1‴), 5.50 (1H slightly br. s, H-1″), 5.67 (1H d, $J_{1',2'}=3.5$ Hz, H-1′).

Anal. Calcd. for $C_{27}H_{51}N_5O_{16}\cdot 2H_2CO_3\cdot H_2O$: C 41.28, H 6.76, N 8.30%.
Found: C 41.43, H 6.82, N 8.37%.

5-*O*-[3-*O*-(6-Amino-6-deoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3′-deoxyparomamine (**11**)

Compound **9** (50 mg) was treated similarly as described for **10** to give a solid of **11**, 15 mg (55% as hemicarbonate-$\frac{3}{2}$ hydrate), $[\alpha]_D^{26}+40°$ (*c* 0.9, water); $Rf_{\text{lividomycin B}}$ 0.57 (*n*-butanol - pyridine - water - acetic acid=6: 4: 3: 1); IR(KBr): 1560, 1640 cm^{-1}; ^1H NMR (D_2O): δ 5.00 (1H d, $J_{1''',2'''}\simeq 1.5$ Hz, H-1‴), ~5.4 (2H, H-1′, 1″).

Anal. Calcd. for $C_{27}H_{51}O_{16}\cdot\frac{1}{2}H_2CO_3\cdot\frac{3}{2}H_2O$: C 43.48, H 7.25, N 9.22%.
Found: C 43.27, H 7.14, N 9.17%.

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