Synthesis of 1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]lividomycin A[1]

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The title compound (**7**), which is active against resistant bacteria producing phosphotransferase I, was synthesized from lividomycin A through a cyclic carbamate derivative (**3**). Selective hydrolysis of the carbamate ring followed by acylation of the free C-1-amino group with (*S*)-2-hydroxy-4-aminobutyric acid by the active ester method led to **7**.

As already described,[2] (*S*)-4-amino-2-hydroxybutyrylation of 1-NH_2 of ribostamycin gives butirosin B which is active against kanamycin-resistant strains such as *E. coli* K-12 ML 1629 and ML 1630 producing neomycin-kanamycin phosphotransferase I[3] (P-ase I). The 5″-hydroxy group of lividomycins,[4] and ribostamycin undergoes this enzyme reaction and these antibiotics do not inhibit growth of these resistant organisms. We designed, therefore, to attach the above-mentioned side chain to the 1-NH_2 of lividomycin A, hoping that this antibiotic could be converted to a derivative with the activity against resistant strains.

Lividomycin A was treated with benzyl chloroformate to give penta-*N*-benzyloxycarbonyl derivative (**1**), which was treated with benzaldehyde dimethyl acetal in the presence of acidic catalyst according to the method described by Bissett, Evans, and Parrish.[5] Chromatographic separation of the products gave the tri-*O*-benzylidene derivative (**2**) in a yield of 48%. It should be noted that cyclohexylidenation of tetra-*N*-benzyloxycarbonylribostamycin with cyclohexanone dimethyl ketal gave a 5″-*O*-(1-methoxycyclohexyl) derivative.[6]

Selective removal of the protecting group at 1-NH_2 was successful through the cyclic carbamate method.[2] Treatment of **2** with sodium hydride in DMF afforded the 1,6-cyclic carbamate (**3**) in a yield of 52%. The presence of the cyclic carbamate was confirmed by the absorption peak[7] at 1765 cm^{-1}. Another cyclic carbamate formation between 2-NH_2 and 3-OH groups in the L-idose moiety was not recognized, suggesting that the 2,6-diamino-2,6-dideoxyidose moiety may be in 1C-like conformation which hinders the carbamate formation.

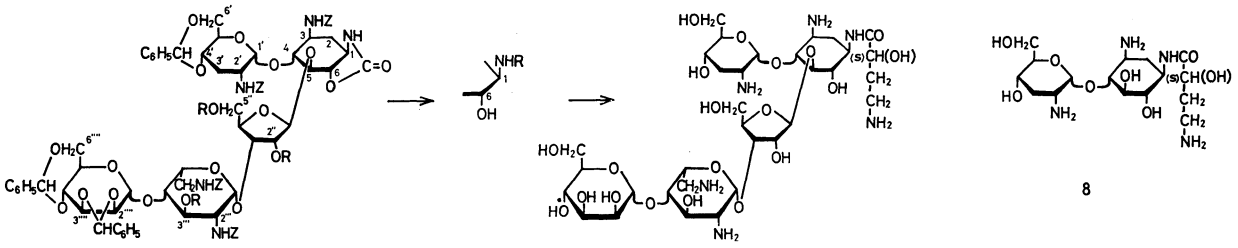
In order to determine the positions of the benzylidene and cyclic carbamate groups, **3** was mesylated in pyridine and the tri-*O*-mesyl derivative (**4**) was subjected to acidic hydrolysis in methanol. On examination of the hydrolyzate by thin-layer chromatography, the formation of 3′-deoxyparomamine[8] and methyl mannoside was clearly discerned, but formation of methyl riboside and methyl 2,6-diamino-2,6-dideoxyidoside was not observed. Since *O*-mesyl group is generally resistant to acid hydrolysis,[9] the above result indicates that the benzylidene groups are attached to 4′,6′-*O*, 2″″,3″″-*O*, and 4″″,6″″-*O*, and the cyclic carbamate is formed between 1-NH_2 and 6-OH groups. Observation of no OMe group of hemiacetal in the NMR spectrum of **3** also supported the structure **3**.

Protection of the hydroxyl groups with benzylidene groups is not required for the formation of 1,6-cyclic carbamate[7]; treatment of **1** with sodium hydride in DMF gave the corresponding cyclic carbamate in a yield of 45%. However, benzylidenation increased the solubility in organic solvents and facilitated later 1-*N*-acylation.

Selective hydrolysis of the cylic carbamate by gradual addition of barium hydroxide in a manner as already described[2] gave the aminol (**5**), which was then condensed with (*S*)-2-hydroxy-4-phthalimidobutyric acid[10] (HPBA) by the active ester method, using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in tetrahydrofurane. The condensation product (**6**) was then treated with hydrazine to remove the phthaloyl group and with palladium black and hydrogen to remove the benzyloxycarbonyl and benzylidene groups to give the titled compound (**7**).

A mild acid hydrolysis of **7** gave 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′-deoxyparomamine (**8**), and this fact confirmed the structure of **7**.

The synthetic 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-lividomycin A showed antibacterial activity[1] against organisms producing P-ase I.



	R
3	H
4	SO_2CH_3

	R
5	H (S)
6	$COCH(OH)CH_2CH_2N$=Pht

7 Z : $CO_2CH_2C_6H_5$, Pht : $(OC)_2C_6H_4$

8

Eoperimental

Thin layer chromatography (tlc) was carried out on silica gel (Silica Rider 5B, Daiichi Pure Chemicals Co., Ltd.)unless otherwise stated. Paper chromatography (ppc) was carried out on Toyo-Roshi paper No. 50, developed with 1-butanol–pyridine–water–acetic acid (6: 4: 3: 1) and detected by ninhydrin.

*1,3,2′,2‴,6‴-Penta-*N*-benzyloxycarbonyllividomycin A (1).* To a mixture of lividomycin A sulfate (21.4 g, approximately 20 mmol of lividomycin A base) and anhydrous sodium carbonate (16 g) in aqueous methanol (1: 4, 500 ml, ice-salt cooled), benzyl chloroformate (22.8 g) was added dropwise under stirring and stirring was continued for 3 hr at −5∼0 °C. The reaction mixture was evaporated to dryness and the residue was extracted with hot acetone (170 ml×3). The combined solution was evaporated to ∼100 ml. Addition of ether (300 ml) gave colorless precipitates, 30 g. On tlc with chloroform–methanol (6: 1), it gave a single spot at R_f 0.57. Purification by column chromatography on silica gel with chloroform–methanol (10: 1) as a solvent gave **1**, 26.07 g (91%); $[\alpha]_D^{22}$ +40 ° (*c* 1, MeOH). Found: C, 57.73; H, 6.14; N, 4.60 %. Calcd for $C_{69}H_{85}N_5O_{28}$: C, 57.86; H, 5.98; N, 4.89%.

*4′,6′; 2‴,3‴; 4‴,6‴-Tri-*O*-benzylidene-1,3,2′,2‴,6‴-penta-*N*-benzyloxycarbonyllividomycin A (2).* To a solution of **1** (5.5 g) in dry DMF (80 ml), anhydrous *p*-toluenesulfonic acid (140 mg) and benzaldehyde dimethyl acetal (8 g) were added and the solution was heated at 30 °C for 6 hr under reduced pressure (10—15 Torr). On tlc with chloroform–methanol (20: 1), the solution showed a major spot (R_f 0.68) and other several minor spots. Methanol (100 ml) was added and the solution was allowed to stand for 30 min. After addition of saturated sodium hydrogen carbonate solution (3 ml), the mixture was evaporated with the aid of toluene. The residue was extracted with chloroform (150 ml×2). The organic layer was washed with water, dried over sodium sulfate and the solvent was removed by evaporation. The residue (7 g) was chromatographed on a column of silica gel with chloroform–ethanol–triethylamine (25: 1: 0.05). The fractions containing **2** was evaporated to give a solid of **2**, 3.14 g (48%); $[\alpha]_D^{25}$ +32.5 °(*c* 1, $CHCl_3$). Found: C, 63.92; H, 5.92; N, 4.11%. Calcd for $C_{90}H_{97}N_5O_{28}$: C, 63.71; H, 5.76; N, 4.13%. Mono and di-*O*-benzylidene derivatives were also obtained (2.1 g).

*4′,6′;2‴,3‴;4‴,6‴-Tri-*O*-benzylidene-3,2′,2‴,6‴-tetra-*N*-benzyloxycarbonyllividomycin A 1,6-Carbamate (3).* To an ice-cold solution of **2** (550 mg) in dry DMF (5 ml) under nitrogen, 50% oily sodium hydride (70 mg) was added and the mixture was stirred for 3 hr under cooling. After addition of acetic acid (0.2 ml), the resulting viscous, pale-brown solution was poured into a mixture of chloroform (70 ml) and saturated sodium chloride solution (70 ml) under vigorous stirring. The organic layer was separated, washed with sodium chloride solution, dried over sodium sulfate and evaporated. The residue was chromatographed on a short column of silica gel with chloroform–methanol–triethylamine (20: 1: 0.1) to give a solid of **3**, 270 mg (52%); $[\alpha]_D^{25}$ +34° (*c* 1, $CHCl_3$); IR (KBr): 1765 cm^{-1} (*trans*-fused cyclic carbamate[7]). R_f 0.36 on tlc with chloroform–methanol (20: 1). Found: C, 62.49; H, 5.53; N, 4.21%. Calcd for $C_{83}H_{89}N_5O_{27}$: C, 62.75; H, 5.65; N, 4.41%.

*4′,6′;2‴,3‴;4‴,6‴-Tri-*O*-benzylidene-3,2′,2‴,6‴-tetra-*N*-benzyloxycarbonyl-2″,5″,3‴-tri-*O*-mesyllividomycin A 1,6-Carbamate (4).* To a solution of **3** (95 mg) in pyridine (2 ml), mesyl chloride (0.1 ml) was added and the solution was allowed to stand at room temperature overnight. Isolation of the product in a usual manner gave a solid of **4**, 105 mg (96%); $[\alpha]_D^{24}$ +28.5° (*c* 1, $CHCl_3$); IR (KBr): 1175 (ν_s SO_2), 1350 (ν_{as} SO_2) ,1770 cm^{-1}; NMR (in $CDCl_3$): δ 2.93 (3H s, SO_2CH_3), 3.06 (6H s, SO_2CH_3). Found: C, 56.39; H, 5.23; N, 3.99; S, 5.54%. Calcd for $C_{86}H_{95}N_5O_{33}S_3$: C, 56.67; H, 5.25; N, 3.84; S, 5.28%.

Acidic Methanolysis of 3 and 4. Procedure 1. A suspension of **3** or **4** in 1 M methanolic hydrogen chloride was heated at 80 °C and the reaction mixture was examined by ppc and ninhydrin coloration. After overnight heating, the reaction mixture of **3** gave a spot of 3′-deoxyparomamine[8] (the R_f-value was taken as 1) and a spot of R_f 3.5. The reaction mixture of **4** gave spots of R_f 4.75 and 3.31 (slight) and 1 (very slight). After 3 days heating, the chromatographic pattern of the reaction mixture of **3** did not changed but that of **4** changed to give a clear spot of 3′-deoxyparomamine. Other spots did not changed.

Procedure 2. A suspension of **3** or **4** in 0.5 M methanolic hydrogen chloride was heated at 80 °C for 2 days and the reaction mixture was examined by tlc (Kieselgel 60 F-254 from E. Merck in Germany) with benzene–methanol (1: 1) and detected by 5% anisaldehyde in ethanolic sulfuric acid. Each of the reaction mixtures gave a spot of methyl mannoside (R_f 0.28).

*4′,6′;2‴,3‴; 4‴,6‴-Tri-*O*-benzylidene-3,2′,2‴,6‴-tetra-*N*-benzyloxycarbonyllividomycin A (5).* To a solution of **3** (600 mg) in dioxane (35 ml), 0.025 M barium hydroxide solution (6 ml) was added and the mixture was stirred at 60 °C for 30 min to the completeness of neutralization. Additional aliquots (6 ml×2) of the barium hydroxide solution were added and treated similarly. Carbon dioxide was introduced and the mixture was filtrated. After evaporation of the filtrate, the residue was taken up in chloroform, washed with water, dried over sodium sulfate and freed of solvent. The crude product (620 mg) was chromatographed on a short column of silica gel with chloroform–methanol–triethylamine (10: 1: 0.03) to give a solid of **5**, 320 mg (54%); $[\alpha]_D^{20}$ +28° (*c* 1, $CHCl_3$). Found: C, 62.75; H, 6.00; N, 4.26%. Calcd for $C_{82}H_{91}N_5O_{26}$: C, 63.03; H, 5.87; N, 4.48%.

*4′,6′; 2‴,3‴;4‴,6‴-Tri-*O*-benzylidene-3,2′,2‴,6‴-tetra-*N*-benzyloxycarbonyl-1-*N*-[(S)-2-hydroxy-4-phthalimidobutyryl]-lividomycin A (6).* To an ice-cold solution of HPBA (42 mg) and *N*-hydroxysuccinimide (19 mg) in THF (1 ml), DCC (37 mg) was added and the mixture was stirred for 1 hr under cooling. To the resulting suspension, a solution of **5** (205 mg) and triethylamine (30 mg) in THF (2 ml) was added and the mixture was stirred for 2 hr under cooling. After filtration, the solution was evaporated and the residue was extracted with acetone. The solvent was removed and the residue (240 mg) was chromatographed on a short column of silica gel with chloroform–ethanol–triethylamine (20: 1: 0.05) to give a solid of **6**, 172 mg (73%); $[\alpha]_D^{22}$ +37° (*c* 1, $CHCl_3$); IR (KBr): 1713, 1655 (sh), 1525 cm^{-1}. Found: C, 62.73; H, 5.62; N, 4.60%. Calcd for $C_{94}H_{100}N_6O_{30}$: C, 62.94; H, 5.62; N, 4.68%.

*1-*N*-[(S)-4-Amino-2-hydroxybutyryl]lividomycin A (7).* To a suspension of **6** (185 mg) in aqueous ethanol (7: 1, 5 ml), hydrazine hydrate (200 mg) was added and the solution was heated at 60°C for 1.5 hr. After evaporation of the solvent, the residue was washed with water and extracted with chloroform. The solvent was removed and the residue was extracted with dioxane. To the solution (4 ml), water (1 ml) and acetic acid (0.3 ml) were added and the mixture was hydrogenated over palladium black at room temperature overnight. The mixture was filtered and evaporated. The residue was extracted with water and, after addition of a few drops of acetic acid, the aqueous solution was again treated with palladium black and hydrogen likewise. The hydrogenation was repeated once again. After filtration, the filtrate was evaporated and the residue was chromatographed on a column of CM-Sephadex C-25 (NH_4 form). Elution with a linear gradient of am-

monia (0—0.2 M) gave a single peak of chromatographically homogeneous **7**, 63 mg (69%); $[\alpha]_D^{22}$ +68° (*c* 1, H_2O); ppc, $R_{f\text{lividonycin A}}$ 0.52; IR (KBr): 1660, 1575 cm^{-1}. Found: C, 44.81; H, 7.13; N, 9.30%. Calcd for $C_{33}H_{62}N_6O_{20}\cdot H_2O$: C, 45.00; H, 7.32; N, 9.54%.

1-N-((S)-4-Amino-2-hydroxybutyryl)-3'-deoxyparomamine (8). A suspension of **7** (100 mg) in 0.4 M methanolic hydrogen chloride (5 ml) in a sealed tube was shaken at 70 °C for 15 hr. Powdered sodium hydrogen carbonate was added and the neutral mixture was filtered and the filtrate was evaporated. The residue was chromatographed on a column of CM-Sephadex C-25 (NH_4 form). Elution with a linear gradient of ammonia (0—0.15 M) gave a single peak of chromatographically homogeneous **8**, 25 mg (47%); $[\alpha]_D^{19}$ +19° (*c* 0.7, H_2O); ppc, $R_{f\ 3'\text{-deoxyparomamine}}$ 0.45; IR (KBr): 1640, 1550 cm^{-1}. Found: C, 43.62; H, 7.41; N, 11.72%. Calcd for $C_{16}H_{32}N_4O_8\cdot H_2CO_3$: C, 43.40; H, 7.28; N, 11.90%.

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