SYNTHESIS OF 1-N-((s)-4-AMINO-2-HYDROXYBUTYRYL) LIVIDOMYCIN A

Sir:

In the foregoing communication[1] we have shown that the combination of 3′, 4′-dideoxygenation and the attachment of the (s)-4-amino-2-hydroxybutyryl residue to 1-NH_2 group caused marked increase and expanse of the antibacterial activity of ribostamycin. Structurally related antibiotic lividomycin A[2] has no 3′-hydroxyl group, but resistant organisms produce an enzyme which phosphorylates[3] 5″-hydroxyl group of this antibiotic. In this communication, we synthesized the titled compound in order to see the effect of the side residue on the antibacterial activity of lividomycin A.

The five amino groups of lividomycin A were protected with benzyloxcarbonyl chloride in aqueous methanol in the presence of sodium carbonate to give penta-N-benzyloxycarbonyl-lividomycin A (**1**) in a yield of 95 % $[\alpha]_D^{22}$ +40° (*c* 1, methanol), which was dissolved in dry DMF and treated with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid at 30°C under reduced pressure (10~15 Torr) for 6 hours. The resulting products were separated by silica gel column chromatography with chloroform-methanol-triethylamine (30:1:0.1) and the main tribenzylidene product (**2**) was isolated in a yield of 28 %, $[\alpha]_D^{25}$ +32.5° (*c* 1, chloroform). [Calcd. for $C_{90}H_{97}N_5O_{28}$: C 63.71, H 5.76, N 4.13; Found: C 63.92, H 5.92, N 4.11]. The hydroxyl groups of **2** benzylidenated were assumed to be at 2, 3 and 4, 6 of mannose moiety and at 4, 6 of 3′-deoxyglucosamine moiety.

In order to make 1, 6-cyclic carbamate, compound **2** was dissolved in dry DMF and treated with sodium hydride similarly as described in the foregoing communications[1,4] and the reaction product (**3**) was purified by a silica gel column chromatography with chloroform-methanol-triethylamine (20 : 1 : 0.1). Tri-O-benzylidene-tetra-N-benzyloxycarbonyl-1, 6-carbamate (**3**) was obtained in a yield of 53 %, mp 155~157°C (decomp.), $[\alpha]_D^{25}$ +34° (*c* 1, chloroform). ir: 1765 cm^{-1}. [Calcd. for $C_{83}H_{89}N_5O_{27}$: C 62.75, H 5.65, N 4.41; Found: C 62.49, H 5.53, N 4.21].

Selective hydrolysis of the cyclic carbamate was achieved similarly as described in a previous paper.[5] To the solution of **3** in dioxane, 0.05N barium hydroxide was added at intervals keeping the solution temperature at 60°C until the solution was weakly alkaline for 30 minutes after the last addition. The reaction product was purified by silica gel column chromatography with chloroform-methanol-triethylamine (10:1:0.03) to give the corresponding

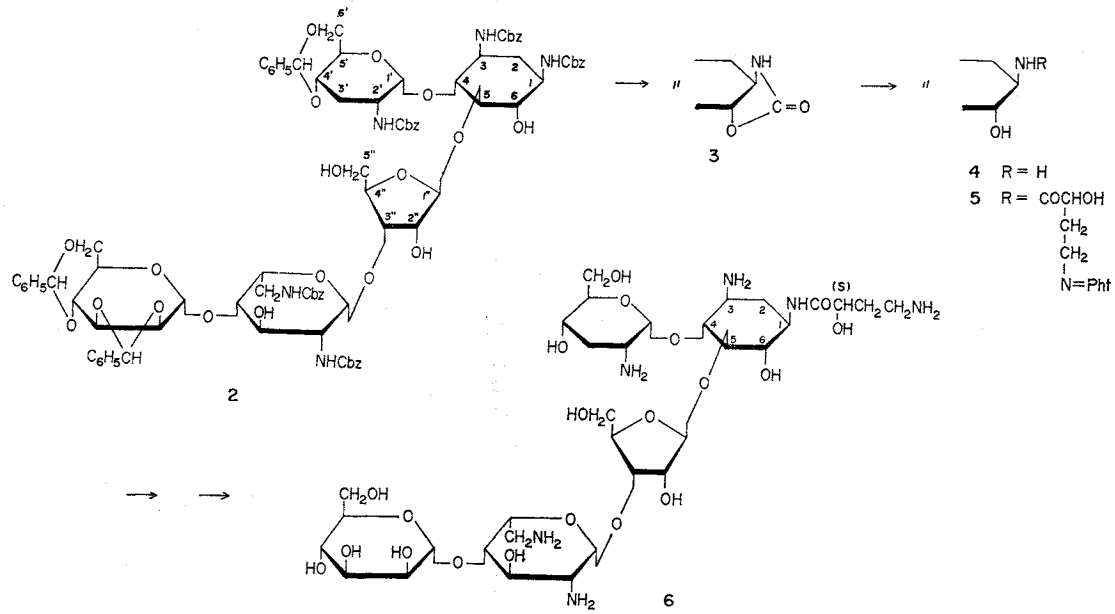

Table 1. Antibacterial spectra of 1-N-((s)-4-amino-2-hydroxybutyryl)lividomycin A (**6**) and lividomycin A

Test organisms*	Minimal inhibitory concentration (mcg/ml)	
	6	Lividomycin A
Staphylococcus aureus FDA 209P	1.56	0.78
Sarcina lutea PCI 1001	>100	50
Bacillus subtilis NRRL B-558	<0.39	<0.39
Klebsiella pneumoniae PCI 602	1.56	1.56
〃 type 22 #3038	3.12	3.12
Salmonella typhosa T-63	0.78	0.78
Escherichia coli NIHJ	3.12	3.12
〃 K-12	1.56	1.56
〃 〃 R5	1.56	1.56
〃 〃 ML 1629	3.12	>100
〃 〃 ML 1630	6.25	>100
〃 〃 ML 1410	1.56	1.56
〃 〃 〃 R 81	12.5	>100
〃 〃 LA 290 R 55	3.12	3.12
〃 〃 〃 R 56	1.56	1.56
〃 〃 〃 R 64	3.12	3.12
〃 〃 C 600 R 135	3.12	1.56
〃 〃 W 677	3.12	1.56
〃 〃 JR 66/W 677	6.25	6.25
〃 J-5 R 11-2	3.12	>100
Pseudomonas aeruginosa A3	12.5	12.5
〃 No. 12	12.5	12.5
〃 GN 315	50	50
〃 TI 13	25	25
〃 99	50	50
Proteus rettgeri GN 311	100	12.5
〃 GN 466	6.25	1.56
Mycobacterium smegmatis ATCC 607**	0.39	0.39

* Agar dilution streak method (nutrient agar, 37°C, 18 hours).
** 48 hours.

1-amino derivative (**4**) in a yield of 54 %, $[\alpha]_D^{22}$ + 27.5° (*c* 1, chloroform). ir: no peak was observed near 1760 cm^{-1}. [Calcd. for $C_{82}H_{91}N_5O_{26}$: C 63.03, H 5.87, N 4.48; Found: C 62.75, H 6.00, N 4.26].

The compound **4** was condensed with (s)-2-hydroxy-4-phthalimido-butyric acid with aid of N-hydroxysuccinimide and dicyclohexylcarbodiimide in THF as described in a previous paper[5]. The 1-N-((s)-2-hydroxy-4-phthalimido-butyryl) derivative (**5**) was obtained in a yield of 73 %, mp 143~147° (decomp.), $[\alpha]_D^{22}$ +37° (*c* 1, chloroform). ir: 1713, 1655 (sh.), 1525 cm^{-1}. [Calcd. for $C_{94}H_{100}N_6O_{30}$: C 62.94, H 5.62, N 4.68; Found: C 62.73, H 5.62, N 4.60].

The compound **5** was then treated with 4 % hydrazine hydrate in 80 % ethanol at 60°C for 1.5 hours to remove the phthaloyl group and then with palladium black and hydrogen in aqueous dioxane to remove the benzyloxycarbonyl and benzylidene groups to give the deblocked product, which was purified by a column of CM-Sephadex C-25 (NH_4^+ form) with ammonia (0~0.2 N). 1-N-((s)-4-Amino-2-hydroxybutyryl) lividomycin A (**6**) was obtained in a yield of 69 % from (**5**) as a monohydrate, $[\alpha]_D^{22}$ +68° (*c* 1, water). ir: 1660 cm^{-1}. $Rf_{\text{lividomycin A}}$ 0.52 (on paper chromatography with 1-butanol-pyridine-water-acetic acid (6:4:3:1)). [Calcd. for $C_{83}H_{62}N_6O_{20}\cdot H_2O$: C 45.00, H 7.32, N 9.54; Found: C 44.81, H 7.13, N 9.30].

To confirm the structure of **6**, namely, to decide the position of the amide group formed by the condensation of the 4-amino-2-hydroxybutyric acid, **6** (100 mg) was hydrolyzed with 0.4 N hydrogen chloride in methanol at 70°C for 15 hours. A compound which showed an $Rf_{\text{2-deoxystreptamine}}$ 0.53 on paper chromatography with the system described above was isolated in a yield of 25 mg and proved to be 1-N-((s)-4-amino-2-hydroxybutyryl)-3′-deoxy-

paromamine,* $[\alpha]_D^{19}$ +19° (*c* 0.7, water). [Cacld. for $C_{16}H_{32}N_4O_8 \cdot H_2O$: C 45.06, H 8.04, N 13.14; Found: C 45.28, H 8.17, N 12.98]. This result confirmed that the cyclic carbamate was formed between 1-amino and 6-hydroxyl groups of 2-deoxystreptamine moiety and not between 2-amino and 3-hydroxyl groups of 2, 6-diamino-2, 6-dideoxyidose moiety.

The synthetic 1-N-((s)-4-amino-2-hydroxybutyryl) lividomycin A was markedly broad antibacterial, inhibiting resistant bacteria as compared with that of lividomycin A (Table 1). However, it is to be noted that the attachment of the (s)-4-amino-2-hydroxybutyryl residue to lividomycin A did not enhance the activity of the parent antibiotic. Whereas, in the case[1,5] of ribostamycin and 3′, 4′-dideoxyribostamycin, the attachment of the residue gave rise to the substances having markedly enhanced activity.

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* 3′-Deoxyparomamine is synonymous with 4-O-(2-amino-2, 3-dideoxy-α-D-ribo-hexopyranosyl)-2-deoxystreptamine.

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