# Synthesis of 6′-Amino-1-*N*-[(*S*)-4-Amino-2-Hydroxybutyryl]-6′-Deoxylividomycin A

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The titled compound (**11**) was prepared from lividomycin A. A partially protected cyclic 1,6-carbamate derivative (**6**), which has free hydroxyl groups at C-4′ and C-6′, was prepared, and the 6′-hydroxyl group was selectively tosylated. Replacement of the tosyl group by azido group, followed by partial hydrolysis of the carbamate ring gave the aminol (**9**). Acylation of the free C-1-amino group with (*S*)-4-amino-2-hydroxybutyric acid and hydrogenation of the azido group led to **11**. Preparation of 4′,6′-di-*O*-methyllividomycin A (**13**) was also described.

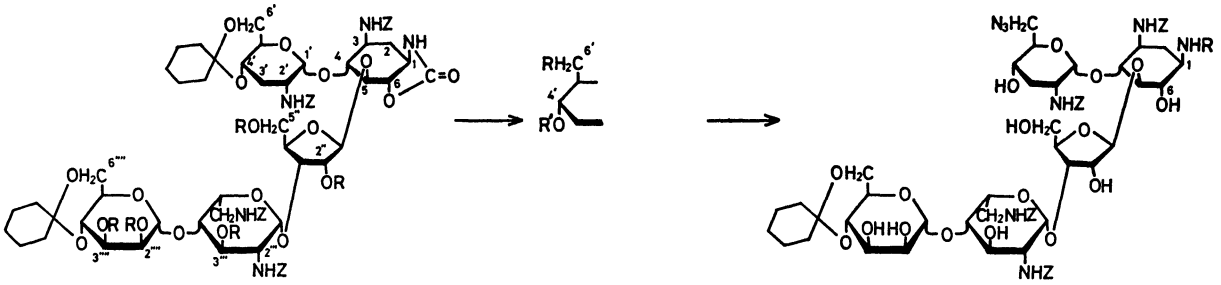
In the foregoing paper,[1] we reported the synthesis of 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)lividomycin A (**1**). The compound had an improved antibiotic spectrum, being active against resistant organisms producing neomycin-kanamycin phosphotransferase I (P-ase I). We have further undertaken to prepare its 6′-amino-6′-deoxy derivative namely the titled compound which is synonymous with 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′-deoxy-4‴-*O*-mannosylneomycin B expecting to obtain a more effective compound, because introduction of an amino group at C-6′ of related aminoglycoside antibiotics generally enhances the antibacterial activity of the parent antibiotic.

Penta-*N*-benzyloxycarbonyllividomycin A (**2**) described in the foregoing paper[1] was treated with 1,1-dimethoxycyclohexane in the presence of acidic catalyst to give di-*O*-cyclohexylidene derivative (**3**) as the major product. It should be noted that the similar reaction of **2** with benzaldehyde dimethyl acetal gave a tri-*O*-benzylidene derivative[1] as the major product. Addition of methanol to the reaction mixture containing di-*O*-cyclohexylidene derivatives as major products caused hydrolysis of the minor products, and this treatment easily afforded the purified **3**. The positions of the cyclohexylidene groups in **3** were proved by periodate oxidation of **3** and by *O*-methylation of **6** that is later described. Periodate oxidation of **3** followed by acidic hydrolysis gave no trace of mannose, indicating the absence of a cyclohexylidene group at C-2 and C-3 of the mannose moiety. It is also noteworthy that **3** contains no 1-methoxycyclohexyl group, which is formed[2] at C-5 of the ribose moiety in the case of similar cyclohexylidenation of tetra-*N*-benzyloxycarbonylribostamycin; this result is compatible with the benzylidenation of penta-*N*-benzyloxycarbonyllividomycin A.[1]

Treatment of **3** with sodium hydride in DMF[1,3] gave the 1,6-cyclic carbamate (**4**) which was then benzoylated. The penta-*O*-benzoyl derivative (**5**) was more soluble in organic solvents than the corresponding tri-*O*-benzylidene derivative of lividomycin A described previously.[1] Controlled hydrolysis of **5** with aqueous acetic acid successfully removed only the cyclohexylidene group at C-4′,6′. Possibly this selectivity is due to participation of the *O*-benzoyl group at C-3‴′. We have experienced the similar phenomenon in the other examples which will be published elswhere. For structural confirmation, the mono-*O*-cyclohexylidene derivative (**6**) was methylated with diazomethane in the presence of $BF_3$-etherate to give the di-*O*-methyl derivative (**12**) and treated successively with barium hydroxide, acetic acid, and palladium and hydrogen to give the 4′,6′-di-*O*-methyllividomycin A (**13**), whose acidic methanolysis did not give 3′-deoxyparomamine in the hydrolyzate, indicating that the hydroxyl groups at C-4′ and C-6′ (not those at C-4‴′ and C-6‴′) were methylated.

Tosylation of **6** gave the 6′-*O*-tosyl derivative (**7**). The selective 6′-*O*-tosylation was supported by the fact that tritylation was difficult for **7**.

Compound **7** was treated with sodium azide in DMF to give the 6′-azido derivative (**8**). Treatment of **8** with dilute solution of barium hydroxide hydrolyzed the 1,6-cyclic carbamate as well as the benzoyl ester



Z : $CO_2CH_2C_6H_5$

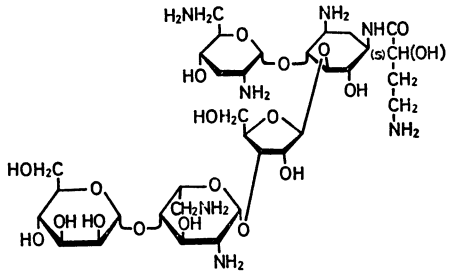
| | R | R′ |
|---|---|---|
| 6 | OH | H |
| 7 | $OSO_2C_6H_4$-*p*-$CH_3$ | H |
| 8 | $N_3$ | H |
| 12 | $OCH_3$ | $CH_3$ |

| | R |
|---|---|
| 4 | H |
| 5 | $COC_6H_5$ |

| | R |
|---|---|
| 9 | H (S) |
| 10 | $COCH(OH)CH_2CH_2NHZ$ |

groups to give **9** which has a free amino group at C-1. Condensation of **9** with (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid[4] by an active ester method using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in THF gave the 1-*N*-acyl derivative (**10**). The cyclohexylidene group of **10** was removed by acidic treatment and the product was catalytically hydrogenated to remove the benzyloxycarbonyl groups and to reduce the azido to the amino group to afford the titled compound (**11**).

The derivative **11** showed antibacterial activity against resistant bacteria producing P-ase I as expected, but the activity did not exceed that of 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)lividomycin A.[1] The 4′,6′-di-*O*-methyllividomycin A (**13**) showed no antibacterial activity.



**11**

## Experimental

General procedure was the same as described in the foregoing paper.[1]

*1,3,2′,2‴,6‴-Penta*-N-*benzyloxycarbonyl-4′,6′;4⁗,6⁗-di*-O-*cyclohexylidenelividomycin A (3).* To a solution of penta-*N*-benzyloxycarbonyllividomycin A (**2**) (7.87 g) in dry DMF (170 ml), *p*-toluenesulfonic acid (200 mg) and 1,1-dimethoxycyclohexane (18 ml) were added and the solution was heated at 30 °C for 3.5 hr under reduced pressure (~30 Torr). Methanol (80 ml) was added and the solution was allowed to stand for 30 min. Examination by tlc using chloroform–methanol (20 : 1) showed that by the methanol treatment, three spots initially observed collapsed to single major one ($R_f$ 0.26). After addition of saturated sodium hydrogen carbonate solution, the solvent was removed by coevaporation with toluene. The residue was chromatographed on a column of silica gel with chloroform–methanol–triethylamine (30 : 1 : 0.1). The portion containing **3** was evaporated to give a solid of **3**, 3.73 g (43%); $[\alpha]_D^{27}$ +43° (*c* 1, $CHCl_3$).

Found: C, 60.92; H, 6.39; N, 4.22%. Calcd for $C_{81}H_{101}N_5O_{28}$: C, 61.08; H, 6.39; N, 4.40%.

*3,2′,2‴,6‴-Tetra*-N-*benzyloxycarbonyl-4′,6′;4⁗,6⁗-di*-O-*cyclohexylidenelividomycin A 1,6-Carbamate (4).* To an ice-cold solution of **3** (300 mg) in dry DMF (3 ml), 50% oily sodium hydride (40 mg) was added under a nitrogen atmosphere and the mixture was stirred for 3 hr in the cold. After neutralization with acetic acid, the resulting viscous solution was poured into a mixture of chloroform and saturated sodium chloride solution under stirring. The separated organic layer was washed with saturated sodium chloride solution and water, and dried over sodium sulfate. The solution was evaporated and the residue (270 mg) was chromatographed on a short column of silica gel with chloroform–methanol–triethylamine (25 : 1 : 0.1). The portion containing **4** was evaporated to give a solid of **4**, 195 mg (70%); $[\alpha]_D^{27}$ +41° (*c* 1, $CHCl_3$); IR (KBr): 1760 $cm^{-1}$.

Found: C, 59.58; H, 6.32; N, 4.45%. Calcd for $C_{74}H_{93}N_5O_{27}$: C, 59.87; H, 6.31; N, 4.72%.

*2″,5″,3‴,2⁗,3⁗,2‴′,6‴′-Penta*-O-*benzoyl-3,2′,2‴,6‴-tetra*-N-*benzyloxycarbonyl-4′,6′;4⁗′,6⁗′-di*-O-*cyclohexylidenelividomycin A 1,6-Carbamate (5).* To a solution of **4** (210 mg) in dry pyridine (4.2 ml), benzoyl chloride (530 mg) was added and the solution was allowed to stand at −10 °C overnight. A few drops of water was added and the solution was allowed to stand for 1 hr. Powdered sodium hydrogen carbonate (300 mg) was added and the mixture was evaporated. The residue was extracted with chloroform and the solution was washed with water, potassium hydrogen sulfate solution, sodium hydrogen carbonate solution, and water and dried over sodium sulfate. After evaporation, the residue was dissolved in a mixture of ethyl acetate–benzene–triethylamine (2 : 5 : 0.02) and the solution was filtered through a short column of silica gel with aid of the above solvent system to remove impurities. Evaporation of the filtrate gave a solid, which was reprecipitated from benzene–*n*-hexane to give **5**, 220 mg (78%); $[\alpha]_D^{27}$ +13° (*c* 1, $CHCl_3$).

Found: C, 65.15; H, 5.70; N, 3.43%. Calcd for $C_{109}H_{113}N_5O_{32}$: C, 65.29; H, 5.68; N, 3.49%.

*2″,5″,3‴,2⁗,3⁗,2‴′,6‴′-Penta*-O-*benzoyl-3,2′,2‴,6‴-tetra*-N-*benzyloxycarbonyl-4⁗′,6⁗′*-O-*cyclohexylidenelividomycin A 1,6-Carbamate (6).* To a solution of **5** (600 mg) in acetone (15 ml), acetic acid (6.25 ml) and water (3.75 ml) were added and the solution was allowed to stand at 37 °C for 8 hr. Sodium hydrogen carbonate (4 g) was added and the mixture was concentrated. The resulting sludge was shaken with chloroform. The separated organic layer was washed with saturated sodium hydrogen carbonate solution, and water, dried over sodium sulfate, and evaporated. On tlc with chloroform–methanol (30 : 1), the residue showed two main spots of $R_f$ 0.71 (**5**) and $R_f$ 0.29 (**6**) along with two faint spots. The residue was chromatographed on a column of silica gel (22 g) successively with chloroform–methanol–triethylamine (50 : 1 : 0.1, totally 120 ml) and chloroform–methanol–triethylamine (30 : 1 : 0.1). From the early fractions, **5** (240 mg, 40%) was recovered. From the later fractions, **6** was obtained; it was reprecipitated from chloroform–*n*-hexane, 290 mg (50%); $[\alpha]_D^{27}$ +11° (*c* 1, $CHCl_3$).

Found: C, 64.47; H, 5.53; N, 3.55%. Calcd for $C_{103}H_{105}N_5O_{32}$: C, 64.27; H, 5.50; N, 3.64%.

*2″,5″,3‴,2⁗,3⁗,2‴′,6‴′-Penta*-O-*benzoyl-3,2′,2‴,6‴-tetra*-N-*benzyloxycarbonyl-4⁗′,6⁗′*-O-*cyclohexylidene-6′*-O-*tosyllividomycin A 1,6-Carbamate (7).* To a cold solution (−10 °C) of **6** (770 mg) in pyridine (16 ml), *p*-toluenesulfonyl chloride (390 mg) was added and the solution was allowed to stand at the temperature overnight and worked up in a similar manner as described for the preparation of **5** from **4**, 764 mg (92%); $[\alpha]_D^{31}$ +12.5° (*c* 2, $CHCl_3$); IR (KBr): 1175 $cm^{-1}$ (Ts); NMR ($CDCl_3$): $\delta$ 2.39 (3H s, $SO_2C_6H_4C\underline{H}_3$).

Found: C, 63.23; H, 5.33; N, 3.14; S, 1.55%. Calcd for $C_{110}H_{111}N_5O_{34}S$: C, 63.55; H, 5.38; N, 3.37; S, 1.54%.

*6′-Azido-2″,5″,3‴,2⁗,3⁗′-Penta*-O-*benzoyl-3,2′,2‴,6‴-tetra*-N-*benzyloxycarbonyl-4⁗′,6⁗′*-O-*cyclohexylidene-6′-deoxylividomycin A 1,6-Carbamate (8).* To a solution of **7** (720 mg) in dry DMF (18 ml), sodium azide (230 mg) was added and the mixture was stirred at 60 °C for 6 hr. On tlc with chloroform–ethanol (30 : 1), **8** ($R_f$ 0.40) appeared instead of **7** ($R_f$ 0.47). Chloroform (200 ml) was added and the solution was washed with saturated sodium chloride solution (50 ml × 4), and water, and dried over sodium sulfate. After evaporation of the solvent, the residue was dissolved in a mixture of chloroform–ethanol–triethylamine (40 : 1 : 0.1)

and the solution was filtered through a short column (20 g) of silica gel with aid of the above solvent system to remove impurities. Evaporation of the filtrate gave **8**, 633 mg (94%); $[\alpha]_D^{28}$ +12.5° (*c* 2, $CHCl_3$); IR (KBr): 2100 ($N_3$), 1775 $cm^{-1}$.

Found: C, 63.26; H, 5.39; N, 5.61%. Calcd for $C_{103}H_{104}N_8O_{11}$: C, 63.44; H, 5.38; N, 5.75%.

*6'-Azido-3,2',2''',6''''-tetra-*N*-benzyloxycarbonyl-4'''',6''''-O-cyclohexylidene-6'-deoxylividomycin A (**9**).* To a solution of **8** (520 mg) in dioxane (32 ml), 0.025 M barium hydroxide solution (16 ml) was added and the mixture was stirred at 60 °C for 30 min. Additional aliquot (16 ml) of the barium hydroxide solution was added and the mixture was stirred for 2 hr until the mixture became neutral. The treatment was repeated once again. Subsequent procedures were carried out as described for the preparation of **5** from **3** in the foregoing paper[1] except for the use of chloroform–methanol–triethylamine (6 : 1 : 0.01) for the chromatography, 340 mg (91%); $[\alpha]_D^{31}$ +48° (*c* 2, $CHCl_3$).

Found: C, 56.53; H, 6.08; N, 7.23%. Calcd for $C_{67}H_{86}N_8O_{25}\cdot 1/2H_2CO_3$: C, 56.52; H, 6.11; N, 7.81%.

*6'-Azido-3,2',2''',6''''-tetra-*N*-benzyloxycarbonyl-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-4'''',6''''-O-cyclohexylidene-6'-deoxylividomycin A (**10**).* To an ice-cold solution of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid[4] (58.5 mg) and *N*-hydroxysuccinimide (26.6 mg) in THF (1 ml), DCC (47 mg) was added and the solution was allowed to stand for 1 hr under cooling. To the resulting suspension containing dicyclohexylurea, a solution of **9** (270 mg) and triethylamine (30 mg) in THF (2.5 ml) was added and the mixture was stirred at 0 °C for 1 hr and then at room temperature overnight. Filtration followed by evaporation gave a solid, which was chromatographed on a column of silica gel with chloroform–ethanol–triethylamine (10 : 1 : 0.2) to give **10**, 126 mg (40%); $[\alpha]_D^{20}$ +35° (*c* 1, $CHCl_3$); IR (KBr): 2090 $cm^{-1}$ ($N_3$).

Found: C, 57.84; H, 6.25; N, 7.40%. Calcd for $C_{79}H_{98}N_9O_{29}$: C, 57.94; H, 6.03; N, 7.70%.

*6'-Amino-1-N-((S)-4-amino-2-hydroxybutyryl)-6'-deoxylividomycin A (**11**).* A solution of **10** (110 mg) in a mixture (10 ml) of acetone–acetic acid–water (3 : 5 : 2) was heated at 60 °C for 2 hr. Evaporation gave a residue, which was dissolved in aqueous dioxane (2 : 5, 4 ml). After addition of a few drops of acetic acid, the solution was hydrogenated over palladium black. Chromatography of the product on a column of CM-Sephadex C-25 ($NH_4$ form) with a linear gradient of aqueous ammonia (0~0.3 M) gave a solid, which showed a single peak of **11**, 41 mg (64%); $[\alpha]_D^{20}$ +68° (*c* 1, $H_2O$); ppc, $R_{f\ \text{lividomycin A}}$ 0.6; IR (KBr): 1650, 1580 $cm^{-1}$.

Found: C, 42.46; H, 7.19; N, 10.19%. Calcd for $C_{33}H_{63}N_7O_{19}\cdot H_2CO_3\cdot 2H_2O$: C,42.54; H, 7.25; N, 10.21%.

*2'',5'',3''',2'''',3''''-Penta-O-benzoyl-3,2',2''',6'''-tetra-N-benzyloxycarbonyl-4'''',6''''-O-cyclohexylidene-4',6'-di-O-methyllividomycin A 1,6-Carbamate (**12**).* To a solution of **6** (140 mg) in dry dichloromethane (3 ml) in an ice-cold bath, boron trifluoride etherate (5 mg) was added, and diazomethane in dichloromethane was added until the solution became yellow. Triethylamine was added and the solution was evaporated to give a residue, which was dissolved in chloroform. The solution was washed with saturated sodium hydrogen carbonate solution, and water, dried over sodium sulfate, and evaporated. The residue was chromatographed on a column of silica gel with chloroform–ethanol–triethylamine (50 : 1 : 0.1); 65 mg (46%); $[\alpha]_D^{20}$ +21° (*c* 1, $CHCl_3$); NMR ($CDCl_3$): $\delta$ 3.22, 3.33 (each 3H s, $OCH_3$).

Found: C, 64.82; H, 5.63; N, 3.50%. Calcd for $C_{105}H_{109}N_5O_{12}$: C, 64.57; H, 5.63; N, 3.59%'

*4'-6'-Di-O-methyllividomycin A (**13**).* A sample of **12** was treated with barium hydroxide solution in a similar manner as described for the preparation of **9**. The product was treated with acetic acid and then with palladium black and hydrogen as described for the preparation of **11** to afford **13** (65%); $[\alpha]_D^{20}$ +83° (*c* 1, $H_2O$); PPC, $R_{f\ \text{lividomycin A}}$ 3.1; NMR ($D_2O$): $\delta$ 3.41 (6H s, $OCH_3$).

Found: C, 43.75; H, 7.10; N, 7.96%. Calcd for $C_{31}H_{59}N_5O_{18}\cdot H_2CO_3\cdot H_2O$: C, 44.19; H, 7.30; N, 8.05%.

*Acidic methanolysis of **13**.* A suspension of **13** in 0.4 M methanolic hydrogen chloride was heated at 80 °C for 10 hr. As a control experiment, lividomycin A was similarly treated. Paper chromatography of the both hydrolyzates gave the following results: Methanolysis of **13**: $R_{f\ \text{DST}}$* 1.4 (faint), 2.1 (3'-deoxy-4',6'-di-*O*-methylparomamine), and 2.9 (methyl 2,6-diamino-2,6-dideoxyidoside?). Methanolysis of lividomycin A: $R_{f\ \text{DST}}$ 0.8 (3'-deoxyparomamine), 1.4 (faint), and 2.9.

## References

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* Relative $R_f$ value, that of 2-deoxystreptamine being taken as 1.