Synthesis of 5″-Deoxy-5″-fluorolividomycin B

Takahiro Torii, Tsutomu Tsuchiya,* Sumio Umezawa, and Hamao Umezawa†

Institute of Bioorganic Chemistry, 1614 Nakahara-ku, Kawasaki 211

† *Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received December 13, 1982)

A masked lividomycin B derivative in which only the C-5″ hydroxyl group remains free has been prepared and treated with diethylaminosulfur trifluoride as the key step to give the title compound. A macrocyclic compound, that is, 2′,5″-carbamate was also obtained by the above reaction, and the structure was determined chemically and by the NMR-spectrum of its deblocked derivative.

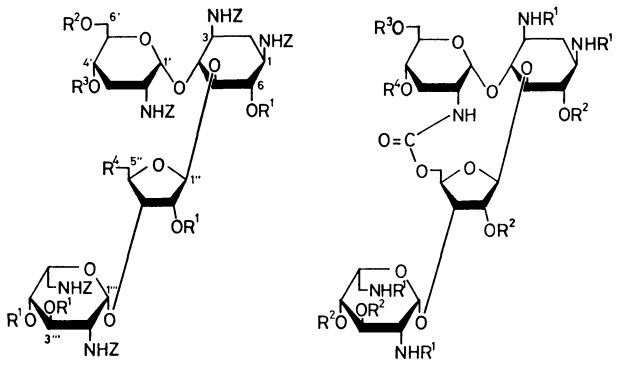
Lividomycin B[1] is an amino glycoside antibiotic produced by *Streptomyces lividus* nov. sp. This antibiotic has strong activity[2] in inhibiting the growth of resistant strains producing 3′-phosphotransferase II which transfers the phosphate of ATP to the 3′-hydroxyl group of paromomycin I (which is synonymous with 3′-hydroxylividomycin B). However, lividomycin B is inactivated[2] by the other resistant strains producing 3′-phosphotransferase I, which transfers the phosphate to the 5″-hydroxyl group.[3] To overcome the inactivation by this enzyme, 5″-deoxylividomycin B[2] had been prepared; however, the derivative had only weak antibacterial activity, suggesting the importance of the role of the 5″-hydroxyl group in the antibacterial action. Recently we prepared several fluoro derivatives[4] of amino glycoside antibiotics in the purpose of developing useful antibacterial amino glycosides. This paper describes the preparation of 5″-deoxy-5″-fluorolividomycin B, to investigate the effect of strongly electronegative fluorine atom instead of the electronegative 5″-hydroxyl group.

First attempts to fluorinate the *N*,*O*-protected 5″-*O*-tosyl derivative[2] (**10**) with cesium fluoride or tetrabutylammonium fluoride, both of which are commonly used for fluorination, were unsuccessful, to give several unidentified products. Therefore, direct fluorination of a 5″-hydroxyl derivative (**6**) with diethylaminosulfur trifluoride[5] (DAST reagent) was attempted. Pentakis (*N*-benzyloxycarbonyl)lividomycin B[2] (**1**) reported earlier was treated with chlorotriphenylmethane in the presence of 2 molar equivalents of phenylboronic acid. Then, 5″-*O*- (**2**, 72%) and 6′-*O*-triphenylmethyl (**3**, 2%) derivatives were produced together with 6′,5″-bis(*O*-triphenylmethyl) derivative (**4**, 8%). Reaction without addition of phenylboronic acid gave **3** as the major product, suggesting that, in the presence of phenylboronic acid, a phenylboronic ester was tentatively formed between the 4′- and 6′-hydroxyl groups.[6] This facilitated the selective 5″-*O*-triphenylmethylation. Acetylation of **2** (to give **5**) followed by acidic hydrolysis of the triphenylmethyl group gave **6** carrying the free 5″-hydroxyl group. The structure of **6** was confirmed by the fact that tosylation of **6** gave the product identical with the derivative (**12**) obtained by debenzylidenation (to give **11**) of an authentic **10**[2] followed by acetylation.

Reaction of **6** with diethylaminosulfur trifluoride proceeded smoothly to give two products, the minor one being the desired 5″-deoxy-5″-fluoro derivative (**7**, 28%) and the major, 2′-*N*:5″-*O*-carbonyl macrocyclic compound (**8**, 61%). Deacetylation of **7** followed by catalytic debenzyloxycarbonylation gave the desired 5″-deoxy-5″-fluorolividomycin B (**9**).

The structure of **9** is evident from the synthetic pathway, but further confirmed by NMR spectral study. In ^{1}H-NMR spectrum, presence of $-CH_2F$ was clarified from the splitting pattern having large $^2J_{H,F}$ (47 Hz) values (see Experimental section). In (proton decoupled) ^{13}C-NMR spectrum, signals of C-5″,-4″, and -3″ appeared as a doublet with a spacing of 168.5 ($=^1J_{C,F}$), 17.7 ($=^2J_{C,F}$), and 6.7 Hz ($=^3J_{C,F}$), respectively (see Table 1), supporting the presence of above fragment.

As a reference compound, 5″-chloro-5″-deoxylividomycin B (**15**) was prepared. A *N*-protected 4′,6′-*O*-benzylidene-5″-chloro-5″-deoxy derivative (**13**) was prepared from **10**[2] according to the method of Watanabe,[7] and it was led to **15** by debenzylidenation (to give **14**) followed by catalytic debenzyloxycarbonylation. One-step deblocking of **13** by the catalytic hydrogenolysis required long reaction-period giving 5″-deoxylividomycin B[2] (**21**). The 5″-chloro structure of **15** was confirmed by the resonance of C-5″ appeared

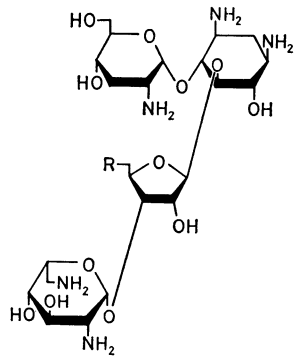


	R^1	R^2 R^3	R^4
1	H	H H	OH
2	H	H H	OTr
3	H	Tr H	OH
4	H	Tr H	OTr
5	Ac	Ac Ac	OTr
6	Ac	Ac Ac	OH
7	Ac	Ac Ac	F
10	H	$C_6H_5CH=$	OTs
11	H	H H	OTs
12	Ac	Ac Ac	OTs
13	H	$C_6H_5CH=$	Cl
14	H	H H	Cl
19	H	$C_5H_{10}C=$	OH
20	H	$C_5H_{10}C=$	OTr

	R^1	R^2 R^3	R^4
8	Z	Ac Ac	Ac
16	H	H H	H
17	H	H H	H
18	Z	$C_5H_{10}C=$	H

Tr: $C(C_6H_5)_3$,
Ts: $O_2SC_6H_4CH_3$(p),
Z: $CO_2CH_2C_6H_5$

at high-field (45.8 ppm) in ^{13}C-NMR spectrum, the value being typical for that of the terminal chloromethyl carbon of carbohydrates reported.[8]



Lividomycin B	R=OH
9	R=F
15	R=Cl
21	R=H

The structure of 2′-*N*:5″-*O*-carbonyl compound (**8**) was determined as follows: Deacetylation of **8** with methanolic aqueous ammonia (to give **16**) followed by catalytic hydrogenolysis gave the 2′-*N*:5″-*O*-carbonyl compound (**17**). The presence of one carbonyl carbon was evidenced by the resonance at down-field (157.8 ppm) in ^{13}C-NMR spectrum. Bridging the 2′-amino and 5″-hydroxyl groups with a carbonyl was verified by the ^{1}H-NMR spectral study. The HC(2′)NH– proton resonances, which should appear at rather down-field ($\delta \approx 3.6$, see Experimental section), were not in the usual methine region (δ=2.5—3.1) of $\underline{\mathrm{H}}\mathrm{CNH}_2$ (measured in 20% ND_3 in D_2O as the free base). Furthermore, one of the proton resonances of $C(5'')H_AH_BO$–, which appeared separately at δ= 4.10 and 5.15, lay beyond the usual region of methylene protons carrying a hydroxyl group. These results led to the conclusion that bridging the 2′-amino and 5″-hydroxyl groups with a carbonyl group occurred to give **8**.

A similar kind of cyclic carbamate formation as that of **8** was also observed[9] on fluorination of tetrakis-(*N*-benzyloxycarbonyl)sporaricin B with the DAST reagent. The tendency to form the carbamates in these cases will be ascribed[9] to the basic nature of fluoride ion liberated during the reactions.

Furthermore, the above conclusion was chemically confirmed. A *N*-protected derivative (**16**, *loc. cit.*) of **17** was treated with 1,1-dimethoxycyclohexane in a usual manner[10] to give the 4′,6′-*O*-cyclohexylidene derivative (**18**), which could not be triphenylmethylated by reaction with chlorotriphenylmethane (in pyridine, 50 °C, 2 d). On the other hand, 4′,6′-*O*-cyclohexylidene derivative (**19**) of **1** gave a mono-*O*-triphenylmethyl (5″-*O*-triphenylmethyl) derivative (**20**) by the same treatment. This result clearly shows that the 5″-hydroxyl group of **17** is blocked. Another structural confirmation was given from the alkaline hydrolysis (2 M aqueous NaOH,** 80 °C, 2 h) of **17**, when lividomycin B was obtained almost quantitatively.

** 1 M=1 mol dm^{-3}.

Table 1. The ^{13}C chemical shifts[A] of **9**, **15**, and lividomycin B (all as free bases) dissolved in 20% ND_3 in D_2O

Carbon	Lividomycin	**15**	**9**	$J_{C,F}$ of **9**
1′	99.7	99.5	99.6	
2′	50.3	50.2	50.2	
3′	35.8	35.3	35.3	
4′	65.4	65.5	65.5	
5′	74.5	74.4	74.4	
6′	61.5	61.6	61.6	
1	51.3[c]	51.2[d]	51.2	
2	36.7	36.7	36.7	
3	51.2[c]	51.1[d]	51.2	
4	84.2	84.0	84.1	
5	85.1	86.2	86.0	
6	78.4	78.5	78.6	
1″	109.3	110.5	110.5	
2″	74.1	74.1	73.9	
3″	77.1	77.6	75.4	$J_{C-3'',F}$=6.7 Hz
4″	82.4	80.7	80.1	$J_{C-4'',F}$=17.7 Hz
5″	62.2	45.8	83.1	$J_{C-5'',F}$=168.5 Hz
1‴	100.3	100.1	100.1	
2‴	53.8	53.6	53.6	
3‴	71.4	71.4	71.4	
4‴	69.4	69.3	69.3	
5‴	77.0	76.9	77.0	
6‴	42.2	42.0	42.0	

a) In ppm downfield from TMS calculated as $\delta^{TMS}=\delta^{dioxane}+67.4$ ppm. b) Assignments were made based on the data of lividomycin B reported,[6] and also on selective proton decoupling on the following cases (H-4, δ=3.44; H-5: 3.6—3.7, H-6: 3.23, H-1′: 5.21, H-1″: 5.33, H-3″: 4.40, H-1‴: 4.93, H-3‴: 4.00, H-5‴: $\approx$3.85). c,d) Figures indicated by the same character may be interconvertible.

Antibacterial spectra of **9** and **15** were shown in Table 2 in comparison with those of lividomycin B and 5″-deoxylividomycin B[2] (**21**). The substitutions of the 5″-hydroxyl group with halogens were shown to diminish the antibacterial activity as in the case of 5″-deoxygenation, although the fluoro compound (**9**) showed the best activity among them (**9**, **15**, **21**). The macrocyclic compound **17** was devoid of antibacterial action.

Experimental

General. ^{1}H-NMR spectra were recorded at 250 MHz at 30 °C (unless otherwise stated) in the FT mode with a Bruker WM 250 spectrometer. ^{13}C-NMR spectra were recorded at 30 °C (unless otherwise stated) in the FT mode with a Bruker WM 250 spectrometer operating at 62.5 MHz. Mass spectrum (field desorption) was recorded with a Hitachi M-80H spectrometer. Thin-layer chromatography(TLC) was performed on precoated Kieselgel 60, Merck. For column chromatography, silica-gel (Wakogel C-200) was used.

1,3,2′,2‴,6‴-Pentakis(N-benzyloxycarbonyl)-5″-O-triphenylmethyllividomycin B (2). A mixture of **1**[2] (5.0 g) and phenylboronic acid (0.94 g, 2 mol equiv. for **1**) in dry pyridine

Table 2. Minimal inhibitory concentration(μg/ml) of **9**, **15**, **21**, and lividomycin B

	Lividomycin B	**9**	**15**	**21**
Staphylococcus aureus FDA 209P	0.78	6.25	12.5	6.25
Staphylococcus aureus AP01	>100	>100	>100	>100
Micrococcus luteus PCI 1001	1.56	6.25	12.5	6.25
Bacillus subtilis NRRL B-558	0.39	3.12	6.25	3.12
Escherichia coli K-12	0.78	12.5	12.5	50
Escherichia coli K-12 ML1629	>100	50	50	100
Escherichia coli W677	1.56	12.5	25	25
Salmonella typhi T-63	0.39	6.25	6.25	25
Pseudomonas aeruginosa A3	0.78	12.5	50	25

(50 ml) was kept for 2 h at room temperature. Chlorotriphenylmethane (5.5 g) was added, and after standing the solution overnight at room temperature, another chlorotriphenylmethane (5.5 g) was added, and the solution was kept overnight at 37 °C. Addition of methanol (15 ml) and 1,3-propanediol (1 ml) followed by concentration gave a residue, which was extracted with chloroform. The chloroform-soluble products were separated by silica-gel column chromatography with chloroform–methanol (30: 1→25: 1→20: 1→10: 1, gradually changed) to give a solid each of **4**, 548 mg (8%), **3**, 136 mg (2%), **2**, 4.30 g (72%), and the starting material (**1**) recovered, 293 mg (6%).

Compound **2**: $[\alpha]_D^{20}+37°$ (*c* 1, chloroform); Found: C, 64.91; H, 5.87; N, 4.48%. Calcd for $C_{82}H_{89}N_5O_{23}$: C, 65.11; H, 5.93; N, 4.63%.

Compound **3**: $[\alpha]_D^{20}+23°$ (*c* 1, chloroform); Found: C, 64.89; H, 5.97; N, 4.63%. Calcd for $C_{82}H_{89}N_5O_{23}$: C, 65.11; H, 5.93; N, 4.63%.

Compound **4**: $[\alpha]_D^{20}+15°$ (*c* 1, chloroform); Found: C, 69.34; H, 5.92; N, 3.87%. Calcd for $C_{101}H_{103}N_5O_{23}$: C, 69.12; H, 5.92; N, 3.99%.

*6,4′,6′,2″,3‴,4‴-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentakis(*N*-benzyloxycarbonyl)-5″-*O*-triphenylmethyllividomycin B (**5**).* A mixture of **2** (3.9 g), acetic anhydride (20 ml) and pyridine (40 ml) was kept at room temperature overnight. Another acetic anhydride (10 ml) was added and the mixture was kept further overnight. Concentraction gave a syrup, which was dissolved in chloroform. The organic solution was washed with 0.4 M aqueous potassium hydrogensulfate solution, then aqueous sodium hydrogencarbonate solution (saturated), dried over sodium sulfate, and concentrated. The residue was reprecipitated from hexane–chloroform to give a solid of **5**, 4.22 g (93%), $[\alpha]_D^{20}+31°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=1.83$, 1.97, 2.00, 2.07, 2.08, and 2.17 (each 3H s, Ac).

Found: C, 63.72; H, 5.86; N, 3.88%. Calcd for $C_{94}H_{101}N_5O_{29}$: C, 63.97; H, 5.77; N, 3.97%.

*6,4′,6′,2″,3‴,4‴-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentakis(*N*-benzyloxycarbonyl) lividomycin B(**6**).* A solution of **5** (4.22 g) in a mixture of acetic acid–acetone–water (2: 1: 1, 120 ml) was heated at 60 °C for 6 h. Concentration gave a syrup, which was dissolved in chloroform, and the organic solution was washed with aqueous sodium hydrogencarbonate (saturated). The product obtained by concentration was purified by silica-gel column chromatography with benzene–ethyl acetate (1: 1→2: 3) to give a solid of **6**, 3.52 g (97%), $[\alpha]_D^{20}+25°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=1.93$ (3H), 2.00(6H), 2.05(3H), 2.08(3H), and 2.16(3H) (each s, Ac).

Found: C, 59.30; H, 5.75; N, 4.50%. Calcd for $C_{75}H_{87}N_5O_{29}$: C, 59.17; H, 5.76; N, 4.60%.

*6,4′,6′,2″,3‴,4‴′-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentakis(*N*-benzyloxycarbonyl)-5″-deoxy-5″-fluorolividomycin B (**7**) and 6,4′,-6′,2″,3‴,4‴-Hexa-*O*-acetyl-1,3,2‴,6‴-tetrakis(*N*-benzyloxycarbonyl)-2′-*N*: 5″-*O*-carbonyllividomycin B (**8**).* To a cold (−15 °C) solution of **6** (300 mg) in dichloromethane (3.5 ml), was added diethylaminosulfur trifluoride (0.1 ml) and the solution was kept at the temperature for 15 min, then at room temperature for 30 min. After addition of water (0.1 ml) followed by standing for 30 min, chloroform (30 ml) was added, and the solution was washed with aqueous sodium hydrogencarbonate (saturated) and water, dried (sodium sulfate), and concentrated to give a syrup. On checking by TLC with benzene–ethyl acetate(1: 1), the syrup showed two spots at R_f 0.2 (**8**) and 0.5 (**7**) (*cf.* **6**: R_f 0.3). Separation of the products by silica-gel column chromatography with benzene–ethyl acetate (3: 2→2: 3) gave a solid each of **7**, 83 mg (28%) and **8**, 171 mg (61%).

Compound **7**: $[\alpha]_D^{20}+28°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=1.77$, 1.92, 2.00, 2.06, 2.09, and 2.18 (each 3H s, Ac), 7.35 (25H s, $NHCO_2CH_2C_6\underline{H}_5$); Found: C, 58.87; H, 5.91; N, 4.49; F, 1.39%. Calcd for $C_{75}H_{86}FN_5O_{28}$: C, 59.09; H, 5.67; N, 4.59; F, 1.25%.

Compound **8**: $[\alpha]_D^{20}+43°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=1.88$ (3H), 1.97 (6H), 2.06 (6H), and 2.13 (3H) (each s, Ac), 7.35 (20H s, $NHCO_2CH_2C_6\underline{H}_5$); Found: C, 57.46; H, 5.62; N, 4.87%. Calcd for $C_{68}H_{79}N_5O_{28}$: C, 57.74; H, 5.63; N, 4.95%.

*5″-Deoxy-5″-fluorolividomycin B (**9**).* A solution of **7** (80 mg) in a mixture of methanol (8 ml) and 28% aqueous ammonia (9 ml) was kept at room temperature overnight. Concentration gave a syrup (de-*O*-acetyl product), which was dissolved in 0.2 M methanolic hydrochloric acid (4 ml), and the solution was hydrogenated under atmospheric pressure of hydrogen in the presence of palladium catalyst for 2 h. The resulting crude product was purified by chromatography first on a column of Dowex 50 W (NH_4^+ form) (the charged column was washed with water, then 1 M aqueous ammonia was used as the developer), and then on a column of CM-Sephadex C-25 (0→0.1→0.3 M aqueous ammonia was used as the developer) to give pure **9** as the carbonate, 29 mg (83%), $[\alpha]_D^{20}+48°$ (*c* 1, water); ^{1}H-NMR (20% ND_3 in D_2O): $\delta=1.18$ (1H q, $J=12.5$ Hz H-2ax), 1.59 (1H q, $J=11.5$ Hz, H-3′ ax), 1.95 (1H dt, $J=12.5$, ≈4.5, ≈4.5 Hz, H-2eq), 2.01 (1H dt, $J=11.5$, ≈4.5 ≈4.5 Hz, H-3′eq) 4.95 (1H d, $J_{1''',2'''}\approx1.3$ Hz, H-1‴), 5.22 (1H d, $J_{1',2'}=3.5$ Hz, H-1′), 5.34 (1H d, $J_{1'',2''}\approx1.7$ Hz, H-1″). The signals for the AB part of an ABX system (A,B=H-5″a, −5″b; X=H-4″) were divided into two groups (each octet) centered at $\delta=4.62$ and 4.81, respectively. The upper quartet of each group was constituted from couplings of 11.5 ($=J_{5''a,5''b}$) and 4 Hz ($=J_{5''a,4''}$), and the lower quartet, from couplings of 11.5 and 2.5 Hz ($=J_{5''b,4''}$) (each 1H in total). These two groups were separated by 47 Hz ($=J_{5''a,F}=J_{5''b,F}$).

Found: C, 43.93; H, 7.17; N, 10.89; F, 2.58%. Calcd for $C_{23}H_{44}FN_5O_{12}\cdot H_2CO_3$: C, 43.43; H, 6.99; N, 10.55; F,

2.87%.

6,4′,6′,2″,3‴,4‴-Hexa-O-acetyl-1,3,2′,2‴,6‴-pentakis(N-benzyloxycarbonyl)-5″-O-tosylliuidomycin B (**12**). *From* **10**[2]: A solution of **10** (282 mg) in a mixture of acetic acid–acetone–water (2: 1: 1, 6 ml) was heated for 3 h at 60 °C. Concentration gave a syrup, which was dissolved in chloroform and the solution was washed with aqueous sodium hydrogencarbonate (saturated). Silica-gel column chromatography of the crude product with chloroform, then with chloroform–methanol (10: 1) gave a solid of **11**, 242 mg (91%), $[\alpha]_D^{20}+29°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=2.32$ (3H s, Ts).

A mixture of **11** (136 mg) and acetic anhydride (2 ml) in dry pyridine (4 ml) was kept overnight at room temperature. Concentration gave a syrup, which was purified by silica-gel column chromatography with benzene–ethyl acetate (3: 1) to give a solid of **12**, 144 mg (90%), $[\alpha]_D^{20}+27°$ (*c* 1, chloroform); ^{1}H-NMR ($CDCl_3$): $\delta=1.80$, 1.87, 2.00, 2.06, 2.08, 2.21 (each 3H s, Ac); 2.42 (3H s, CH_3 of Ts).

Found; C, 58.45; H, 5.56; N, 4.13; S, 1.87%. Cacld for $C_{82}H_{93}N_5O_{31}S$: C, 58.74; H, 559; N, 4.18; S, 1.91%.

From **6**: A mixture of **6** (72 mg) and *p*-toluenesulfonyl chloride (105 mg) in pyridine (3.5 ml) was kept overnight at 37 °C. Concentration gave a syrup, which was purified as described above for **12** to give a solid of **12**, 72 mg (91%).

4′,6′-O-Benzylidene-1,3,2′,2‴,6‴-pentakis(N-benzyloxycarbonyl)-5″-chloro-5″-deoxylividomycin B (**13**). Prepared from **10**[2] (500 mg) with lithium chloride (580 mg) in *N,N*-dimethylformamide (95 °C, overnight) in the presence of calcium sulfate (Drierite), according to Watanabe[6]; a solid, 422 mg (93%), $[\alpha]_D^{20}+59°$ (*c* 1, chlornform).

1,3,2′,2‴,6‴-Pentakis(N-benzyloxycarbonyl)-5″-chloro-5″-deoxylividomycin B (**14**). A solution of **13** (300 mg) in a mixture of acetone–water–acetic acid (1: 1: 2, 6 ml) was heated at 60 °C for 3 h. Concentration gave a syrup, which was purified by silica-gel column chromatography first with chloroform, then with chloroform–methanol (10: 1) as the developers to give a solid of **14**, 261 mg (92%), $[\alpha]_D^{20}+35°$ (*c* 1, chloroform).

Found: C, 57.74; H, 5.76; N, 5.15; Cl, 2.95%. Calcd for $C_{63}H_{74}ClN_5O_{22}\cdot H_2O$: C, 57.90; H, 5.71; N, 5.36; Cl, 2.71%.

5″-Chloro-5″-deoxylividomycin B (**15**). A solution of **14** (monohydrate, 102 mg) in 0.2 M methanolic hydrochloric acid (10 ml) was hydrogenated in the presence of palladium black under atmospheric pressure of hydrogen for 2 h and the product was purified as described for **9** to give a solid of **15** as the carbonate, 48 mg (88%), $[\alpha]_D^{20}+51°$ (*c* 1, water); ^{1}H-NMR (20% ND_3 in D_2O): $\delta=1.18$ (1H q, $J=12.5$ Hz, H-2ax), 1.59 (1H q, $J=11.5$ Hz, H-3′ax), 1.94 (1H dt, $J=12.5$, ≈4.5, ≈4.5 Hz, H-2eq), 2.00 (1H dt, $J=11.5$, ≈4.5, ≈4.5 Hz, H-3′eq), 4.93 (1H d, $J_{1‴,2‴}\approx2$ Hz, H-1‴), 5.25 (1H d, $J_{1′,2′}=3.5$ Hz, H-1′), 5.32 (1H d, $J_{1″,2″}\approx2$ Hz, H-1″).

Found: C, 41.69; H, 6.77; N, 9.79; Cl, 5.35%. Calcd for $C_{23}H_{44}ClN_5O_{12}\cdot H_2CO_3\cdot H_2O$: C, 41.29; H, 6.93; N, 10.03; Cl 5.07%.

1,3,2‴,6‴-Tetrakis(N-benzyloxycarbonyl)-2′-N:5″-O-carbonyllividomycin B (**16**). A solution of **8** (103 mg) in a mixture of methanol–28% aqueous ammonia (1: 1, 10 ml) was kept at room temperature overnight. The solid precipitated was filtered, washed with aqueous methanol (1: 1), and dired to give a solid of **16**, 78 mg (92%), $[\alpha]_D^{20}+41°$ (*c* 1, dimethyl sulfoxide).

Found: C, 57.57; H, 5.73; N, 5.85%. Calcd for $C_{56}H_{67}N_5O_{22}$: C, 57.87; H, 5.81; N, 6.03%.

2′-N:5″-O-Carbonyllividomycin B (**17**). A suspended mixture of **16** (70 mg) in 70% aqueous oxolane (10 ml) containing 0.1 ml of 2 M aqueous hydrochloric acid was hydrogenated in the presence of palladium black under atmospheric pressure of hydrogen at room temperature for 3 h with occasional additions of 0.1 M aqueous hydrochloric acid (2 ml×2). Filtration of the reaction mixture followed by concentration of the filtrate gave a residue, which was chromatographed on CM-Sephadex C-25 column with water, then with 0.3 M aqueous ammonia to give a ninhydrin-positive solid of **17**, 33 mg (71%), $[\alpha]_D^{20}+86°$ (*c* 1, water); ν_{max}^{KBr} 1700 cm^{-1}; *m/z* 626 (M^+), 600, 466 ($M^+-C_6H_{12}N_2O_3$), 440 ($600^+-C_6H_{12}N_2O_3$). ^{1}H-NMR (20% ND_3 in D_2O measured at 4 °C): $\delta=1.19$ (1H q, H-2a), 1.74 (1H q, H-3′a), 1.95 (1H apparent dt, H-2e), 2.09 (1H m, H-3′e), 2.6—3.05 (5H, H-1, 3, 2‴, 6‴a, 6‴b), 3.29 (1H t, H-6), 3.47 (1H t, H-4), 4.01 (1H t, H-3‴), 4.10 (1H d, H-5″a), 4.14 (1H dd, H-4″), 4.31 (1H d, H-2″), 4.51 (1H dd, H-3″; broad HOD signal at $\delta=4.2$—4.6 was suppressed by homogated decoupling), 4.97 (1H apparent s, H-1‴), 5.15 (1H dd, with small splittings, H-5″b), 5.96 (1H s, H-1″), 6.08 (1H d, H-1′); $J_{1,2a}=J_{2a,2e}=J_{2a,3}\approx12$, $J_{1,2e}=J_{2e,3}\approx4$, $J_{3,4}=J_{4,5}=J_{5,6}=J_{6,1}\approx9.5$, $J_{1′2′}=3.5$, $J_{2′,3′a}=J_{3′a,3′e}=J_{3′a,4′}\approx12$, $J_{1″,2″}=0$, $J_{2″,3″}=4$, $J_{3″,4″}=9$, $J_{4″,5″a}=0$, $J_{4″,5″b}\approx4$, $J_{5″a,5″b}=13$, $J_{1‴,2‴}\approx1$, $J_{2‴,3‴}=J_{3‴,4‴}\approx3$ Hz. Irradiation of H-3″ caused H-2″ d to s, and H-4″ dd to a narrow-space d; irr. of H-5″ b caused H-4″ dd→a large-space d, and H-5″ a d →s; irr. at $\delta=2.7$ (H-3) caused H-2a q→t, H-2e dt→dd, and H-4 t→d; irr. at $\delta=2.87$ (H-1) caused H-2a q→t, H-2e dt→dd, and H-6 t→d; irr. at $\delta=2.95$ (H-2‴) caused H-3‴ t→d; irr. at $\delta=3.63$ (H-2′, 4′, 4‴) caused H-3‴ t→d, H-1′ d→s, H-3′a q→d, and H-3′e q→d, the latter two doublets forming an AB quartet.

^{13}C-NMR (20% ND_3 in D_2O, measured at 4 °C): 31.7 (C-3′), 36.1 (C-2), 42.2 (C-6‴), 50.3, 50.6, 51.3, 53.6 (C-2‴), 60.2, 61.4 (C-6′), 64.7, 69.1 (C-4‴), 71.4 (C-3‴), 72.8, 73.6, 74.5, 76.4, 77.0 (C-5‴), 78.1, 80.3, 81.4, 92.8, 98.9, 107.2, 157.8 (C=0) ppm.

Found: C, 40.42; H, 6.56; N, 9.21%. Calcd for $C_{24}H_{43}N_5O_{14}\cdot 2H_2CO_3\cdot H_2O$: C, 40.68; H, 6.43; N, 9.12%.

1,3,2‴,6‴-Tetrakis(N-benzyloxycarbonyl)-2′-N:5″-O-carbonyl-4′,6′-O-cyclohexylidenelividomycin B (**18**). To a solution of **16** (50 mg) in *N,N*-dimethylformamide (10 ml) were added 1,1-dimethoxycyclohexane (0.5 ml) and anhydrous *p*-toluenesulfonic acid (10 mg) and the mixture was heated at 30 °C under reduced pressure (10—15 Torr, 1 Torr≈133.322 Pa) for 6 h. Addition of aqueous sodium hydrogencarbonate (saturated, 0.5 ml) followed by evaporation gave a residue, which was washed with water. The water-insoluble product was washed with ether to give a solid, 51 mg (94%), $[\alpha]_D^{20}+38°$ (*c* 1, dimethyl sulfoxide).

Found: C. 59.33; H, 6.03; N, 5.12%. Calcd for $C_{62}H_{75}N_5O_{22}\cdot H_2O$: C, 59.08; H, 6.16; N, 5.56%.

1,3,2′,2‴,6‴-Pentakis(N-benzyloxycarbonyl)-4′,6′-O-cyclohexylidenelividomycin B (**19**). Compound **1** was treated with 1,1-dimethoxycyclohexane as described for **18** to give a solid of **19** in 90% yield, $[\alpha]_D^{20}+46°$ (*c* 1, chloroform).

Found: C, 60.96; H, 6.12; N, 5.01%. Calcd for $C_{69}H_{83}N_5O_{23}\cdot 1/2\,H_2O$: C, 60.96; H, 6.23; N, 5.15%.

1,3,2′,2‴,6‴-Pentakis(N-benzyloxycarbonyl)-4′,6′-O-cyclohexylidene-5″-O-triphenylmethyllividomycin B (**20**). Compound **19** (202 mg) was treated with chlorotriphenylmehtane (200 mg) in pyridine (4 ml) at 50 °C for 2 d. After usual work-up, the product was chromatographed on a silica-gel column with chloroform–methanol (30: 1) to give a solid of **20**, 158 mg (66%), $[\alpha]_D^{20}+28°$ (*c* 1, chloroform).

Found: C, 65.93; H, 6.20; N, 4.09%. Cacld for $C_{88}H_{97}N_5O_{23}\cdot H_2O$: C, 65.62; H, 6.20; N, 4.35%.

The authors wish to thank Mr. Saburo Nakada of Keio University for the microanalysis, Dr. Hiroshi Naganawa of Institute of Microbial Chemistry for measurement of a mass spectrum, and Miss Yukiko Suzuki of our Institute for measurements of NMR spectra. Thanks are also due Miss Junko Yamamoto of our Institute for preparation of the manuscript.

References

1) T. Mori, Y. Kyotani, I. Watanabe, and T. Oda, *J. Antbiot.*, **25**, 149 (1972).
2) S. Umezawa, I. Watanabe, T. Tsuchiya, H. Umezawa, and M. Hamada, *J. Antbiot.*, **25**, 617 (1972).
3) S. Kondo, H. Yamamoto, H. Naganawa, H. Umezawa, and S. Mitsuhashi, *J. Antbiot.*, **25**, 483 (1972).
4) T. Tsuchiya, T. Torii, S. Umezawa, and H. Umezawa, *J. Antbiot.*, **35**, 1245 (1982).
5) L. N. Markovskij, V. E. Pashinnik, and A. V. Kirsanov, *Synthesis*, 787 (1973); W. J. Middleton, *J. Org. Chem.*, **40**, 575 (1975).
6) T. Torri, T. Tsuchiya, I. Watanabe, and S. Umezawa *Bull., Chem. Soc. Jpn.*, **55**, 1178 (1982).
7) I. Watanabe, Thesis, Keio University "Synthetic Studies on Lividomycin and Butirosin Derivatives," 1979.
8) W. A. Szarek, D. M. Vyas, S. D. Gero, and G. Lukacs, *Can. J. Chem.*, **52**, 3394 (1974).
9) T. Torii, T. Tsuchiya, and S. Umezawa, *Carbohydr. Res.*, under Submission.
10) For example see: T. Jikihara, T. Tsuchiya, H. Umezawa, and S. Umezawa, *Carbohydr. Res.*, **89**, 91 (1981).