for the synthesis of compound **2**. The compound was isolated by lyophilization to give 0.0234 g (23%) of a yellow solid. The indole **3** was immediately stored under argon: mp 107–109 °C dec (lit.[10] mp 108–109 °C); NMR (D_2O) δ 7.05 (m, 1 H, CH=C*H*N), 6.90 (s, 1 H, aromatic), 6.45 (m, 1 H, C*H*=CHN), 2.35 (s, 3 H, CH_3).

5,6-Dihydroxyindole (4). 5,6-Bis(benzyloxy)indole was debenzylated in a manner similar to that described by Benigni and Minnis.[25]

Acknowledgment. The authors gratefully acknowledge support of this project by research grants from the National Institutes of Neurological Diseases and Stroke (NS-10918 and NS-15692). The support provided by the Center for Biomedical Research, University of Kansas, is also gratefully acknowledged.

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Novel Dimeric Derivatives of Leucomycins and Tylosin, Sixteen-Membered Macrolides

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The reductive amination of an aldehyde group on the aglycon moiety of leucomycins A_3 and A_5 and tylosin with sodium cyanoborohydride in the presence of $NH(CH_3)_2$ or NH_2CH_3 afforded the corresponding amine derivative. The use of NH_3 as an amine source in the reduction of leucomycin A_3 and tylosin afforded a novel dimeric derivative, 18,18′-dideoxo-18,18′-iminodileucomycin A_3 and 20,20′-dideoxo-20,20′-iminoditylosin, respectively. The structures of the dimers were elucidated by field desorption mass spectral analysis. The dimeric derivative of tylosin possesses considerable antibacterial activity. The binding activity of the dimer for *Escherchia coli* ribosome was approximately the same as for tylosin.

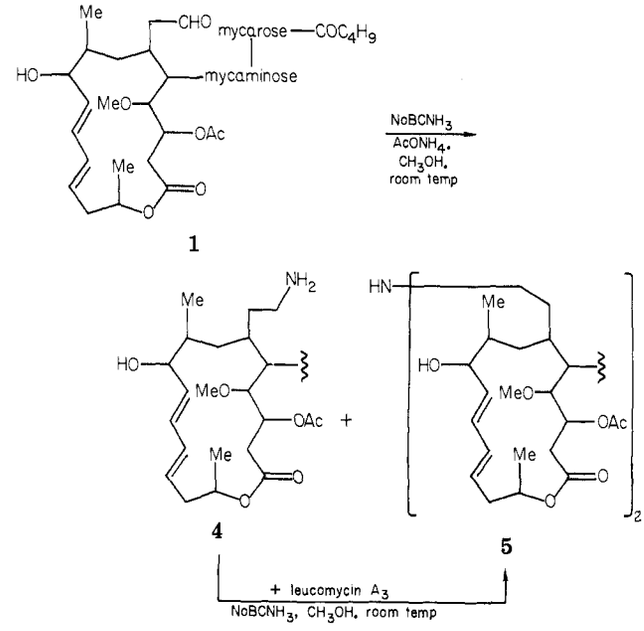
In previous structure–activity correlations of 16-membered macrolide antibiotics, leucomycins, carbomycins, tylosin, and chalcomycin, it was revealed that the presence of an aldehyde group on the lactone ring and a dimethylamino group on the mycaminose moiety may be important for antimicrobial activity.[1] We have also found that when a carbonyl group at the 9 position of the lactone ring is present, an aldehyde group at the 18 position is not necessary for antimicrobial activity. Therefore, we have focused on the modification of the aldehyde group on the aglycon moiety of leucomycins and tylosin. In this paper we describe the reductive amination of an aldehyde group and the structure of a dimer of the amine derivative coupled together through an amino function attached to the methylenic group at the 18 position of the aglycon moiety. In addition, antimicrobial activity (MIC) and binding to ribosomes were evaluated.

Results and Discussion

The reductive amination of leucomycins and tylosin was carried out according to the procedure of Jacquensy et al.[2] Reduction of leucomycin A_3 (1) with sodium cyanoborohydride and dimethylamine hydrochloride gave 18-deoxo-18-(dimethylamino)leucomycin A_3 (**2**).

The reductive amination of 1 with sodium cyanoborohydride and methylamine hydrochloride afforded 18-deoxo-18-(methylamino)leucomycin A_3. The use of ammonium acetate as an amine source gave three products differing from those obtained from the reactions described above. Three compounds, 18-amino-18-deoxoleucomycin A_3 (**4**), 18,18′-dideoxo-18,18′-iminodileucomycin A_3 (**5**), and 18-deoxoleucomycin A_3 (**6**), can also be obtained by using ammonium chloride instead of ammonium acetate. Their IR spectra showed the lack of an absorption band due to an aldehyde group (ν_{CH} 2920 and 2720 cm^{-1}). The structure

Scheme I



of **4** was easily assigned from the mass spectral data.

Elemental analysis of compound **5** gave a compositional formula, $C_{84}H_{141}N_3O_{28}\cdot H_2O$, suggesting the possibility of the dimerized structure of 1. The ^{13}C chemical-shift values of **5** are approximate to those of **4**, except for that of each C-18 methylenic carbon at δ 38.7 and 47.1 in 4 and **5**, respectively. The field desorption (FD) mass spectrum of **5** showed a distinct molecular ion peak at m/z 1639 ($C_{84}H_{141}N_3O_{28}$) and a fragment peak, m/z 827, due to the aglycon moiety involving the di-*N*,*N*-ethylenic amino group, being the symmetric center in the molecule of **5**, clearly demonstrating that compound **5** has the structure of two molecules of leucomycin A_3 (1) condensed through an aminomethyl group introduced at the 18 position by amination. Compound **5** was also obtained by the con-

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(2) M.-H. Boutigue and R. Jacquesy, *Bull. Soc. Chim. Fr.*, 750 (1973).

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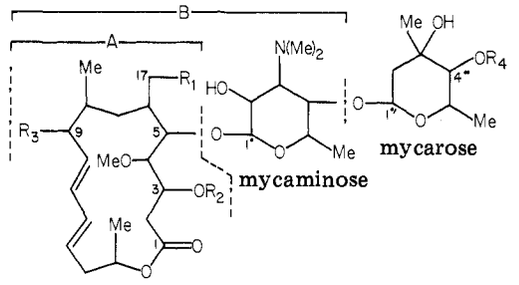
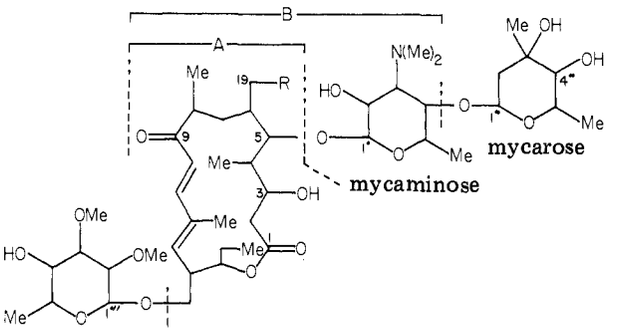
Table I. Structures of Leucomycin A_3 (1), Leucomycin A_5 (7), and Tylosin (10) and Their Amino Derivatives



leucomycin group (1-8, 14-16)



tylosin group (10, 11)

no.	formula	R_1	R_2	R_3	R_4	EIMS M^+ (m/z)	EIMS A (m/z)	EIMS B (m/z)
1	$C_{42}H_{69}NO_{15}$	CHO	$COCH_3$	OH	$COCH_2CH(CH_3)_2$	827	409	540
2	$C_{44}H_{76}N_2O_{14}$	$CH_2N(CH_3)_2$	$COCH_3$	OH	$COCH_2CH(CH_3)_2$	856	438	612
3	$C_{43}H_{74}N_2O_{14}$	CH_2NHCH_3	$COCH_3$	OH	$COCH_2CH(CH_3)_2$	842	424	598
4	$C_{42}H_{72}N_2O_{14}$	CH_2NH_2	$COCH_3$	OH	$COCH_2CH(CH_3)_2$	828	410	582
6	$C_{42}H_{71}NO_{14}$	CH_3	$COCH_3$	OH	$COCH_2CH(CH_3)_2$	813	395	567
7	$C_{39}H_{65}NO_{14}$	CHO	H	OH	$COCH_2CH_2CH_3$	771	367	498
8	$C_{39}H_{68}N_2O_{13}$	CH_2NH_2	H	OH	$COCH_2CH_2CH_3$	772	368	499
10	$C_{46}H_{77}NO_{17}$	CHO				915	390	563
11	$C_{46}H_{78}N_2O_{16}$	CH_2NH_2				916	391	564
14	$C_{44}H_{74}N_2O_{14}$	$CH_2N(CH_3)_2$	$COCH_3$	=O	$COCH_2CH(CH_3)_2$	854	436	610
15	$C_{43}H_{72}N_2O_{14}$	CH_2NHCH_3	$COCH_3$	=O	$COCH_2CH(CH_3)_2$	840	422	596
16	$C_{42}H_{69}N_2O_{14}$	CH_2NH_2	$COCH_3$	=O	$COCH_2CH(CH_3)_2$	826	408	580

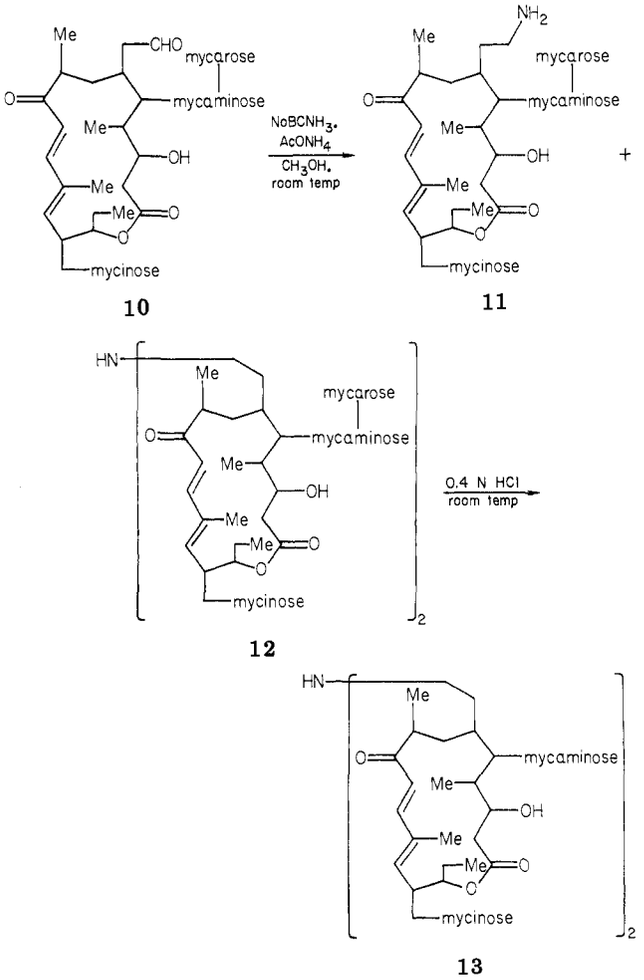
densation of 1 with 4 in the presence of sodium cyanoborohydride, indicating that the formation of the dimer **5** proceeded via **4** as an intermediate, as shown in Scheme I.

The structure of compound **6** was easily assigned to 18-deoxoleucomycin A_3 from the mass and ^1H NMR data. Treatment of leucomycin A_5 (**7**), in which the hydroxy and butyryl groups are situated at the 3 and 4″ position, respectively, with sodium cyanoborohydride and ammonium chloride afforded 18-amino-18-deoxoleucomycin A_5 (**8**) and 18,18′-dideoxo-18,18′-iminodileucomycin A_5 (**9**). Thus, it is very interesting that the macrolide dimer was formed by reductive amination using sodium cyanoborohydride and ammonium acetate.

In a similar manner as leucomycins, dimerization of tylosin (**10**) afforded two products, 20-amino-20-deoxotylosin (**11**) and 20,20′-dideoxo-20,20′-iminoditylosin (**12**). The structures of compounds **11** and **12** were easily determined from the ^{13}C NMR and the mass spectral data. The observation of each fragment peak at m/z 872, 886, 900, and 915 in the FD mass spectrum of **12** well explains the structure in which two molecules of **10** condensed through the amine attached to the methylenic carbon at the 20 position.

On the basis of the information that removal of the mycarose moiety from tylosin resulted in an increase of antimicrobial activity,[3] we carried out acidic hydrolysis of 20,20′-dideoxo-20,20′-iminoditylosin (**12**). Treatment of **12** with 0.4 N HCl afforded a corresponding didemycarosyl derivative (**13**) (Scheme II). The ^{13}C NMR spectrum of **13** revealed the lack of carbon signals arising from the mycarose moiety and a considerable upfield shift of the C-4 carbon of mycaminose accompanying removal of the

Scheme II



10 11 12 13

(3) H. Matsubara, K. Miyano, A. Nakagawa, and S. Ōmura, *Chem. Pharm. Bull.*, in press.

Table II. Antimicrobial Activities (MIC) of Leucomycin A_3 (1), Leucomycin A_5 (7), Tylosin (10), and Their Derivatives [a]

no.	compd	MIC, μg/mL *Staph. aureus* FDA 209P	*B. subtilis* PCI 219	*Sarc. lutea* PCI 1001	*E. coli* NIHJ	*K. pneumoniae* PCI 602
1	leucomycin A_3	0.4	0.4	<0.1	50	50
4	18-amino-18-deoxoleucomycin A_3	>100	50	>100	>100	>100
5	18,18′-dideoxo-18,18′-iminodileucomycin A_3	100	6.25	25	>100	>100
6	18-deoxoleucomycin A_3	6.25	6.25	1.56	>100	>100
7	leucomycin A_5	<0.1	0.2	<0.1	25	25
8	18-amino-18-deoxoleucomycin A_5	>100	>100	25	>100	>100
9	18,18′-dideoxo-18,18′-iminodileucomycin A_5	50	12.5	12.5	>100	>100
10	tylosin	0.78	0.78	<0.1	50	100
11	20-amino-20-deoxotylosin	50	25	6.25	>100	>100
12	20,20′-dideoxo-20,20′-iminoditylosin	1.56	0.78	0.2	>100	>100
13	didemycarosyl-20,20′-dideoxo-20,20′-iminoditylosin	3.12	25	0.78	50	100

[a] Medium: nutrient agar (peptone 0.5%, meat extract 0.5%, agar 1.0%, pH 7.0), 37 °C, 20 h.

mycarose moiety. The FD mass spectrum of **13** showed a molecular ion peak at m/z 1527 and the fragment peak arising from the same aglycon moiety as that of **12** at m/z 737.

Taking into account the importance of the ketone carbonyl at the 9 position for appearance of antimicrobial activity, we oxidized the C-9 hydroxy group of the *N,N*-dimethylamino derivative (**2**) with activated manganase dioxide in chloroform to obtain 9-dehydro-18-deoxo-18-(dimethylamino)leucomycin A_3 (**14**). The UV and IR spectra of **14** revealed the formation of a $\alpha,\beta,\gamma,\delta$-dienone system. Similarly, the oxidation of the C-18 methylamino derivative (**3**) and the amine **14** afforded 9-dehydro-18-deoxo-18-(methylamino)leucomycin A_3 (**15**) and 9-dehydro-18-amino-18-deoxoleucomycin A_3 (**16**), respectively.

The antimicrobial activities (MIC) of leucomycins A_3 (**1**) and A_5 (**7**), tylosin (**10**), and their derivatives are shown in Table II. The reductive aminated compounds **2–4** and **8** have very little or no antimicrobial activity against Gram-positive and -negative bacteria, except for *Sarcina lutea* PCI 1001. The compounds **14–16** did not also show any significant antimicrobial activities in spite of the presence of the carbonyl function at the 9 position. It should be noted that the tylosin dimer (**12**) possesses the same amount of activity as compound **10**, in spite of the disappearance of antimicrobial activity in the amine derivative (**11**). Previously, we reported that the antimicrobial activities of leucomycins and their derivatives correlate with ribosomal binding activity.[4,5] We investigated the binding activity of novel dimers **5**, **9**, and **12** for *Escherichia coli* ribosomes, taking into account the permeability of these compounds to cell membranes. The ribosomal binding activity is reported as the concentrations (ID_{50}) at which [10,11,12-13-^{3}H]tetrahydroleucomycin A_3 binding was inhibited 50% by the antibiotics and their related compounds. The procedure of the ribosomal binding assay will be published in detail elsewhere.[6] The effect of leucomycin A_3, tylosin, and their dimeric derivatives on [^{3}H]tetrahydroleucomycin A_3 binding to *E. coli* ribosome is shown in Figure 3. The dimers **5** and **9** showed

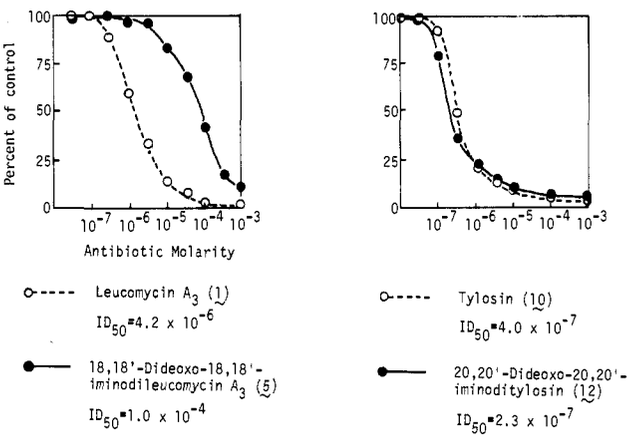


Figure 3. Effect of leucomycin A_3 (**1**), tylosin (**10**), and their dimeric derivatives on [10,11,12,13-^{3}H]tetrahydroleucomycin A_3 binding to *E. coli* ribosomes.

a reduction in their ribosomal binding activity (ID_{50} = 1.0 × 10^{-4} in **5**, 1.2 × 10^{-6} in **7**, and 2.0 × 10^{-5} in **9**) compared with **1** (ID_{50} = 4.2 × 10^{-6}). On the other hand, the dimerized derivative of tylosin, 20,20′-dideoxo-20,20′-iminoditylosin (**12**; ID_{50} = 2.3 × 10^{-7}), revealed a ribosomal binding activity on the same level as that of tylosin (ID_{50} = 4.0 × 10^{-7}). Furthermore, it is very interesting that the dimer of tylosin showed the same antimicrobial activity and ribosomal binding activity as tylosin, while those of the leucomycin group did not.

Experimental Section

All melting points were measured on a Mitamura Riken Prismscope melting point determinator and were uncorrected. IR spectra were recorded on a JASCO IRA-1, and UV spectra were measured on a Shimadzu UV-210 A spectrometer. Electron-impact (EI) mass spectra were performed on a JEOL JMS-D-100. FD mass spectra were measured on a JMS-D 300/JMA2000H. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL PS-100 and a PFT-100 spectrometer, respectively, with deuteriochloroform as solvent and tetramethylsilane as internal standard. MIC (minimum inhibitory concentration; in micrograms per milliliter) was determined by the agar dilution method in nutrient agar (peptone 0.5%, meat extract 0.5%, agar 1.0%, pH 7.0) incubated at 37 °C for 20 h.

18-Deoxo-18-(dimethylamino)leucomycin A_3 (2). To a stirred solution of sodium cyanoborohydride (250 mg, 4 mmol) and dimethylamine hydrochloride (810 mg, 10 mmol) in absolute methanol (6 mL) was added a solution of leucomycin A_3 (**1**; 827 mg, 1 mmol) in absolute methanol (20 mL). After stirring for 3

(4) S. Pestka, A. Nakagawa, and S. Ōmura, *Antimicrob. Agents Chemother.*, **6**, 606 (1974).
(5) S. Ōmura, A. Nakagawa, H. Sakakibara, O. Okekawa, R. Brandsch, and S. Pestka, *J. Med. Chem.*, **20**, 732 (1977).
(6) S. Ōmura, H. Tanaka, and J. Inokoshi, *J. Antibiot.*, in press.

h at room temperature, the solution was poured into 5% aqueous sodium bicarbonate and then extracted with ethyl acetate. The organic layer was washed twice with saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. After the solvent was removed, the residue was subjected to preparative TLC on Kieselgel GF_{254}, developed with chloroform–methanol–concentrated ammonia water (100:10:0.2, v/v). The major band was scratched off from the silica gel plate and eluted with chloroform–methanol (3:1, v/v). The eluate was evaporated in vacuo to obtain a white powder of **2**: yield 695 mg (81%); UV (CH_3OH) λ_{max} 230 nm (ϵ 20500); ^1H NMR δ 1.00 (3 H, d, $J_{8,19}$ = 7 Hz, C_{18} CH_3), 1.28 (3 H, d, $J_{15,16}$ = 7 Hz, C_{15} CH_3), 2.11 (3 H, s, C_3 $OCOCH_3$), 2.22 [6 H, s, C_{18} $N(CH_3)_2$], 2.48 [6 H, s, $C_{3'}$ $N(CH_3)_2$], 3.20 (1 H, d, $J_{4,5}$ = 9 Hz, C_9 H), 3.54 (3 H, s, C_4 OCH_3), 3.72 (1 H, d, $J_{4,5}$ = 9 Hz, C_5 H), 4.20 (1 H, dd, $J_{8,9}$ = 4 Hz, $J_{9,10}$ = 10 Hz, C_9 H), 4.40 (1 H, d, $J_{2,3}$ = 10 Hz, C_3 H), 5.08 (1 H, m, C_{15} H), 5.63 (1 H, m, $J_{12,13}$ = 14 Hz, C_{13} H), 5.65 (1 H, dd, $J_{9,10}$ = 10 Hz, $J_{10,11}$ = 14 Hz, C_{10} H), 6.04 (1 H, ddd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14 Hz, $J_{12,14}$ = 1 Hz, C_{12} H), 6.50 (1 H, dd, $J_{10,11}$ = 14 Hz, $J_{11,12}$ = 10 Hz, C_{11} H); ^{13}C NMR δ 84.4 (d, C-4), 78.1 (d, C-5), 29.6 (d, C-6), 31.4 (t, C-7), 33.9 (d, C-8), 41.9 (t, C-17), 57.6 (t, C-18), 44.9 [q, C_{18} $N(CH_3)_2$]. Anal. ($C_{44}H_{76}N_2O_{14}$) C, H, N.

18-Deoxo-18-(methylamino)leucomycin A_3 (3). A solution of 1 (1.65 g, 2 mmol), sodium cyanoborohydride (0.50 g, 8 mmol), and methylamine hydrochloride (1.34 g, 20 mmol) in absolute methanol (30 mL) was stirred for 3 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate and then evaporated to dryness. The residue was subjected to preparative TLC on silica gel, developed with chloroform–methanol–concentrated ammonia water (100:5:0.1, v/v), to obtain **3** as a white powder: yield 1.03 g (61%); UV (CH_3OH) λ_{max} 231 nm (ϵ 26500); ^1H NMR δ 1.00 (3 H, d, $J_{8,19}$ = 7 Hz, C_{17} CH_3), 1.26 (3 H, d, $J_{15,16}$ = 7 Hz, C_{15} CH_3), 2.11 (3 H, s, C_3 $OCOCH_3$), 2.48 [6 H, s, C_3, $N(CH_3)_2$], 2.63 (3 H, s, C_{18} $NHCH_3$), 3.18 (1 H, d, $J_{4,5}$ = 9 Hz, C_4 H), 3.54 (3 H, s, C_4 OCH_3), 3.68 (1 H, d, $J_{4,5}$ = 9 Hz, C_5 H), 4.20 (1 H, dd, $J_{8,9}$ = 4 Hz, $J_{9,10}$ = 10 Hz, C_9 H), 4.40 (1 H, d, $J_{2,3}$ = 10 Hz, C_3 H), 5.08 (1 H, m, C_{15} H), 5.63 (1 H, m, $J_{12,13}$ = 14 Hz, C_{13} H), 5.64 (1 H, dd, $J_{9,10}$ = 10 Hz, $J_{10,11}$ = 14 Hz, C_{10} H), 6.03 (1 H, ddd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14 Hz, $J_{12,14}$ = 1 Hz, C_{12} H), 6.45 (1 H, dd, $J_{10,11}$ = 14 Hz, $J_{11,12}$ = 10 Hz, C_{11} H); ^{13}C NMR δ 29.6 (d, C-6), 31.6 (t, C-7), 33.9 (d, C-8), 41.9 (t, C-17), 47.9 (t, C-18), 41.9 (t, $NHCH_3$). Anal. ($C_{43}H_{74}N_2O_{14}$) C, H, N.

Reductive Amination of Leucomycin A_3 (1) with Sodium Cyanoborohydride and Ammonium Acetate. A solution of 1 (1.65 g, 2 mmol), sodium cyanoborohydride (0.50 g, 8 mmol), and ammonium acetate (2.00 g, 26 mmol) in absolute methanol (30 mL) was stirred for 3 h at room temperature. The solution was worked up as described above. The residual pale yellowish powder was subjected to preparative TLC on silica gel, developed with chloroform–methanol–concentrated ammonia water (100:10:0.2, v/v), to obtain three compounds.

The most polar compound [R_f 0.58; Kieselgel GF_{254}; chloroform–methanol–concentrated ammonia water (100:10:0.01, v/v)] was purified by chromatography using Kieselgel GF_{254} plates and chloroform–methanol (12:1, v/v) as eluent to give a white powder of 18-amino-18-deoxoleucomycin A_3 (**4**): yield 462 mg (28%); UV (CH_3OH) λ_{max} 232nm (ϵ 25200). ^{13}C NMR δ 84.1 (d, C-4), 78.4 (d, C-5), 29.6 (d, C-6), 31.8 (t, C-7), 33.9 (d, C-8), 41.9 (t, C-17), 38.7 (t, C-18). Anal. ($C_{42}H_{72}N_2O_{14}$) C, H, N.

The second compound (R_f 0.78; the same developing solvent used for **4**) was also chromatographed on Kieselgel GF_{254} plates, using chloroform–methanol (15:1 v/v) as developing solvent, to obtain **5** as an amorphous powder: yield 454 mg (33%); mp 130–131 °C; UV (CH_3OH) λ_{max} 231 nm (ϵ 50600); FDMS, m/z 1639 (M^+); IR (KBr) 3480, 1735, 1378, 1254, 1160, 1045 cm^{-1}; ^1H NMR δ 1.00 (6 H, d, $J_{8,19}$ = 7 Hz, C_8 CH_3 × 2), 1.28 (6 H, d, $J_{15,16}$ = 7 Hz, C_{15} CH_3 × 2), 2.12 (6 H, s, C_3 $OCOCH_3$ × 2), 3.26 (2 H, d, $J_{4,5}$ = 9 Hz, C_4 H × 2), 3.56 (6 H, s, C_4 OCH_3 × 2), 3.77 (2 H, d, $J_{4,5}$ = 9 Hz, C_5 H × 2), 4.20 (2 H, dd, $J_{8,9}$ = 4 Hz, $J_{9,10}$ = 10 Hz, C_9 H × 2), 4.40 (2 H, d, $J_{2,3}$ = 10 Hz, C_3 H × 2), 5.08 (2 H, m, C_{15} H × 2), 5.64 (2 H, m, $J_{12,13}$ = 14 Hz, C_{13} H × 2), 5.66 (2 H, dd, $J_{9,10}$ = 10 Hz, $J_{10,11}$ = 14 Hz, C_{10} H × 2), 5.97 (2 H, ddd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14 Hz, $J_{12,13}$ = 1 Hz, C_{12} H × 2), 6.48 (2 H, dd, $J_{10,11}$ = 14 Hz, $J_{11,12}$ = 10 Hz, C_{11} H × 2); ^{13}C NMR δ 84.1 (d, C-4), 78.4 (d, C-5), 29.8 (d, C-6), 31.8 (t, C-7), 33.6 (d, C-8), 41.7 (t, C-17), 47.1 (t, C-18). Anal. ($C_{84}H_{141}N_3O_{28}$) C, H, N.

The third compound was further purified by preparative TLC, using chloroform–4% methanol (25:1, v/v), to obtain a white powder of 18-deoxoleucomycin A_3 (**6**; 8.3 mg, 0.5%): UV (CH_3OH) λ_{max} 231 nm (ϵ 25800); IR (KBr) 1735, 1720, 1377, 1165, 1048 cm^{-1}; ^1H NMR δ 0.87 (3 H, t, $J_{17,18}$ = 7 Hz, C_{17} CH_3). Anal. ($C_{42}H_{71}NO_{14}$) C, H, N.

Dimerization of Leucomycin A_3 (1) and 18-Amino-18-deoxoleucomycin A_3 (4) with Sodium Cyanoborohydride. To a stirred solution of 1 (200 mg) and **4** (200 mg) in methanol (5 mL) was added sodium cyanoborohydride (25 mg). After stirring for 3 h at room temperature, the reaction mixture was poured into saturated aqueous sodium bicarbonate, extracted with ethyl acetate, and then washed with saturated aqueous sodium chloride. The extract was dried over anhydrous sodium sulfate and then evaporated to dryness. The residue was subjected to preparative TLC on Kieselgel GF_{254}, using chloroform–methanol (15:1, v/v) as eluent, to afford 18,18′-dideoxo-18,18′-iminodileucomycin A_3 (**5**; 167 mg, 42%) as a white powder.

18-Amino-18-deoxoleucomycin A_5 (8) and 18,18′-Dideoxo-18,18′-iminodileucomycin A_5 (9). In a manner similar to 1, leucomycin A_5 (**7**; 7.0 g, 9.1 mmol) was treated with sodium cyanoborohydride (1.06 g, 16.9 mmol) and ammonium acetate (8.4 g, 0.19 mmol) in absolute methanol (106 mmol). The mixture was worked up as described above. The products were chromatographed on a silica gel column [Kieselgel 60, chloroform–methanol–concentrated ammonia water (10:1:0.1, v/v)]. The first fraction was collected, and removal of the solvents gave a pale yellowish powder. The crude product was purified on a silica gel preparative TLC, using chloroform–methanol–concentrated ammonia water (10:2:0.1, v/v) as eluent, to obtain **8** as an amorphous powder: yield 1.8 g (26%); UV (CH_3OH) λ_{max} 232 nm (ϵ 25840); EIMS, m/z 772 (M^+), 677 ($M^+ - C_3H_7CO_2$), 541 (677 – mycarose), 404 (M^+ – aglycon), 368 (aglycon); IR (KBr) 3480, 1725, 1375, 1245, 1155, 1045 cm^{-1}; ^1H NMR δ 2.50 [6 H, s, $N(CH_3)_2$], 3.07 (1 H, d, $J_{4,5}$ = 8 Hz, C_4 H), 3.46 (3 H, s, C_4 OCH_3), 3.69 (1 H, d, $J_{4,5}$ = 8 Hz, C_5 H), 3.90 (1 H, dd, $J_{8,9}$ = 2 Hz, $J_{9,10}$ = 10 Hz, C_9 H), 4.35 (1 H, d, $J_{2,3}$ = 9 Hz, C_3 H), 4.52 (1 H, c, $J_{1',2'}$ = 9 Hz, $C_{1'}$ H), 5.07 (1 H, d, $J_{1'',2''}$ = 4 Hz, $C_{1''}$ H), 5.20 (1 H, m, C_{15} H), 5.63 (1 H, m, $J_{12,13}$ = 14.5 Hz, C_{13} H), 5.78 (1 H, dd, $J_{9,10}$ = 10 Hz, $J_{10,11}$ = 14.5 Hz, C_{10} H), 6.03 (1 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14.5 Hz, C_{12} H), 6.43 (1 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14.5 Hz, C_{11} H); ^{13}C NMR: δ 85.5 (d, C-4), 78.5 (d, C-5), 29.4 (d, C-6), 31.3 (t, C-7), 34.2 (d, C-8), 41.7 (t, C-17), 38.2 (t, C-18). Anal. ($C_{39}H_{68}N_2O_{13}$) C, H, N.

From the second fraction was obtained the dimeric derivative (**9**) of **7** as a white powder: yield 2.1 g (30%); mp 128–129 °C; UV (CH_3OH) λ_{max} 231 (ϵ 51600); FDMS, m/z 1527 (M^+); ^1H NMR δ 2.48 [12 H, s, $N(CH_3)_2$ × 2], 3.07 (2 H, d, $J_{4,5}$ = 8 Hz, C_4 H × 2), 3.44 (6 H, s, C_4 OCH_3 × 2), 3.67 (2 H, d, $J_{4,5}$ = 8 Hz, C_5 H × 2), 3.90 (2 H, dd, $J_{8,9}$ = 2 Hz, $J_{9,10}$ = 10 Hz, C_9 H × 2), 4.33 (2 H, d, $J_{2,3}$ = 9 Hz, C_3 H × 2), 4.52 (2 H, d, $J_{1',2'}$ = 9 Hz, $C_{1'}$ H × 2), 5.05 (2 H, d, $J_{1'',2''}$ = 4 Hz, $C_{1''}$ H × 2), 5.18 (2 H, m, C_{15} H × 2), 5.61 (2 H, m, $J_{12,13}$ = 14.5 Hz, C_{13} H × 2), 5.76 (2 H, dd, $J_{9,10}$ = 10 Hz, $J_{10,11}$ = 14.5 Hz, C_{10} H × 2), 6.03 (2 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14.5 Hz, C_{12} H × 2), 6.42 (2 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14.5 Hz, C_{11} H × 2); ^{13}C NMR δ 85.8 (d, C-4), 78.5 (d, C-5), 29.4 (d, C-6), 31.3 (t, C-7), 34.0 (d, C-8), 41.9 (t, C-17), 46.6 (t, C-18). Anal. ($C_{78}H_{133}N_3O_{26}$) C, H, N.

20-Amino-20-deoxotylosin (11) and 20,20′-Dideoxo-20,20′-iminoditylosin (12). In a fashion similar to the leucomycins, the reaction of tylosin (**10**; 9.2 mg, 10 mmol) with sodium cyanoborohydride (1.25 g, 19.9 mmol) and ammonium acetate (10.0 g, 0.23 mol) in absolute methanol (125 mL) afforded amorphous 20-amino-20-deoxotylosin (**11**; 1.9 g, 21%) and 20,20′-dideoxo-20,20′-iminoditylosin (**9**; 2.0 g, 22%). Compound **11**: UV (CH_3OH) λ_{max} 283 nm (ϵ 13500); EIMS, m/z 916 (M^+), 755 (M^+ – mycarose), 725 (M^+ – mycinose), 582 (M^+ – mycarosylmycaminose), 564 (M^+ – mycarose – mycinose); ^{13}C NMR δ 44.9 (d, C-4), 81.7 (d, C-5), 33.6 (d, C-6), 32.8 (t, C-7), 40.7 (d, C-8), 37.8 (t, C-19), 41.9 (t, C-20). Anal. ($C_{46}H_{78}N_2O_{16}$), C, H, N. Compound **12**: mp 102–103 °C; UV (CH_3OH) λ_{max} 282 nm (ϵ 54400); ^1H NMR δ 0.98 (6 H, t, $J_{16,17}$ = 7 Hz, C_{16} CH_3 × 2), 1.02 (6 H, d, $J_{4,18}$ = 7 Hz, C_4 CH_3 × 2), 1.20 (6 H, d, $J_{5',6'}$ = 7 Hz, $C_{5'}$ CH_3 × 2), 1.24 (6 H, s, $C_{3''}$ CH_3 × 2), 1.81 (6 H, s, C_{12} CH_3 × 2), 2.36 (2 H, dd, $J_{2',3'}$ = 11 Hz, $J_{3',4'}$ = 3 Hz, $C_{3'}$ H × 2), 2.50 [12 H, s, $N(CH_3)_2$ × 2], 3.46 (6 H, s, OCH_3 × 2),

3.60 (6 H, s, $OCH_3 \times 2$), 3.72 (2 H, d, $J_{4,5}$ = 10.5 Hz, C_5 H × 2), 3.68 (4 H, m, C-23 × 2), 3.90 (2 H, dd, $J_{2a,3}$ = 2 Hz, $J_{2b,3}$ = 13 Hz, C_3 H × 2), 4.26 (2 H, d, $J_{1',2'}$ = 8 Hz, $C_{1'}$ H × 2), 4.56 (2 H, d, $J_{1''',2'''}$ = 10 Hz, $C_{1'''}$ H × 2), 5.02 (2 H, br t, C_{15} H × 2), 5.07 (2 H, d, $J_{1'',2''}$ = 4 Hz, $C_{1''}$ H × 2), 5.90 (2 H, dd, $J_{13,14}$ = 12 Hz, $J_{13,22}$ = 1 Hz, C_{13} H × 2), 6.26 (2 H, d, $J_{10,11}$ = 15 Hz, C_{10} H × 2), 7.30 (2 H, d, $J_{10,11}$ = 15 Hz, C_{11} H × 2); ^{13}C NMR δ 81.7 (d, C-5), 44.9 (d, C-4), 40.9 (d, C-8), 33.6 (d, C-6), 32.6 (t, C-7), 4.10 (t, C-10), 46.0 (t, C-20). Anal. ($C_{92}H_{157}N_3O_{32}$) C, H, N.

Didemycarosyl-20,20′-dideoxo-20,20′-iminoditylosin (13). A solution of **12** (90 mg) in 0.4 N HCl (2 mL) was allowed to stand for 30 min at room temperature. The reaction mixture was extracted with ethyl acetate at pH 2.0 to remove the mycarose moiety. The water layer was adjusted to pH 9 with saturated sodium bicarbonate solution and then extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After the ethyl acetate was removed, the residue was subjected to preparative TLC on Kieselgel GF_{254}, developed with chloroform–methanol–concentrated ammonia water (20:3:0.1, v/v), to obtain **13** as an amorphous powder: yield 60 mg (82%); mp 124–125 °C; UV (CH_3OH) λ_{max} 283 nm (ϵ 65800); FDMS, m/z 1527 (M^+), $C_{78}H_{133}N_3O_{26}$), 1353 (M^+ – mycaminose), 1336 (M^+ – mycinose), 1179 (M^+ – mycaminose × 2), 1149 (M^+ – mycinose × 2); ^1H NMR δ 0.97 (6 H, t, $J_{16,17}$ = 7 Hz, C_{16} CH_3 × 2), 1.04 (6 H, d, $J_{4,18}$ = 7 Hz, C_4 CH_3 × 2), 1.22 (6 H, d, $J_{5',6'}$ = 7 Hz, $C_{5'}$ H × 2), 1.80 (6 H, s, C_{12} CH_3 × 2), 2.35 (2 H, dd, $J_{2',3'}$ = 11 Hz, $J_{3',4'}$ = 3 Hz, $C_{3'}$ H × 2), 2.51 [12 H, s, $N(CH_3)_2$ × 2], 3.50 (6 H, s, OCH_3 × 2), 3.63 (6 H, s, OCH_3 × 2), 3.72 (2 H, d, $J_{4,5}$ = 10.5 Hz, C_5 H × 2), 3.80 (4 H, m, C_{23} H × 2), 3.91 (2 H, dd, $J_{2a,3}$ = 2 Hz, $J_{2b,3}$ = 13 Hz, C_3 H × 2), 4.30 (2 H, d, $J_{1',2'}$ = 8 Hz, $C_{1'}$ H × 2), 4.56 (2 H, d, $J_{1''',2'''}$ = 9 Hz, $C_{1'''}$ H × 2), 5.00 (2 H, m, $J_{14,15}$ = 4 Hz, $J_{15,16}$ = 8 Hz, C_{15} H × 2), 5.90 (2 H, dd, $J_{13,14}$ = 12 Hz, $J_{13,22}$ = 1 Hz, C_{13} H × 2), 6.28 (2 H, d, $J_{10,11}$ = 15 Hz, C_{10} H × 2), 7.30 (2 H, d, $J_{10,11}$ = 15 Hz, C_{11}H × 2); ^{13}C NMR δ 41.7 (t, C-19), 43.5 (t, C-20). Anal. ($C_{78}H_{133}N_3O_{26}$) C, H, N.

9-Dehydro-18-deoxo-18-(dimethylamino)leucomycin A_3 (14). A suspension of 18-deoxo-18-(dimethylamino)leucomycin A_3 (**2**; 560 mg, 0.65 mmol) and activated manganase dioxide (6.0 g) in chloroform (60 mL) was stirred for 1 h at room temperature. The reaction mixture was filtered through Celite. The filtrate was evaporated to dryness. The residue was subjected to preparative TLC on Kieselgel GF_{254}, using chloroform–methanol–concentrated ammonia water (100:5:0.1, v/v), to give 9-dehydro-18-dihydro-18-(dimethylamino)leucomycin A_3 (**14**): yield 117 mg (21%); UV (CH_3OH) λ_{max} 279 nm (ϵ 18900); IR (KBr) 1730, 1657, 1630, 1600, 1375, 1175, 1065 cm^{-1}; EIMS, m/z 854 (M^+), 753 (M^+ – isovaleryl), 609 (753 – mycarose), 436 (aglycon), 402 (M^+ – aglycon); ^1H NMR δ 0.99 (3 H, d, $J_{8,19}$ = 7 Hz, C_8 CH_3), 2.19 [6 H, s, C_{18} $N(CH_3)_2$], 4.92 (1 H, ddd, $J_{14a,15}$ = 10 Hz, $J_{14b,15}$ = 3 Hz, $J_{15,16}$ = 7 Hz, C_{15} H), 6.03 (1 H, m, $J_{12,13}$ = 14 Hz, $J_{11,13}$ = 4 Hz, C_{13} H), 6.15 (1 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14 Hz, C_{12}H), 6.28 (1 H, d, $J_{10,11}$ = 14 Hz, C_{10} H), 7.34 (1 H, ddd, $J_{10,11}$ = 14 Hz, $J_{11,12}$ = 10 Hz, $J_{11,13}$ = 4 Hz, C_{11} H); ^{13}C NMR δ 84.8 (d, C-4), 78.9 (d, C-5), 30.3 (d, C-6), 33.6 (t, C-7), 44.2 (d, C-8), 203.0 (s, C-9), 41.8 (t, C-17), 57.3 (t, C-18), 45.2 [q, C_{18} $N(CH_3)_2$]. Anal. ($C_{44}H_{74}N_2O_{14}$) C, H, N.

9-Dehydro-18-deoxo-18-(methylamino)leucomycin A_3 (15). To a solution of 18-deoxo-18-(methylamino)leucomycin A_3 (**3**; 820 mg, 0.97 mmol) in chloroform (92 mL) was added activated manganase dioxide (9.2 g). After the solution was stirred for 1 h at room temperature, the suspension was filtered through Celite. After the solvent was removed, the residue was chromatographed on silica gel preparative TLC, developed with chloroform–methanol (93:7, v/v), to obtain **15** as a white amorphous powder: yield 139 mg (17%); UV (CH_3OH) λ_{max} 281 nm (ϵ 19083); EIMS, m/z 840 (M^+), 739 (M^+ – isovaleryl), 595 (739 – mycarose), 422 (aglycon), 402 (M^+ – aglycon); IR (KBr) 1735, 1657, 1630, 1595, 1365, 1175, 1065 cm^{-1}; ^1H NMR δ 1.00 (3 H, d, $J_{8,19}$ = 7 Hz, C_8 CH_3), 2.48 (3 H, s, $NHCH_3$), 6.04 (1 H, m, $J_{11,13}$ = 4 Hz, $J_{12,13}$ = 14 Hz, C_{12} H), 6.16 (1 H, dd, $J_{11,12}$ = 10 Hz, $J_{12,13}$ = 14 Hz, C_{12} H), 6.30 (1 H, d, $J_{10,11}$ = 14 Hz, C_{10} H), 7.36 (1 H, d, $J_{10,11}$ = 14 Hz, C_{11} H); ^{13}C NMR δ 202.8 (s, C-9), 47.9 (t, C-18), 41.9 (q, $NHCH_3$). Anal. ($C_{43}H_{72}N_2O_{14}$) C, H, N.

18-Amino-9-dehydro-18-deoxoleucomycin A_3 (16). A suspension of **4** (560 mg, 0.68 mmol) and activated manganase dixode (6.3 g) in chloroform (65 mL) was stirred for 1 h at room temperature. Similar workup as described above gave **16** as an amorphous powder: yield 170 mg (31%); UV (CH_3OH) λ_{max} 279 nm (ϵ 21070); ^{13}C NMR δ 30.1 (d, C-6), 44.0 (d, C-8), 41.9 (t, C-17), 38.6 (t, C-18), 202.5 (s, C-9). Anal. ($C_{42}H_{69}N_2O_{14}$) C, H, N.

Supplementary Material Available: Two figures showing the FD mass spectral fragments for compounds **5** and **12** (2 pages). Ordering information is given on any current masthead page.