N-SUBSTITUTED DERIVATIVES OF 23-AMINO-4′,23-DIDEOXYMYCAMINOSYL TYLONOLIDE

SYNTHESIS AND ANTIBACTERIAL ACTIVITY

SHUICHI SAKAMOTO, TSUTOMU TSUCHIYA, AKIHIRO TANAKA, SUMIO UMEZAWA, MASA HAMADA† and HAMAO UMEZAWA†

Institute of Bioorganic Chemistry,
1614 Ida, Nakahara-ku, Kawasaki 211, Japan
†Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication December 1, 1984)

23-Amino-4′,23-dideoxymycaminosyl tylonolide diethyl acetal (**5**) has been prepared from 4′,23-dideoxy-23-iodomycaminosyl tylonolide diethyl acetal (**3**) by treatment with sodium azide followed by selective reduction of the resulting azide (**4**). 23-Acylamino-23-deoxy (**6**～**8**) and 23-deoxy-23-urethane-type compounds (**12**～**15**) were further prepared. Treatment of the 23-alkylamino-4′,23-dideoxymycaminosyl tylonolides (**9, 10**) with chloroformates gave 23-*N*-alkyl-23-deoxy-23-urethane-type compounds (**16**～**21, 29, 30**). 23-*N*-Alkyl-23-deoxy-23-(2-hydroxyethylamino and 2-methoxyethylamino)-4′,23-dideoxymycaminosyl tylonolides (**22**～**25, 27, 28**) were prepared from **3** and the corresponding amines. Antibacterial activities and toxicities (for **23** and **27**) of these compounds are described.

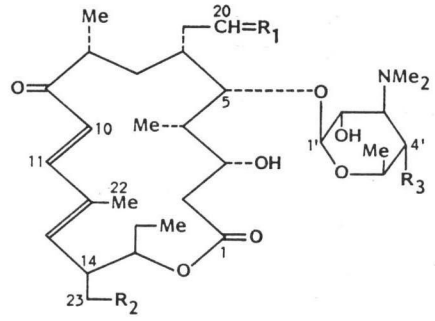
As previously reported[1], 23-dialkylamino-23-deoxymycaminosyl tylonolides and their 4′-deoxy analogs had considerable antibacterial activities against Gram-negative bacteria. This suggested that nitrogen at C-23 might play an important role in bestowing activity against Gram-negative bacteria. This paper describes the synthesis of *N*-substituted derivatives of 23-amino-4′,23-dideoxymycaminosyl tylonolide and their antibacterial activities. Since the derivatives of 4′-deoxymycaminosyl tylonolide[2] (**2**) were found to show much improved antibacterial activities than the starting compound (**1**), we have dealt here with derivatives of the 4′-deoxy macrolide.

Treatment of 4′,23-dideoxy-23-iodomycaminosyl tylonolide diethyl acetal[3] (**3**) with sodium azide in *N*,*N*-dimethylformamide gave the 23-azido derivative (**4**) in good yield. Reduction of **4** with chromium (II) chloride[4] under weak acidic conditions gave the 23-amino-23-deoxy derivative (**5**) in a moderate yield with the carbonyl at C-9 and the formyl protecting groups at C-20 remaining intact. The ability resistance of the acetal group to remain intact under the above acidic conditions was necessary to give **5**, because, if hydrolyzed, the free formyl group reacted with the amino group at C-23 intermolecularly to give intractable complex products. Some other reducing reagents tested failed to give **5**.

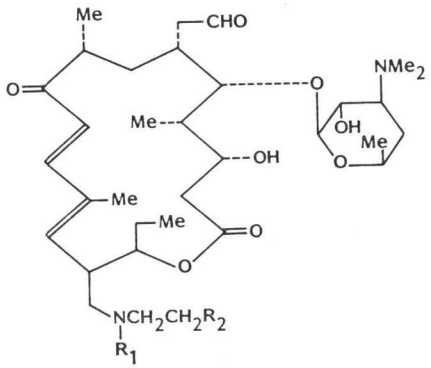
Usual acylation of **5** with acetic anhydride or benzoyl chloride in aqueous methanol gave the corresponding 23-*N*-acyl derivatives, which, after removal of the formyl protecting groups, afforded the 23-acetamido and 23-benzamido derivatives (**6** and **7**). *N*,*N*-Dimethylglycylamido derivative (**8**) was prepared by coupling *N*,*N*-dimethylglycine with **5** by the active ester method followed by deblocking.

Derivatives (**12**～**15**) having urethane-type groups at C-23 were prepared by treating **5** with methyl, ethyl, phenyl, and benzyl chloroformates followed by deblocking.

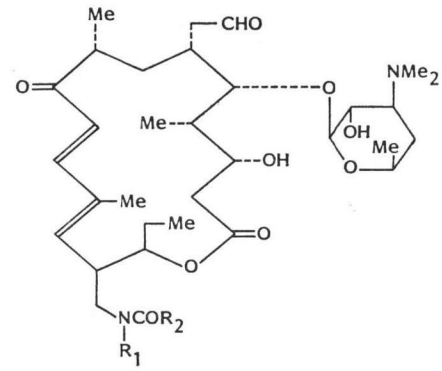
Urethane-type derivatives having *N*-alkyl groups at C-23 were next prepared. These derivatives were expected to have slight increase in basicity of the nitrogen at C-23, and the basicity is considered to



	R_1	R_2	R_3
1	O	OH	OH
2	O	OH	H
3	$(OEt)_2$	I	H
4	$(OEt)_2$	N_3	H
5	$(OEt)_2$	NH_2	H
9	$(OEt)_2$	NHMe	H
10	$(OEt)_2$	NHEt	H



	R_1	R_2
11	Et	H
22	Me	OH
23	Et	OH
24	Pr	OH
25	Bu	OH
26	CH_2CH_2OH	OH
27	Et	OMe
28	Pr	OMe



	R_1	R_2
6	H	Me
7	H	Ph
8	H	CH_2NMe_2
12	H	OMe
13	H	OEt
14	H	OPh
15	H	OCH_2Ph
16	Me	OMe
17	Me	OCH_2Ph
18	Et	OMe
19	Et	OEt
20	Et	OPh
21	Et	OCH_2Ph
29	Et	$O(CH_2)_2NMe_2$
30	Et	$O(CH_2)_3NMe_2$

be correlated[5] with the antibacterial activity. Starting material, 23-deoxy-23-methylamino diethyl acetal (**9**) was prepared from **3** and methylamine according to the method reported[5]. This **9** and the *N*-ethyl analog[5] (**10**) were then treated with several chloroformates as described before to give, after deblocking, the desired *N*-alkyl urethanes (**16**~**21**).

Another group of urethane-type compounds having *N*,*N*-dimethylaminoethyl and *N*,*N*-dimethylaminopropyl groups were further prepared. The compound **10** were treated with 2-bromoethyl chloroformate or 3-bromopropyl chloroformate and the resulting bromo derivatives were reacted with dimethylamine to give, after deblocking the formyl protecting groups, the desired derivatives (**29** and **30**).

A series of derivatives having *N*-alkyl-2-hydroxyethylamino groups at C-23 were further prepared in order to compare the effect of the β-oxygens with those of related compounds (for example, see **16b** and **17b** of a literature[1]), on antibacterial activities against Gram-negative bacteria. Treatment of **3** with commercially available *N*-methyl-2-hydroxyethylamine, *N*-ethyl-2-hydroxyethylamine, *N*-propyl-

Table 1. Yields, optical rotations, molecular weights (EI-MS) and elemental analysis.

Compound	Yield (%)	$[\alpha]_D^{25}$ (c 1, $CHCl_3$)	MS (m/z)	Formula	C		H		N	
					Calcd	Found	Calcd	Found	Calcd	Found
6		+57°	622 (M^+)	$C_{33}H_{54}N_2O_9$	63.66	63.94	8.68	8.90	4.50	4.30
7		+77°	684 (M^+)	$C_{38}H_{56}N_2O_9$	66.66	66.47	8.19	8.38	4.09	4.25
8		+48°		$C_{35}H_{59}N_3O_9$	63.16	63.22	8.87	8.74	6.32	6.14
12	68	+50°	638 (M^+)	$C_{33}H_{54}N_2O_{10}$	62.07	62.27	8.46	8.45	4.39	4.53
13	72	+48°		$C_{34}H_{56}N_2O_{10}$	62.58	62.31	8.59	8.43	4.29	4.04
14	51	+71°		$C_{38}H_{56}N_2O_{10}$	65.14	64.89	8.00	8.07	4.00	4.17
15	49	+47°		$C_{39}H_{58}N_2O_{10}$	65.55	65.81	8.12	8.11	3.92	3.75
16	83	+61°		$C_{34}H_{56}N_2O_{10}\cdot\frac{1}{2}H_2O$	61.72	61.78	8.62	8.36	4.23	3.98
17	68	+64°	728 (M^+)	$C_{40}H_{60}N_2O_{10}$	65.93	66.11	8.24	8.17	3.85	3.82
18	90	+68°		$C_{35}H_{58}N_2O_{10}$	63.06	62.80	8.71	8.58	4.20	4.06
19	85	+80°		$C_{36}H_{60}N_2O_{10}$	63.53	63.66	8.82	8.80	4.12	4.06
20	66	+105°	728 (M^+)	$C_{40}H_{60}N_2O_{10}$	65.93	66.17	8.24	8.24	3.85	3.97
21	63	+80°		$C_{41}H_{62}N_2O_{10}$	66.31	66.29	8.36	8.27	3.77	3.84
22	90	+23°	639 ($M+H^+$)	$C_{34}H_{58}N_2O_9$	63.92	64.12	9.15	9.04	4.38	4.49
23	71	+30°	653 ($M+H^+$)	$C_{35}H_{60}N_2O_9$	64.39	64.48	9.26	9.14	4.29	4.30
24	83	+38°	667 ($M+H^+$)	$C_{36}H_{62}N_2O_9$	64.86	64.60	9.31	9.22	4.20	4.46
25	43	+32°	681 ($M+H^+$)	$C_{37}H_{64}N_2O_9$	65.29	65.00	9.41	9.23	4.12	4.07
27		+27°	666 (M^+)	$C_{36}H_{62}N_2O_9\cdot\frac{3}{2}H_2O$	62.34	62.40	9.38	8.88	4.04	3.79
28		+28°	680 (M^+)	$C_{37}H_{64}N_2O_9\cdot\frac{1}{2}H_2O$	64.44	64.63	9.43	9.08	4.06	3.92

Table 2. Antibacterial activity (MIC μg/ml) of **6**~**15** [C(23)NHCOR].

Test organism*	**6** R=Me	**7** Ph	**8** CH_2NMe_2	**12** OMe	**13** OEt	**14** OPh	**15** OCH_2Ph	**2**[2)]
Staphylococcus aureus 193	1.56	0.39	1.56	<0.2	<0.2	<0.2	<0.2	0.39
〃 EMf**	>100	25	>100	>100	>100	100	100	>100
〃 209P	1.56	0.39	0.78	<0.2	<0.2	<0.2	<0.2	0.39
〃 MS 9610	>100	>100	>100	>100	>100	>100	>100	>100
Micrococcus luteus PCI 1001	0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Escherichia coli NIHJ	25	3.12	12.5	3.12	3.12	3.12	6.25	3.12
〃 K-12	50	6.25	12.5	12.5	12.5	6.25	6.25	6.25
〃 〃 ML 1629	>100	25	100	25	50	25	25	50
〃 〃 ML 1410 R81	>100	25	50	50	50	50	50	25
〃 〃 LA 290 R55	25	3.12	3.12	3.12	3.12	6.25	1.56	1.56
Klebsiella pneumoniae PCI 602	3.12	3.12	3.12	1.56	3.12	1.56	1.56	1.56
Salmonella enteritidis 1891	3.12	3.12	1.56	1.56	3.12	1.56	1.56	3.12
S. typhi T-63	100	100	50	25	50	25	25	25
Proteus vulgaris OX-19	25	12.5	25	6.25	12.5	6.25	6.25	12.5
Pseudomonas aeruginosa A3	50	50	100	12.5	50	50	100	25

* Agar dilution streak method (nutrient agar 37°C, 17 hours); the same in Tables 2 and 3.
** Erythromycin-resistant strain; the same in Tables 2 and 3.

2-hydroxyethylamine (newly prepared), and *N*-butyl-2-hydroxyethylamine gave, after deblocking the formyl group, the desired products (**22**~**25**). In a similar way, the bis(2-hydroxyethyl)amino derivative (**26**) was prepared by treating **3** with bis(2-hydroxyethyl)amine. Dialkylamino derivatives (**27** and **28**) having an ether group, that is, *N*-ethyl-2-methoxyethylamino and *N*-propyl-2-methoxyethylamino groups

Table 3. Antibacterial activity (MIC μg/ml) of **16**~**21**, **29** and **30** [C(23)$NR_1CO_2R_2$].

Test organism	**16** R_1=Me R_2=Me	**17** Me CH_2Ph	**18** Et Me	**19** Et Et	**20** Et Ph	**21** Et CH_2Ph	**29** Et $(CH_2)_2$-NMe_2	**30** Et $(CH_2)_3$-NMe_2
S. aureus 193	0.39	<0.2	<0.2	0.39	0.39	<0.2	0.78	0.78
〃 EMf	>100	>100	>100	>100	100	100	>100	>100
〃 209P	0.39	<0.2	<0.2	<0.2	<0.2	<0.2	0.39	0.78
〃 MS 9610	>100	>100	>100	>100	>100	>100	>100	>100
M. luteus PCI 1001	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.78	1.56
E. coli NIHJ	6.25	25	6.25	6.25	12.5	12.5	6.25	1.25
〃 K-12	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25
〃 〃 ML 1629	100	100	50	50	50	50	50	100
〃 〃 ML 1410 R81	100	100	100	50	50	50	100	100
〃 〃 LA 290 R55	6.25	12.5	12.5	6.25	3.12	3.12	1.56	25
K. pneumoniae PCI 602	3.12	3.12	12.5	3.12	3.12	3.12	1.56	3.12
S. enteritidis 1891	3.12	3.12	6.25	6.25	12.5	6.25	1.56	3.12
S. typhi T-63	50	100	25	50	50	50	25	100
P. vulgaris OX-19	25	25	12.5	6.25	6.25	12.5	12.5	25
P. aeruginosa A3	25	50	50	50	100	100	25	25

Table 4. Antibacterial activity (MIC μg/ml) of **22**~**28** [C(23)$NR_1(CH_2CH_2OR_2)$].

Test organism	**22** R_1=Me R_2=H	**23** Et H	**24** Pr H	**25** Bu H	**26** CH_2CH_2OH H	**27** Et Me	**28** Pr Me	**11**[1]
S. aureus 193	0.39	0.39	0.39	<0.2	3.12	0.39	0.39	0.39
〃 EMf	>100	100	50	12.5	>100	50	50	50
〃 209P	0.39	0.39	<0.2	<0.2	3.12	<0.2	<0.2	0.39
〃 MS 9610	>100	>100	>100	>100	>100	>100	>100	>100
M. luteus PCI 1001	<0.2	<0.2	<0.2	<0.2	0.78	<0.2	<0.2	<0.2
E. coli NIHJ	3.12	3.12	1.56	3.12	50	3.12	3.12	1.56
〃 K-12	3.12	3.12	3.12	3.12	50	3.12	3.12	3.12
〃 〃 ML 1629	12.5	12.5	6.25	12.5	>100	12.5	12.5	6.25
〃 〃 ML 1410 R81	12.5	12.5	6.25	25	>100	12.5	12.5	12.5
〃 〃 LA 290 R55	1.56	3.12	3.12	3.12	100	3.12	3.12	0.78
K. pneumoniae PCI 602	1.56	0.78	1.56	0.78	25	3.12	1.56	0.78
S. enteritidis 1891	1.56	0.78	1.56	0.78	3.12	1.56	1.56	1.56
S. typhi T-63	6.25	6.25	6.25	12.5	100	12.5	12.5	6.25
P. vulgaris OX-19	6.25	6.25	3.12	3.12	100	6.25	6.25	3.12
P. aeruginosa A3	100	50	25	50	100	25	25	25

were also prepared by treating **3** with *N*-ethyl-2-methoxyethylamine and *N*-propyl-2-methoxyethylamine.

The antibacterial spectra of the new derivatives were shown in Tables 2~4. As shown in Tables 2 and 3, the amido- and urethane-type compounds gave similar antibacterial activities as compared with the activity of 4′-deoxymycaminosyl tylonolide (**2**) taken as the reference, although the urethanes have somewhat better activities than amides, and among them **12** was found to be the best. Table 4 shows that there is no notable difference in antibacterial activities among the oxygen-containing dialkylamino derivatives as compared with **11** taken as the reference, except **26**, the bis(2-hydroxyethyl)amino derivative, being much less effective. Interestingly **23** and **27** showed fairly different acute toxicities [LD_{50} 85 (**23**) and 175 mg/kg (**27**) in mice, by iv injection], suggesting the dramatic effect of the presence of methoxy group.

Experimental

General

^{1}H NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer, or at 250 MHz in the FT mode with a Bruker WM250 spectrometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Hitachi M-80 spectrometer. Thin-layer chromatography (TLC) were performed on Kieselgel 60 F-254 silica gel with detection by spraying with sulfuric acid, followed by heating. Column chromatography was performed on Kieselgel 60, 230~400 mesh (E. Merck).

23-Azido-4′,23-dideoxymycaminosyl Tylonolide Diethyl Acetal (4)

A mixture of **3** (1.04 g) and sodium azide (0.27 g) in dry *N*,*N*-dimethylformamide (20 ml) was stirred at 80°C for 6.5 hours. Evaporation gave a residue, that was extracted with $CHCl_3$. The organic solution was washed with satd aq sodium hydrogencarbonate, satd aq sodium sulfate, dried (Na_2SO_4), and evaporated to give a solid (0.86 g). Purification by column chromatography with $CHCl_3$ - MeOH - 28% aq NH_3 (30: 1: 0.1) gave a solid of **4**, 635 mg (69%), $[\alpha]_D^{25}$ +32° (*c* 1, $CHCl_3$); IR 2100 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H, 22-Me), 2.30 (s, 6H, NMe_2), 4.29 (d, 1H, H-1′), 5.77 (d, 1H, H-13), 6.30 (d, 1H, H-11).

Anal Calcd for $C_{35}H_{60}N_4O_9$: C 61.74, H 8.88, N 8.23.
Found: C 61.48, H 8.82, N 8.18.

23-Amino-4′,23-dideoxymycaminosyl Tylonolide Diethyl Acetal (5)

To an ice-cold solution of **4** (1.554 g) in Me_2CO (31 ml) was added chromium (II) chloride[4] (10 ml of 1.43 M solution in 0.6 M aq HCl), and the deep blue mixture was stirred at 0°C for 5 minutes under argon atmosphere. Nitrogen gas was evolved during the period. The mixture was poured into ice-cold half-satd aq sodium hydrogencarbonate (150 ml), filtered with aid of Celite, and the filtrate was extracted with $CHCl_3$. The organic solution was washed with satd aq sodium carbonate, satd aq sodium sulfate, dried (Na_2SO_4), and concentrated. Column chromatography of the residue (1.35 g) with $CHCl_3$ - MeOH - 28% aq NH_3 (20: 1: 0.1) gave a solid of **5**, 862 mg (58%), $[\alpha]_D^{25}$ +30° (*c* 1, $CHCl_3$); MS *m/z* 655 (M+H); ^{1}H NMR ($CDCl_3$) δ 1.85 (s, 3H, 22-Me), 2.31 (s, 6H, NMe_2), 4.30 (d, 1H, H-1′), 5.72 (d, 1H, H-13), 6.36 (d, 1H, H-10).

Anal Calcd for $C_{35}H_{62}N_2O_9 \cdot \frac{1}{4}H_2CO_3$: C 63.16, H 9.40, N 4.18.
Found: C 63.05, H 9.26, N 4.12.

23-Acetamido- and Benzamido-4′,23-dideoxymycaminosyl Tylonolides (6 and 7)

A mixture of **5** (65 mg), sodium hydrogencarbonate (25 mg), and acetic anhydride (22 mg) (for **6**) or benzoyl chloride (27 mg) (for **7**) in 60% aq MeOH (3.2 ml) was stirred at 0°C for 10 minutes and then at room temp for 2 hours. After addition of satd aq sodium hydrogencarbonate (1 ml), the mixture was extracted with $CHCl_3$ (1 ml×3). The organic solution was washed with satd aq sodium sulfate, dried (Na_2SO_4), and concentrated. A solution of the crude acylated product dissolved in a mixture of 0.1 M aq HCl (4 ml) - acetonitrile (1.5 ml) was kept at room temp for 1 hour, neutralized with sodium hydrogencarbonate, and extracted with $CHCl_3$ (1 ml×3). The organic solution was washed with satd aq sodium sulfate and concentrated. Column chromatography of the residue with $CHCl_3$ - MeOH - 28% aq NH_3 (30: 1: 0.1) gave a solid of **6**, 58 mg (88%) and **7**, 56 mg (85%).

4′,23-Dideoxy-23-*N*,*N*-dimethylglycylamidomycaminosyl Tylonolide (8)

N-(*N*,*N*-Dimethylglycyloxy)succinimide was prepared by treating *N*,*N*-dimethylglycine hydrochloride (1.4 g) with *N*-hydroxysuccinimide (1.15 g) and dicyclohexylcarbodiimide (2.05 g) in tetrahydrofuran (34 ml) containing triethylamine (10 ml) at 5°C for 1 day. To the resulting supernatant solution (8 ml), **5** (44.5 mg) was added and the mixture was kept at room temp for 2 hours. Evaporation gave a residue, that was extracted with $CHCl_3$ (2 ml). Usual work-up gave a syrup. Purification by column chromatography with $CHCl_3$ - MeOH - 28% aq NH_3 (30: 1: 0.1) gave a solid (38 mg). The acylated product was then deblocked by the procedure as described for **6** to give a solid of **8**, 29 mg (64%); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H, 22-Me), 2.27 (s, 6H, 3′-NMe_2), 2.30 (s, 6H, $COCH_2NMe_2$), 2.93 (s, 2H,

$COCH_2NMe_2$), 4.23 (d, 1H, H-1′), 4.83 (dt, 1H, H-15), 5.73 (d, 1H, H-13), 6.33 (d, 1H, H-10), 7.40 (d, 1H, H-11), 9.83 (s, 1H, H-20).

4′,23-Dideoxy-23-methylaminomycaminosyl Tylonolide Diethyl Acetal (**9**)

A solution of **3** (678 mg) and methylamine (17 ml of 5 M solution in acetonitrile) in acetonitrile (50 ml) was heated at 80°C for 1 hour. Additional methylamine (8.5 ml of the above solution) was added, and the reaction was continued for further 1.5 hours. Evaporation gave a residue, that was purified by column chromatography with $CHCl_3$ - MeOH - 28% aq NH_3 (20: 1: 0.1) to give a solid of **9**, 375 mg (63%), $[\alpha]_D^{24}$ +34° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.83 (s, 3H, 22-Me), 2.30 (s, 6H, NMe_2), 2.43 (s, 3H, NH*Me*), 4.28 (d, 1H, H-1′), 5.68 (d, 1H, H-13), 6.35 (d, 1H, H-10).

Anal Calcd for $C_{36}H_{64}N_2O_9 \cdot \frac{1}{2}H_2O$: C 63.81, H 9.60, N 4.14.
Found: C 63.73, H 9.29, N 3.97.

General Procedure for the Syntheses of 23-Alkyl-, Aralkyl- and Aryloxycarbonylamino-4′,23-dideoxymycaminosyl Tylonolides (**12**～**15**)

A mixture of **5** (～60 mg), sodium hydrogencarbonate (3 equiv for **5**) and the acylating reagents (1.2 equiv for **5**), that is, methyl chloroformate (for the preparation of **12**), ethyl chloroformate (for **13**), phenyl chloroformate (for **14**), or benzyl chloroformate (for **15**) in 60% aq MeOH (3 ml) was stirred at 0°C for 10 minutes, then at room temp overnight. After addition of satd aq sodium hydrogencarbonate (2 ml), the mixture was extracted with $CHCl_3$ (1 ml×3). The products obtained were then deblocked as described for **6** to give the desired products.

General Procedure for the Syntheses of 23-*N*-(Alkyl-, Aralkyl- and Aryloxycarbonyl)-4′,23-dideoxy-23-(ethyl and methylamino)mycaminosyl Tylonolides (**16**～**21**)

Compound **9** (60 mg) or **10** (55 mg) was treated with methyl chloroformate, ethyl chloroformate, benzyl chloroformate, or phenyl chloroformate (each 1.2 equiv for **9** or **10**) as described for **12** to give, after deblocking, solids of **16**～**21**.

General Procedure for the Syntheses of 23-*N*-Alkyl-4′,23-dideoxy-23-(2-hydroxyethylamino)mycaminosyl Tylonolides (**22**～**25**)

To a solution of **3** (～100 mg) in acetonitrile (2 ml) was added the respective amine (10 equiv for **3**), that is, *N*-methyl-2-hydroxyethylamine (for the preparation of **22**), *N*-ethyl-2-hydroxyethylamine (for **23**), *N*-propyl-2-hydroxyethylamine (for **24**) (prepared from *N*-(2-hydroxyethyl)propionamide and LAH in oxolane) and *N*-butyl-2-hydroxyethylamine (for **25**), and the solution was heated at 80°C or ～15 hours. Work-up including hydrolysis as described for **26** gave the desired *N*-alkyl-23-(2-hydroxyethylamino) derivatives.

4′,23-Dideoxy-23-bis(2-hydroxyethyl)aminomycaminosyl Tylonolide (**26**)

A solution of **3** (87 mg) and bis(2-hydroxyethyl)amine (120 mg) in acetonitrile (1.7 ml) was heated at 80°C for 10 hours. Evaporation gave a residue, that was extracted with $CHCl_3$ (6 ml). The organic solution was washed with satd aq sodium hydrogencarbonate, satd aq sodium sulfate, dried (Na_2SO_4), and evaporated to give a solid (83 mg). A mixture of the solid in acetonitrile (1.6 ml) and 0.1 M aq HCl (3.3 ml) was kept at room temp for 1 hour. Neutralization with satd aq sodium hydrogencarbonate was followed by extraction with $CHCl_3$. The organic solution was washed with satd aq sodium sulfate, dried (Na_2SO_4), and concentrated. Column chromatography of the residue with $CHCl_3$ - MeOH - 28% aq NH_3 (12: 1: 0.1) gave a solid of **26**, 70 mg (91%), $[\alpha]_D^{20}$ +34° (*c* 0.5, $CHCl_3$); MS *m/z* 669 (M+H), 118 [$CH_2N(CH_2CH_2OH)_2$]; ^1H NMR ($CDCl_3$) δ 1.85 (s, 3H, 22-Me), 2.30 (s, 6H, NMe_2), 4.22 (d, 1H, H-1′), 4.78 (dt, 1H, H-15), 5.83 (d, 1H, H-13), 6.36 (d, 1H, H-10), 9.81 (s, 1H, H-20).

Anal Calcd for $C_{35}H_{60}N_2O_{10}$: C 62.85, H 9.04, N 4.19.
Found: C 63.02, H 9.11, N 3.99.

4′,23-Dideoxy-23-*N*-ethyl-23-(2-methoxyethylamino)mycaminosyl Tylonolide (**27**)

A solution of **3** (100 mg) and *N*-ethyl-2-methoxyethylamine (68 mg) in acetonitrile (1 ml) was heated at 80°C for 2 hours. Work-up in a usual manner and hydrolysis as described for **26** followed by column

chromatography ($CHCl_3$ - MeOH - 28% aq NH_3, 15: 1: 0.1) gave a solid of **27**, 78 mg (89%), MS *m/z* 666 (M, $C_{36}H_{62}N_2O_9$), 116 [$CH_2NEt(CH_2CH_2OMe)$]; ^1H NMR ($CDCl_3$) δ 3.33 (s, 3H, OMe), 4.75 (dt, 1H, H-15), 5.66 (d, 1H, H-13), 6.28 (d, 1H, H-10), 7.36 (d, 1H, H-11), 9.75 (s, 1H, H-20).

4′,23-Dideoxy-23-(2-methoxyethylamino)-23-*N*-propylmycaminosyl Tylonolide (**28**)

A solution of **3** (280 mg) and *N*-propyl-2-methoxyethylamine (224 mg) in acetonitrile (2.8 ml) was heated at 80°C for 6 hours. Work-up as described for **27** gave a solid of **28**, 187 mg (72%), MS *m/z* 130 [$CH_2NPr(CH_2CH_2OMe)$]; ^1H NMR ($CDCl_3$) δ 3.32 (s, 3H, OMe), 4.22 (d, 1H, H-1′), 4.73 (m, 1H, H-15), 5.77 (d, 1H, H-13), 6.26 (d, 1H, H-10), 7.36 (d, 1H, H-11), 9.74 (s, 1H, H-20).

4′,23-Dideoxy-23-ethylamino-23-*N*-[2-(*N*,*N*-dimethylamino)ethoxycarbonyl]mycaminosyl Tylonolide (**29**)

To an ice-cold mixture of **10** (30 mg) and sodium hydrogencarbonate (11 mg) in 60% aq MeOH (1.5 ml) was added 2-bromoethyl chloroformate (12.4 mg) and the mixture was stirred for 1.5 hours at 0°C. Addition of satd aq sodium hydrogencarbonate (1 ml) was followed by extraction with $CHCl_3$ (0.5 ml×3). The organic solution was washed with satd aq sodium sulfate, dried (Na_2SO_4), and concentrated. Column chromatography of the residue with $CHCl_3$ - MeOH - 28% aq NH_3 (40: 1: 0.1) gave the 23-*N*-(2-bromoethoxycarbonyl) derivative (26 mg) as a solid. A mixture of the solid and dimethylamine (190 mg; used as the acetonitrile solution) in acetonitrile (1 ml) was heated at 80°C for 30 minutes, followed by an additional same amount of dimethylamine, and further heating for 30 minutes. Evaporation, extraction of the residue with $CHCl_3$ (0.5 ml×3), washing the organic solution as usual, and concentration gave a solid of the dimethylamino derivative. A mixture of the solid in acetonitrile (0.52 ml) and 0.1 M aq HCl (1 ml) was kept at room temp for 30 minutes to cleave the formyl protecting groups. Neutralization with satd aq sodium hydrogencarbonate was followed by usual purification procedure gave crude **29**, that was further purified by column chromatography with $CHCl_3$ - MeOH - 28% aq NH_3 (30: 1: 0.1) to give a solid, 13.2 mg (43%), $[\alpha]_D^{22}$ +66° (*c* 0.5, $CHCl_3$); MS *m/z* 724 (M+H); ^1H NMR ($CDCl_3$) δ 1.80 (s, 3H, 22-Me), 2.30 (s, 12H, NMe_2 ×2), 4.83 (dt, 1H, H-15), 5.80 (d, 1H, H-13), 9.82 (s, 1H, H-20).

Anal Calcd for $C_{35}H_{65}N_3O_{10}\cdot H_2O$: C 61.53, H 9.04, N 5.67.
Found: C 61.16, H 8.57, N 5.55.

4′,23-Dideoxy-23-ethylamino-23-*N*-[3-(*N*,*N*-dimethylamino)propoxycarbonyl]mycaminosyl Tylonolide (**30**)

A mixture of **10** (99 mg), 3-bromopropyl chloroformate (44 mg) and sodium hydrogencarbonate (36 mg), in 60% aq MeOH (5 ml) was treated similarly as described for **29**. Successive processes were also carried out in a manner as described for **29** to give **30**, 26 mg (24%) and a by-product 14 mg: **30**: $[\alpha]_D^{22}$ +66° (*c* 1, $CHCl_3$); MS *m/z* 737 (M, $C_{39}H_{67}N_3O_{10}$); ^1H NMR ($CDCl_3$) δ 1.77 (s, 3H, 22-Me), 2.22 (s, 6H, $(CH_2)_3NMe_2$), 2.27 (s, 6H, 3′-NMe_2), 4.78 (dt, 1H, H-15), 5.75 (d, 1H, H-13), 6.30 (d, 1H, H-10), 9.73 (s, 1H, H-20). The by-product was possibly a derivative having the partial structure of NEt ($CH_2CH_2CH_2CONMe_2$) as confirmed by its ^1H NMR spectrum.

Anal Calcd for $C_{29}H_{67}N_3O_{10}\cdot 2H_2O$: C 60.54, H 9.18, N 5.43.
Found: C 60.75, H 8.43, N 5.36.

N-Ethyl-2-methoxyethylamine

To a mixture of 2-methoxyethylamine (1 g) and triethylamine (2 ml) in acetonitrile (5 ml) was added acetic anhydride (1.5 g), and the solution was kept at room temp for 30 minutes. Evaporation, extraction of the residue with $CHCl_3$ (15 ml), washing the organic solution with satd aq sodium chloride, satd aq sodium hydrogencarbonate and H_2O, dried over sodium sulfate, and evaporation gave a solid of *N*-(2-methoxyethyl)acetamide 1.6 g. A mixture of the solid and lithium aluminium hydride (780 mg) in tetrahydrofuran (12 ml) was refluxed for 2 hours. Addition of sodium sulfate 10 hydrate to decompose the excess lithium aluminium hydride was followed by filtration with aid of dichloromethane. The organic solution was extracted with aq HCl and to the aq acidic layer was added sodium hydroxide and the mixture was extracted with dichloromethane. Evaporation gave a liquid of the title amine, 48 mg (34%).

N-Propyl-2-methoxyethylamine

N-(2-Methoxyethyl)propionamide (1.25 g) was prepared by treating 2-methoxyethylamine (1 g) with propionic anhydride (1.7 ml). Reduction of the solid with lithium aluminium hydride (0.54 g) in oxolane (12 ml) as described above gave the title amine, 0.85 g (54%).

2-Bromoethyl Chloroformate

The compound was obtained by treating (0°C for 30 minutes, then at room temp for 1 hour) 2-bromoethanol (2 ml) with trichloromethyl chloroformate (2.5 ml) (ref[6] used phosgen), 1.5 g (90%), bp 175~176°C.

3-Bromopropyl Chloroformate

The compound was obtained by treating 3-bromopropanol (2 ml) with trichloromethyl chloroformate (2 ml) in a manner as described above, 1.7 g (92%), bp 110~112°C/4 mmHg.

References

1) TANAKA, A.; T. TSUCHIYA, Y. OKADA, S. UMEZAWA & H. UMEZAWA: Syntheses of 23-dialkylamino derivatives of mycaminosyl tylonolide and 4'-deoxymycaminosyl tylonolide effective against Gram-negative bacteria. J. Antibiotics 35: 113~116, 1982
2) TANAKA, A.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 4'-deoxymycaminosyl tylonolide. J. Antibiotics 34: 1374~1376, 1981
3) TANAKA, A.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Syntheses of derivatives of 4'-deoxymycaminosyl tylonolide and mycaminosyl tylonolide modified at C-23. J. Antibiotics 34: 1377~1380, 1981
4) KIRK, D. N. & M. A. WILLSON: Novel route to D-homoandrostane derivatives, including new methods for the preparation and reduction of hydroxy azide. J. Chem. Soc., Dalton Trans. 1970: 64~65, 1970
5) SAKAMOTO, S.; T. TSUCHIYA, A. TANAKA, S. UMEZAWA, M. HAMADA & H. UMEZAWA: Syntheses of 23-deoxy-23-*N*-ethyl-23-(2-fluoro-, 2,2-difluoro-, and 2,2,2-trifluoroethyl)amino derivatives of mycaminosyl tylonolide and 4'-deoxymycaminosyl tylonolide. J. Antibiotics 37: 1628~1634, 1984
6) HAEGEL, G.; H. FROEHLICH, D. BISCHOFF & K. HAMANN: Preparation and polymerization of carbamic acid vinyl esters. Makromol. Chem. 75: 98~111, 1964