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Note

Selective methylations of the 2′-hydroxy and C-2 positions of 3-deoxy-5-*O*-(4-deoxymycaminosyl)tylonolide

Shunji Kageyama [a], Tsutomu Tsuchiya [b,*]

[a] *Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba City, Ibaraki 305, Japan*
[b] *Institute of Bioorganic Chemistry, 1614 Ida. Nakahara-ku, Kawasaki 211, Japan*

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Recently we reported [1,2] the synthesis and antibacterial activity of 3-deoxy-5-*O*-(4-deoxymycaminosyl)tylonolide (**1**). This study demonstrated that removal of both the 3- and 4′-hydroxy groups of 5-*O*-mycaminosyltylonolide greatly enhances the activity. To prepare a more-active derivative than **1**, introduction of a methyl group at C-2 in the macrolactone ring of **1** has been undertaken. This modification was expected to give a derivative having longer duration in the blood through restriction of the approach of enzymes hydrolyzing the lactone ring (ring opening inactivates **1**) by the presence of the methyl group. However, conventional methylation was expected to cause simultaneous methylation of the 2′-hydroxy and 3′-dimethylamino groups. In relation to this synthesis, we were also interested in preparing the 2′-*O*-methyl derivative **6**, as **6** was expected to be a key probe for determining whether the 2′-esters of **1** are themselves active or not (that is, they show activity without or after hydrolysis). In erythromycin, a macrolide antibiotic similar to **1**, the 2′-esters were inactive until hydrolyzed [3,4], but in human plasma, they were hydrolyzed enzymatically to restore the activity, the hydrolytic state being inverse-proportionally related to the lipophilicity of the 2′-acyl groups attached. This character suggests that erythromycin 2′-esters might be useful as prodrugs. In our compound **1**, if the 2′-*O*-methyl derivative **6** has antibacterial activity (in vitro, for example) similar to that of **1**, the free 2′-hydroxyl would not be judged essential for activity and thus the 2′-*O*-acyl derivatives (**7–11**), especially the simple 2′-*O*-acyl derivative **7**, would predictably have antibacterial activity. However, a lack of activity in **6** would indicate the free 2′-OH group to be important, and that the 2′-*O*-acyl derivatives would show activity after hydrolysis.

* Corresponding author.

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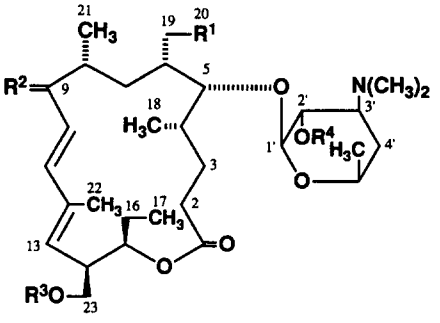
This paper describes the selective methylation of the 2′-hydroxy group of a 9,20,23-triprotected derivative (**4**) of **1** having a free 3′-dimethylamino group, as well as selective methylation at C-2 of **4** in the presence of 2′-hydroxy and 3′-dimethylamino groups. No purely 2′-*O*-methylated derivatives of macrolide antibiotics not having methylation of other hydroxy groups have ever been reported to the best of our knowledge. The synthesis and antibacterial activities of some 2′-*O*-acyl derivatives of **1** [acetyl (**7**), benzoyl (**8**), pentanoyl (**9**), 3-methylbutanoyl (**10**), and hexadecanoyl (**11**)] are also described. In these syntheses, the 2′-hydroxyl groups were selectively acetylated, without protection of the primary alcohol groups at C-23 [5].



	R^1	R^2	R^3	R^4
1	CHO	O	H	H
2	$CH(OCH_3)_2$	O	H	H
3	$CH(OCH_3)_2$	O	$Si(CH_3)_2(t\text{Bu})$	H
4	$CH(OCH_2)_2$	$(OCH_2)_2$	$Si(CH_3)_2(t\text{Bu})$	H
5	$CH(OCH_2)_2$	$(OCH_2)_2$	$Si(CH_3)_2(t\text{Bu})$	CH_3
6	CHO	O	H	CH_3
7	CHO	O	H	CH_3CO
8	CHO	O	H	C_6H_5CO
9	CHO	O	H	$CH_3(CH_2)_3CO$
10	CHO	O	H	$(CH_3)_2CHCH_2CO$
11	CHO	O	H	$CH_3(CH_2)_{14}CO$

The zigzag solid line for the skeleton of macrolactone ring lies on the same plane.

Synthesis of **6** is described first. 23-*O*-*tert*-Butyldimethylsily-3,4′-dideoxymycaminosyltylonolide dimethyl acetal (**3**), prepared from **1** through the dimethyl acetal **2**, was treated with trimethylsilyldiazomethane in acetonitrile, but no methylation occurred. A subsequent trial using CH_2N_2 in CH_2Cl_2 or CH_2N_2–$BF_3 \cdot Et_2O$ in CH_2Cl_2 also failed to give the desired derivative, giving only unexpected products (the macrolactone ring might be methylated randomly in the former case) or recovered starting material along with a small amount of quaternized product (for the latter case). 23-*O*-*tert*-Butyldimethylsilyl-3,4′-dideoxymycaminosyltylonolide 9,20-bis(ethylene acetal) [6] (**4**)

Table 1
Minimal inhibitory concentration (μg/mL) of **6–11, 13,** and **14** with **1**

Test organism [a]	**1**	**6**	**7**	**8**	**9**	**10**	**11**	**13**	**14**
Staphylococcus aureus FDA209P JC-1	0.1	3.13	0.2	0.05	0.1	0.2	0.78	0.05	0.39
S. epidermidis IID 866	0.1	3.13	0.2	0.1	0.1	0.2	1.56	0.05	0.39
Streptococcus pyogenes Cook	0.2	6.25	0.1	0.39	0.39	0.39	3.13	0.1	0.78
S. pneumoniae IID 552	0.1	3.13	0.1	0.2	0.1	0.1	0.78	0.05	0.39
Enterococcus faecalis IID 682	0.39	12.5	0.39	0.78	0.39	0.78	6.25	0.2	1.56
Corynebacterium diphteriae A-7	0.2	12.5	0.1	0.39	0.39	0.39	3.13	0.1	1.56
Branhamella catarrhalis CAY 1267	0.39	3.13	0.39	0.39	0.39	0.39	1.56	0.2	1.56
Escherichia coli 0-1	6.25	> 25	6.25	25	12.5	25	> 25	6.25	12.5
Citrobacter freundii NIH 10018-68	3.13	> 25	3.13	12.5	6.25	12.5	> 25	3.13	6.25
Shigella sonnei II 37148	6.25	> 25	6.25	25	12.5	25	> 25	6.25	12.5
Salmonella enteritidis 1891	3.13	> 25	3.13	6.25	3.13	3.13	> 25	1.56	3.13
Klebsiella pneumoniae ATCC 10031	1.56	25	1.56	1.56	0.78	1.56	25	0.78	1.56
Proteus vulgaris OX-19	6.25	> 25	6.25	25	6.25	25	> 25	12.5	12.5
Pseudomonas aeruginosa NCTC 10490	25	> 25	25	25	25	25	> 25	> 25	> 25

[a] Mueller–Hinton agar, inoculum size 10^6 cfu/mL, incubation 18 h at 37°C.

was next treated with CH_3I–NaH in DMF, but quaternization of the 3′-dimethylamino group occurred in preference to *O*-methylation. Treatment of **4** with Meerwein's salt [$(CH_3)_3O^+BF_4^-$] [7] in CH_2Cl_2 in the presence or absence of a proton sponge [8] also gave unknown products but quaternized ones. After many unsuccessful trials, however, we discovered that treatment of **4** with a limited amount of potassium bis(trimethylsilyl)amide ($KN[Si(CH_3)_3]_2$) in a molar ratio of 1:1:1.1 **4**: the amide: CH_3I in tetrahydrofuran successfully gave the 2′-*O*-methyl derivative **5** in good yield, accompanied with only small amounts of quaternized products. Deprotection of **5** gave the desired 2′-*O*-methyl derivative **6**. The ^1H and ^{13}C NMR spectra of **6** are shown in Tables 2 and 3. As shown in Table 1, **6** showed much lower antibacterial activity as compared to **1** or the 2′-*O*-acyl derivatives. This suggests that complete blocking of the 2′-OH group significantly decreases the activity, although it does not abolish the activity of **1** altogether. As an *O*-methyl group is sterically smaller than an *O*-acetyl group, its steric influence on the activity may be negligible. It is thus suggested that the 2′-*O*-acyl derivatives exhibit activity mainly after hydrolysis, although they are presumed to show weak intrinsic activity.

Synthesis of the 2-*C*-methyl derivative [6] of **1** is described next. Treatment of **4** with conventional basic reagents and CH_3I gave predominantly the quaternized products, along with the 2′-*O*-methyl derivative without formation of any of the desired compound. However, after many trials, we found that use of 10 and 3 molar equivalents (based on **4**) of lithium diisopropylamide (LDA, prepared by reacting equimolar amounts of diisopropylamine and BuLi in hexane) and CH_3I, respectively, in tetrahydrofuran provided a successful procedure. By this method, a mixture of two 2-*C*-methyl derivatives **12** was produced in good yield in the ratio of 2*R*:2*S* 2:1, as determined by ^1H NMR spectroscopy. The initially formed 2-*C*,2′-*O*-dilithio intermediate of **4** should react preferentially with CH_3I at C-2 because the C-2 carbanion is more reactive than

Table 2
^{1}H NMR [a] chemical shifts [b] of **6**, **13**, **14** and **1** in $CDCl_3$ at 27°C

Proton	6	13	14	1
H-2	1.84, 2.44	2.27	2.49	1.84, 2.44
CH_3-2	—	1.20	0.98	—
H-3	1.28, 1.41	1.13, 1.88	1.10, 1.53	1.26, 1.43
H-4	1.69	1.54	1.85	1.73
H-5	3.35	3.43	3.40	3.36
H-6	1.99	1.88	2.00	1.97
H-7	1.48, 1.68	1.48, 1.85	1.58, 1.58	1.52, 1.73
H-8	2.54	2.55	2.54	2.53
H-10	6.36	6.32	6.37	6.35
H-11	7.30	7.20	7.18	7.30
H-13	5.85	5.85	5.75	5.83
H-14	2.91	2.88	2.97	2.90
H-15	4.88	4.88	4.89	4.88
H-16	1.61, 1.81	1.61, 1.80	1.63, 1.83	1.61, 1.86
CH_3-17	0.94	0.93	0.93	0.94
CH_3-18	1.01	1.08	1.02	1.05
H-19	2.46, 2.48	2.42, 2.91	2.51, 3.01	2.45, 3.00
H-20	9.69	9.71	9.70	9.70
CH_3-21	1.26	1.20	1.23	1.21
CH_3-22	1.86	1.82	1.87	1.85
H-23	3.71, 3.74	3.67, 3.74	3.72, 3.72	3.73, 3.73
H-1′	4.21	4.14	4.23	4.19
H-2′	2.91	3.20	3.20	3.19
H-3′	2.60	2.47	2.46	2.49
H-4′	1.23, 1.69	1.21, 1.64	1.23, 1.63	1.23, 1.60
H-5′	3.35	3.44	3.42	3.43
CH_3-6′	1.15	1.20	1.20	1.20
OCH_3-2′	3.56	—	—	—
$N(CH_3)_2$-3′	2.35	2.27	2.26	2.27

[a] Measured at 500 MHz with a JEOL Alpha 500.
[b] In ppm downfield from Me_4Si. The shifts were confirmed by the ^{1}H–^{1}H correlated 2D spectra with aid of, in some cases, the HOHAHA method.

the oxyanion. However, the reaction mechanism seems rather complex. Use of 1.1–3 molar equivalents LDA gave **5** in low yields, together with **4**, but using more than 4 molar equivalents use of LDA gave **12** together with **4** without formation of **5** (see Experimental section). Repeat experiments gave similar results. Surprisingly, with all proportions of LDA–CH_3I examined, no 2-*C*,2′-*O*-dimethyl derivative was produced (monitored by TLC and ^{1}H NMR spectra). Deblocking followed by chromatography gave the 2*R* (**13**) and 2*S* (**14**) isomers in 60–70% total yield (based on **4**).

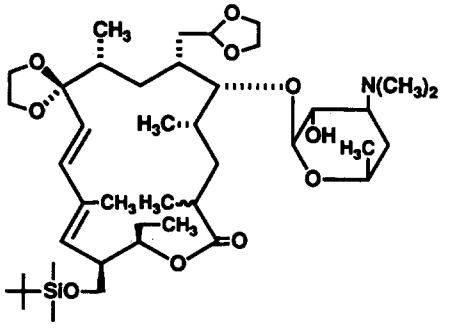
Another method to increase the yield of **13** was sought, and was accomplished by use of the 2′-*O*-silyl derivative **15**. Treatment of **15** with CH_3I in the presence of excess lithium bis(trimethylsilyl)amide [$LiN[Si(CH_3)_3]_2$] in tetrahydrofuran gave the 2*R* isomer **16** in strong preference to the 2*S* isomer. Deprotection gave **13** identical with the product obtained by the former method. The ^{1}H and ^{13}C NMR spectra of **13** and **14** are shown in Tables 2 and 3.

Table 3
^{13}C NMR [a] chemical shifts [b] of **6**, **13**, **14** and **1** in $CDCl_3$ at 27°C

Carbon	**6**	**13**	**14**	**1**
1	173.4	176.7	175.4	173.4
2	32.3	38.2	36.6	32.3
CH_3-2	—	20.4	13.1	—
3	27.9	38.8	35.2	27.7
4	36.2	37.0	32.8	36.1
5	83.0	83.6	84.5	83.6
6	31.5	35.3	31.1	31.3
7	31.6	33.0	31.2	31.8
8	44.7	44.4	44.2	44.9
9	203.5	204.3	204.2	203.7
10	118.8	120.4	120.3	118.5
11	147.8	147.3	147.4	147.9
12	136.5	136.2	137.0	136.4
13	141.3	140.6	139.1	141.4
14	47.2	46.9	46.3	47.1
15	74.2	74.2	73.7	74.2
16	25.8	25.7	25.9	25.6
17	9.6	9.5	9.1	9.5
18	15.2	15.7	14.2	15.2
19	43.9	44.8	42.8	43.5
20	202.3	202.6	202.5	202.4
21	17.6	17.4	17.3	17.5
22	13.3	12.9	13.6	13.2
23	62.7	62.8	63.0	62.6
1′	104.8	104.3	104.7	104.2
2′	80.8	70.4	70.3	70.3
3′	63.8	65.6	65.6	65.6
4′	32.1	28.5	28.3	28.4
5′	66.8	69.6	69.6	69.5
6′	20.9	21.1	21.2	21.0
OCH_3-2′	59.9	—	—	—
N-$(CH_3)_2$	41.0	40.3	40.2	40.2

[a] Measured at 125 MHz with a J Alpha 500 instrument.
[b] In ppm downfield from Me_4Si. The shifts were confirmed by the ^1H–^{13}C corrected 2D spectra with aid of, in some cases, the HMBC method.

The configurations at C-2 of **13** and **14** were determined by the ROESY method; in the spectrum, **14** showed cross peaks between 2-H and 18-CH_3, and between 2-CH_3 and 4-H. As C-4 of **1** is known to have the *S* configuration, the 2*S*,4*S* structure agrees with the foregoing NOE result. If **14** would have the 2*R*,4*S* structure, it would be sterically impossible to bring both 2- and 4-methyl groups close to H-4 and H-2, respectively. The configuration at C-2 of **14** was thus determined *S* and that for **13** to be *R*. The observation of NOE only between 2-H and 18-CH_3 in **13** also supported this conclusion. Circular dichroism measurements attempted for **13** and **14** showed no reliable mode of differentiation.



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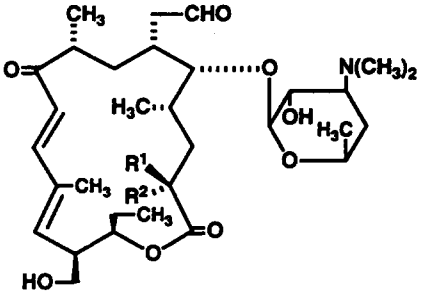
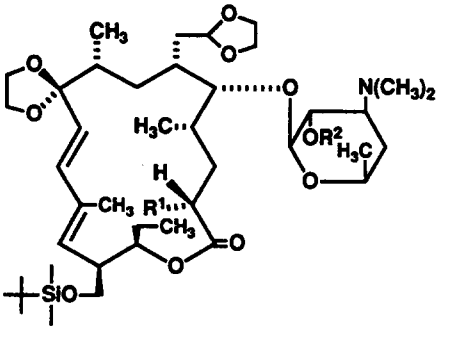


	R^1	R^2
13	H	CH_3
14	CH_3	H



	R^1	R^2
15	H	$Si(CH_3)_3$
16	CH_3	$Si(CH_3)_3$

As reported [6], **13** showed enhanced antibacterial activity as compared to **1**, but **14** showed much lower activity. Interestingly, the C-2 configuration of **13** is coincident with that of erythromycin.

In summary, we have successfully prepared methylated products at C-2 (next to the lactone link) and 2′-OH of **1**, respectively, by choosing appropriate reagents and reaction conditions.

1. Experimental

General methods.—Optical rotations were determined with a Horiba SE PA-200 or Perkin–Elmer 241 polarimeter. ^1H NMR spectra were recorded with Bruker WM 250 (250 MHz), J EX 400 (400 MHz), and J Alpha 500 (500 MHz) spectrometers, the

chemical shifts (δ) being measured downfield from internal Me_4Si. Mass spectra (MS) were measured by the fast-atom bombardment method with a JMS-DX300 (HF) mass spectrometer unless otherwise stated. EI mass spectra (EI-MS) were recorded with a Hitachi M-80 spectrometer. For high-resolution fast-atom bombardment measurements (HRFAB-MS), a VG ZAB-VSE mass spectrometer (in the presence of thioglycerol–glycerol–Me_2SO was used. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck) and column chromatography, on Kieselgel 60, 230 ≈ 400 mesh (Merck).

3-Deoxy-5-O-(4-deoxymycaminosyl)tylonolide dimethyl acetal (**2**).—A mixture of **1** (300 mg, 0.53 mmol) and *p*-toluenesulfonic acid monohydrate (150 mg) in dry MeOH (5 mL) was kept for 1 h at room temperature, poured into aq $NaHCO_3$ (saturated, 15 mL), and the resulting precipitate was collected and chromatographed with $CHCl_3$ → 10:1:0.1 $CHCl_3$–MeOH–28% aq NH_3 to give **2** as a solid, 306 mg (94%), $[\alpha]_D^{24}$ + 8° (*c* 2, $CHCl_3$); EI-MS *m/z* 611 (M^+), ^1H NMR ($CDCl_3$) δ 2.27 [s, 6 H, $N(CH_3)_2$], 3.22 and 3.31 (each s, 3 H, 2 OCH_3). Anal. Calcd for $C_{33}H_{57}NO_9 \cdot 1/2H_2O$: C, 63.84; H, 9.42; N, 2.25. Found: C, 63.95; H, 9.26; N, 2.20.

23-O-tert-Butyldimethylsily-3-deoxy-5-O-(4-deoxymycaminosyl)tylonolide dimethyl acetal (**3**).—A mixture of **2** (1.00 g, 1.64 mmol), *tert*-butylchlorodimethylsilane (296 mg, 1.96 mmol) and imidazole (167 mg) in dry DMF (10 mL) was kept overnight at room temperature. Toluene (80 mL) was added and the organic solution was washed with aq NaCl (saturated) and aq $NaHCO_3$ (saturated), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography with 15:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3 to give **3** as a solid, 1.18 g (99%), $[\alpha]_D^{20}$ + 2° (*c* 1, $CHCH_3$); MS *m/z* 726 $(M + 1)^+$, ^1H NMR ($CDCl_3$) δ 0.03 and 0.04 [each s, 3 H, $Si(CH_3)_2$], 0.88 (s, 9 H, Si*t*Bu), 1.81 (s, 3 H, CH_3-22), 2.27 (s, 6 H, $(CH_3)_2$N-3′), 3.22 and 3.32 (each s, 3 H, 2 OCH_3), 3.68 (m, 2 H, 2 H-23), 4.26 (d, 1 H, $J_{1'2'}$ 7.3 Hz, H-1′), 5.83 (d,1 H, H-13), 6.33 (d,1 H, H-10), and 7.28 (d, 1 H, H-11). Anal. Calcd for $C_{39}H_{71}NO_9Si$: C, 64.51; H, 9.86; N, 1.93. Found: C, 64.25; H, 9.91; N, 1.92.

23-O-tert-Butyldimethylsilyl-3-deoxy-5-O-(4-deoxy-2-O-methylmycaminosyl)tylonolide 9.20-bis(ethylene acetal) (**5**).—To a cooled (5 ~ 10°C) solution of **4** [6] (250 mg, 0.33 mmol) in tetrahydrofuran (2.5 mL) was added 0.5 M $KN[Si(CH_3)_3]_2$ in toluene (0.65 mL, 0.33 mmol as the amide) and the solution was kept for 1 h at the same temperature. After cooling to 0°C, MeI (22 μL,0.35 mmol) was added and the mixture was stirred for 1 h. The resulting suspended mixture was poured into a cold aq NH_4Cl (saturated,20 mL) and the products were extracted with $CHCl_3$. The organic solution was washed with water, dried ($MgSO_4$), and concentrated to give a residue, which was chromatographed with 30:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3 to give **5** as a solid, 157 mg (62%), along with **4** (39 mg) recovered, TLC of **5** (15:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) R_f 0.43 (*cf* **4**: R_f 0.33), $[\alpha]_D^{20}$ −36° (*c* 1, $CHCl_3$); MS *m/z* 782 $(M + 1)^+$ and 172 (sugar portion, $C_9H_{18}NO_2$), ^1H NMR ($CDCl_3$) δ 0.03 (s, 6 H, $Si(CH_3)_2$), 0.88 (s, 9 H, Si*t*Bu), 0.96 (d, 3 H, CH_3-18), 1.02 (d, 3 H, CH_3-21), 1.20 (d, 3 H, CH_3-6′), 1.74 (s, 3 H, CH_3-22), 2.35 (s, 6 H, $(CH_3)_2$N-3′), 3.55 (s, 3 H, CH_3O-2′), 4.28 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.99 (br s, 1 H, H-20), 5.38 (d, 1 H, $J_{13,14}$ 10.7 Hz, H-13), 5.62 (d, 1 H, $J_{10,11}$ 15.6 Hz, H-10), and 6.39 (d, 1 H, H-11). Anal. Calcd for $C_{42}H_{75}NO_{10}Si \cdot 1/2H_2O$: C, 63.76; H, 9.68; N, 1.77. Found: C, 64.10; H, 9.70; N, 1.77.

3-Deoxy-5-O-(4-deoxy-2-O-methylmycaminosyl)tylonolide (**6**).—Compound **5** (96 mg) in a mixture of tetrahydrofuran (1 mL) and aq 0.5 M HCl (1 mL) was kept for 2 h at room temperature. Neutralization with aq $NaHCO_3$ followed by concentration gave a residue, which was extracted with chloroform. Purification by chromatography (15:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) of the product gave **6** as a solid, 61 mg (86%), $[\alpha]_D^{20}$ −18° (*c* 1, $CHCl_3$); MS *m/z* 580 $(M+1)^+$ and 172 (sugar portion, $C_9H_{18}NO_2$). Anal. Calcd for $C_{32}H_{53}NO_8 \cdot 1/2H_2O$: C, 65.27; H, 9.24; N, 2.38. Found: C, 65.09; H, 9.27; N,2.34

General procedure to prepare 2′-O-acyl derivatives (**7-11**) *of* **1**.—To a solution of **1** (0.4 mmol) in MeCN (2.5 mL) was added the acid anhydride (0.5 mmol of Ac_2O, $(C_6H_5CO)_2O$, $(CH_3(CH_2)_3CO)_2O$, $[(CH_3)_2CHCH_2CO_2]_2O$, or hexadecanoic anhydride) and the solution was kept overnight at room temperature (for **11**, the mixture was stirred overnight at 50°C). The resulting solution showed, on TLC (10:1:0.1 $CHCl_3$–MeOH–28% aq NH_3), a single spot of product (cf. **1**: R_f 0.35), respectively. Toluene (30 mL) was added and the solution was poured into a mixture of aq $NaHCO_3$ (saturated, 15 mL)–aq NaCl (saturated, 15 mL). The organic layer separated was washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography with $CHCl_3$–MeOH (10:1 for **7** and 15:1 for the other products) to give a solid of the various 2′-*O*-acyl derivatives.

Compound **2**: yield 92%, $[\alpha]_D^{20}$ −1° (*c* 1, $CHCl_3$); MS *m/z* 608 $(M+1)^+$ and 200 (sugar portion, $C_{10}H_{18}NO_3$), ^1H NMR ($CDCl_3$) δ 1.85 (s, 3 H, CH_3-22), 2.06 (s, 3 H, Ac-2′), 2.26 (s, 6 H, $(CH_3)_2$N-3′), 4.26 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.75 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2′), 5.80 (d, 1 H, $J_{13,14}$ 10.5 Hz, H-13), 6.35 (d, 1 H, $J_{10,11}$ 16 Hz, H-10), 7.28 (d, 1 H, H-11), and 9.68 (s, 1 H, H-20). Anal. Calcd for $C_{33}H_{53}NO_9 \cdot H_2O$: C, 63.33; H, 8.86; N, 2.24. Found: C, 63.40; H, 8.47; N 2.12.

Compound **8**: yield 95%, $[\alpha]_D^{20}$ +6° (*c* 1, $CHCl_3$); MS *m/z* 670 $(M+1)^+$ and 262 (sugar portion, $C_{15}H_{20}NO_3$), ^1H NMR ($CDCl_3$) δ 1.78 (s, 3 H, CH_3-22), 2.26 (s, 6 H, $(CH_3)_2$N-3′), 4.40 (d, 1 H, H-1′), 5.03 (dd, 1 H, H-2′), 5.77 (d, 1 H, H-13), 6.29 (d, 1 H, H-10), 7.25 (d, 1 H, H-11), 7.44, 7.56, and 8.05 (2 H, 1 H, and 2 H short-range m, respectively, C_6H_5CO), and 9.69 (s, 1 H, H-20). Anal. Calcd for $C_{38}H_{55}NO_9$: C, 68.14; H, 8.28; N 2.09. Found: C, 67.96; H, 8.46; N 1.95.

Compound **9**: yield 85%, $[\alpha]_D^{20}$ −2° (*c* 1, $CHCl_3$); MS *m/z* 650 $(M+1)^+$ and 242 (sugar portion, $C_{13}H_{24}NO_3$), ^1H NMR ($CDCl_3$) δ 0.93 (each t, 3 H × 2, CH_3-17 and $CH_3(CH_2)_3CO_2$-2′), 1.85 (3 H, CH_3-22), 2.24 (s, 6 H, $(CH_3)_2$N-3′), 4.25 (1 H, H-1′), 4.75 (1 H, H-2′), 5.81 (1 H, H-13), 6.34 (1 H, H-10), 7.29 (1 H, H-11), and 9.67 (1 H, H-20). Anal. Calcd for $C_{36}H_{59}NO_9 \cdot 1/2H_2O$: C, 65.62; H, 9.18; N, 2.13. Found: C, 65.52; H, 9.05; N, 2.06.

Compound **10**: yield 87%, $[\alpha]_D^{20}$ −1° (*c* 1, $CHCl_3$), MS *m/z* 650 $(M+1)^+$ and 242 (sugar portion, $C_{13}H_{24}NO_3$), ^1H NMR ($CDCl_3$) δ 0.92 and 0.94 (each t, 3 H, $COCH_2CH(CH_3)_2$), 1.85 (CH_3-22), 2.24 (6 H, $(CH_3)_2$N-3′), 4.26 (H-1′), 4.76 (H-2′), 5.81 (H-13), 6.33 (H-10), 7.29 (H-11), and 9.67 (H-20). Anal. Calcd for $C_{36}H_{59}NO_9 \cdot 1/2H_2O$: C, 65.62; H, 9.18; N, 2.13. Found: C, 65.78; H, 9.16; N,2.04.

Compound **11**: yield 97%, TLC, R_f (compound 1) 1.15, $[\alpha]_D^{20}$ +2° (*c* 1, $CHCl_3$); MS *m/z* 804 $(M+1)^+$ and 396 (sugar portion, $C_{24}H_{46}NO_3$), HRFAB-MS *m/z* 804.6037 $(M+H)^+$, Calcd for $C_{47}H_{82}NO_9$: 804.5990; ^1H NMR ($CDCl_3$) δ 1.84

(CH_3-22), 2.33 (s, 6 H, $(CH_3)_2$N-3′), 4.27 (H-1′), 4.82 (dd, 1 H, H-2′), 4.83 (H-13), 6.33 (H-10), 7.29 (H-11), and 9.67 (H-20).

23-O-tert-*Butyldimethylsilyl-3-deoxy-5*-O-*(4-deoxymycaminosyl)-2*-C-*methyltylonolide 9.20-bis(ethylene acetal)* (**12**).—To a cold (−70°C) solution of diisopropylamine (275 μL, 1.96 mmol) in dry tetrahydrofuran (1.5 mL) was added 1.6 M BuLi in hexane (1.22 mL, 1.95 mmol) under argon, and after stirring for 30 min, **4** (150 mg, 0.196 mmol) in dry tetrahydrofuran (1.5 mL) was gradually added. The mixture was warmed to −20°C, MeI (28 μL, 0.45 mmol) and HMPA (44 μL) [9] were added, and stirring was continued for 2 h at 0°C. Another MeI (10 μL) was added, and the mixture was stirred for further 1 h. TLC (15:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) of the solution showed three spots at R_f 0.27 (minor), 0.30 (minor, **4**), and 0.33 (major, **12**). Aqueous NH_4Cl (saturated, 20 mL) and aq NaCl (saturated, 10 mL) were added, and the organic layer isolated was dried ($MgSO_4$) and concentrated. The residue was chromatographed (15:1:0.1 $CHCl_3$–MeOH–aq 28% NH_3) to give **12** as a solid, 128 mg (84%).

Preliminary reactions of **4** *with MeI in the presence of LDA.*—Approximately 150 mg of **4** was added to a cold (−70°C) LDA solution prepared by changing the amounts of equimolar diisopropylamine and BuLi, then HMPA (45 μL) and MeI were added. After stirring for 2 h at 0°C the mixture was treated as before. The procedure was carried out similarly as described for **12**. The crude solid obtained was subjected to TLC and the ^{1}H NMR and MS (if necessary) spectra were measured. The results were as follows [shown in the order of the amounts of LDA–MeI (molar equivalents for **4**) and the yields (%) of the products or, in some cases, the ratio of products determined by the ^{1}H NMR spectra (shown in brackets)]: 1.1–2.0, **4** (54) and **5** (7); 3.0–2.0 [**4:5** 3:1]; 4.0–3.0 [**4:5:12** 1.6:0:1; **12** (2*R*): **12** (2*S*) 1.3:1]; 5.0–2.0, **12** (83) [**4:5:12** 1:0:1.3; **12** (2*R*): **12** (2*S*) 1.3:1]; 10–15, **12** (84) [**12** (2*R*): **12** (2*S*) 3:2].

*(2*R *And 2*S*)-3-deoxy-5*-O-*(4-deoxymycaminosyl)-2*-C-*methyltylonolide* (**13** *and* **14**). —A solution of **12** (120 mg) in a mixture of 2:1 aq 1 M HCl–tetrahydrofuran (1.8 mL) was kept for 2 h at 40°C. After neutralization with aq 0.12 M K_2CO_3 (10 mL), the mixture was extracted with $CHCl_3$. The crude products obtained were chromatographed ($CHCl_3$ → 20:1:0.5 $CHCl_3$–MeOH–aq 28% NH_3) to give **13** as a solid, 22.5 mg (25%), **14** as a solid, 16.5 mg (19%), and a mixture of **13** and **14** (31.5 mg).

Compound **13**: TLC, R_f 0.2 (10:1:0.5 $CHCl_3$–MeOH–aq 28% NH_3), $[\alpha]_D^{20}$ + 2° (*c* 1, $CHCl_3$); MS *m*/*z* 580 $(M + 1)^+$. Anal. Calcd for $C_{32}H_{53}NO_8$: C, 66.29; H, 9.21; N, 2.42. Found; C, 66.16; H, 9.42; N, 2.33.

Compound **14**: TLC, R_f 0.25 (10:1:0.5 $CHCl_3$–MeOH–aq 28% NH_3), $[\alpha]_D^{20}$ + 15° (*c* 1, $CHCl_3$); MS *m*/*z* 580 $(M + 1)^+$. Anal. Calcd for $C_{32}H_{53}NO_8 \cdot 1/2H_2O$: C, 65.27; H, 9.24; N, 2.38. Found: C, 65.43; H, 9.26; N, 2.33.

23-O-tert-*Butyldimethylsilyl-3-deoxy-5*-O-*(4-deoxy-2*-O-*trimethylsilylmycaminosyl)-tylonolie 9.20-bis(ethylene acetal)* (**15**).—A mixture of **4** [6] (2.00 g, 2.61 mmol), chloro trimethylsilane (0.60 mL, 4.7 mmol), and pyridine (1.05 mL) in CH_2Cl_2 (20 mL) was kept overnight at room temperature. After addition of CH_2Cl_2 (20 mL) and ice-cold water (10 mL), it was neutralized with aq $NaHCO_3$ (saturated). The organic solution isolated was dried ($MgSO_4$) and concentrated. The residue was chromatographed (7:3 hexane–acetone) to give **15** as a solid, 1.94 g (89%), $[\alpha]_D^{20}$ −38° (*c* 1, $CHCl_3$), TLC (7:3 hexane–acetone) R_f 0.45 (cf. **4**, R_f 0.15); MS *m*/*z* 840 $(M + 1)^+$ and 230 (sugar

portion, $C_{11}H_{24}NO_2Si$), ^1H NMR ($CDCl_3$) δ 0.02 and 0.03 (each s, 3 H, tBu(CH_3)$_2$SiO-23), 0.11 (s, 9 H, (CH_3)$_3$SiO-2′), 0.88 (s, 9 H, *tBu*(CH_3)$_2$SiO-23), 1.75 (s, 3 H, CH_3-22), 2.23 (s, 6 H, (CH_3)$_2$N-3′), 3.24 (dd, 1 H, H-2′), 4.24 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 5.36 (H-13), 6.34 (H-10), and 7.28 (H-11). Anal. Calcd for $C_{44}H_{81}NO_{10}Si_2$: C, 62.89; H, 9.72; N, 1.67. Found: C, 62.72; H, 9.82; N, 1.67.

*(2R)-23-*O-tert-*Butyldimethylsilyl-3-deoxy-5-*O-*(4-deoxy-2-*O-*trimethylsilylmycaminosyl)-2-*C-*methyltylonolide 9.20-bis(ethylene acetal)* (**16**).—To a cold (−50°C) solution of **15** (300 mg, 0.36 mmol) in tetrahydrofuran (3 mL) was added 1.0 M lithium bis(trimethylsilyl)amide (3.6 mmol) in tetrahydrofuran (3.6 mL) under argon, and the solution was kept for 1 h at −10°C. After cooling to −20°C, MeI (34 μL, 0.55 mmol) was added and the solution was kept for 2 h at 0°C. TLC (7:3 hexane–acetone) showed three spots at R_f 0.37, 0.45 (**15**), and 0.52 (**16**). After addition of toluene (20 mL) the solution was poured into a mixture of aq NH_4Cl (saturated, 10 mL)–aq NaCl (saturated, 10 mL) and the organic solution separated was washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (9:2 hexane–acetone) to give **16** as a solid, 165 mg (54%). A mixture (122 mg) of **15** and a by-product (R_f 0.37) was also obtained.

Compound **16**: $[\alpha]_D^{20}$ −42° (c 1, $CHCl_3$): MS m/z 854 $(M+1)^+$, ^1H NMR ($CDCl_3$) δ 0.01 (s, 6 H, tBu(CH_3)$_2$SiO-23), 0.03 (s, 9 H, (CH_3)$_3$SiO-2′), 0.87 (s, 9 H, *tBu*(CH_3)$_2$SiO-23), 1.15 and 1.19 (each d, 3 H, CH_3-2 and -6′), 1.71 (s, 3 H, CH_3-22), 2.22 (s, 6 H, (CH_3)$_2$N-3′), 4.19 (d, 1 H, $J_{1',2'}$ 6.7 Hz, H-1′), 5.47 (H-13), 5.54 (H-10), and 6.36 (d, 1 H, H-11). Anal. Calcd for $C_{45}H_{83}NO_{10}Si_2 \cdot 1/2H_2O$: C, 62.60; H, 9.80; N, 1.62. Found: C, 62.52; H, 9.65; N, 1.51.

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References

[1] S. Kageyama, T. Tsuchiya, S. Umezawa, and T. Takeuchi, *J. Antibiotics*, 45 (1992) 144–146.
[2] S. Kageyama T. Tsuchiya, S. Umezawa, and M. Orita, *Bull. Chem. Soc. Jpn.*, 65 (1992) 3405–3411.
[3] P.L. Tardrew, J.C.H. Mao, and D. Kenney, *Appl. Microbiol.*, 18 (1969) 159–165.
[4] J. Taskinen and P. Ottoila, *J. Antimicrob. Chemother.*, 21 (1988) Suppl. D, 1–8.
[5] H. Sakakibara and S. Omura, in *Macrolide Antibiotics*, Academic Press, Orlando, 1984, pp 85–125.
[6] S. Kageyama and T. Tsuchiya, *J. Antibiotics*, 47 (1994) 955–956.
[7] H. Meerwein, *Org. Synth.*, 46 (1966) 120–121.
[8] R.W. Alder, P.S. Bowman, W.R.S. Steele, and D.R. Winterman, *Chem. Commun.*, (1968) 723–724; M.J. Diem, D.F. Burow, and J.L. Fry, *J. Org. Chem.*, 42 (1977) 1801–1802.
[9] H.O. House, in *Modern Synthetic Reactions*, W.A. Benjamin, New York, 1972, pp 492–628.