CHEMICAL MODIFICATION OF ERYTHROMYCINS

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF *O*-ALKYL DERIVATIVES OF ERYTHROMYCIN A

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A series of *O*-alkyl erythromycin A derivatives have been synthesized and their antibacterial activities compared with those of erythromycin A (**1**) and 6-*O*-methylerythromycin A (**3**).

Methylation of the hydroxyl groups of erythromycin A analogue proceeded stepwise by the two main pathways beginning at the C-6 and C-11 positions, individually. *O*-Alkylation, other than methylation, took place at the C-11 hydroxyl group exclusively.

Among *O*-alkyl derivatives, 6,12-di-*O*-methylerythromycin A (**5**) showed comparable *in vitro* antibacterial activity to those of **1** and **3**. 11-*O*-Methylerythromycin A (**8**) was slightly less active than **1**. *O*-Methylation at the C-4″ position resulted in a decrease of antibacterial activity.

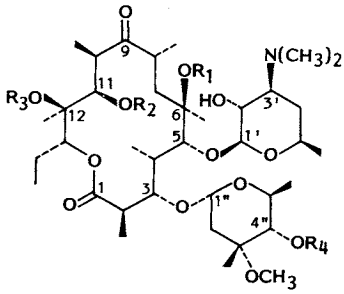
A great number of derivatives of the natural macrolide antibiotic erythromycin A (**1**) have been synthesized and the structure-activity relationship described.[1] 6-*O*-Methylerythromycin A (**3**; clarithromycin) and 6,11-di-*O*-methylerythromycin A (**4**), both possessing higher acid-stability compared to **1**, exhibit excellent *in vitro* and *in vivo* antibacterial activities against Gram-positive bacteria and *Mycoplasma pneumoniae*.[2] These results attracted us to study other *O*-alkyl derivatives. In this paper we describe the synthesis and antibacterial activity of several *O*-alkylerythromycin derivatives, other than **3** and **4**.

Results and Discussion

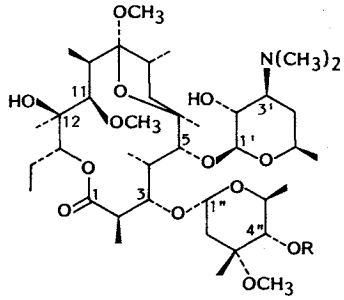
Chemistry

In the development of synthetic routes for **3**, *O*-alkylation of 2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-*N*-demethylerythromycin A (**2**)[3] has been investigated. *O*-Methylation of **2** with methyl iodide and 1 equiv of potassium hydroxide (KOH) in DMSO-1,2-dimethoxyethane gave 6-*O*-methyl derivative (**3z**, 39%) and 11-*O*-methyl derivative (**8z**, 51%). Compound **8z** was also obtained by the *O*-methylation of **2** using sodium hydride (NaH, 1.2 equiv) in DMF in 71% yield. According to the usual deprotection and reductive *N*-methylation sequence,[3] **3z** and **8z** were converted into the free bases **3** and **8**, respectively. HPLC analysis indicated that the crude **3z**, prepared using 1.2 equiv of base, contained another methylated product other than **3z** and 6,11-di-*O*-methyl derivative **4z**. After deprotection and subsequent *N*-methylation, the corresponding unknown compound was isolated and identified as 6,12-di-*O*-methylerythromycin A (**5**). Further methylation of **3z** using 2.3 equiv of KOH gave **4z** and 6,11,4″-tri-*O*-methyl derivative **6z**.

O-Methylation of **2** with excess of methyl iodide and NaH (6 equiv) gave 6,11,12,4″-tetra-*O*-methyl (**7z**), 11,12,4″-tri-*O*-methyl (**10z**), and 9,11,4″-tri-*O*-methyl derivative (**12z**). A reaction of **2** with dimethyl sulfate and NaH (2.1 equiv) in DMF gave **8z** and 11,4″-di-*O*-methyl derivative (**9z**). When methylated using



1 $R_1=R_2=R_3=R_4=H$
3 $R_1=CH_3$ $R_2=R_3=R_4=H$
4 $R_1=R_2=CH_3$ $R_3=R_4=H$
5 $R_1=R_3=CH_3$ $R_2=R_4=H$
6 $R_1=R_2=R_4=CH_3$ $R_3=H$
7 $R_1=R_2=R_3=R_4=CH_3$
8 $R_1=R_3=R_4=H$ $R_2=CH_3$
9 $R_1=R_3=H$ $R_2=R_4=CH_3$
10 $R_1=H$ $R_2=R_3=R_4=CH_3$
13 $R_1=R_3=R_4=H$ $R_2=C_2H_5$
14 $R_1=R_3=R_4=H$ $R_2=C_3H_7$



11 R=H
12 $R=CH_3$

(**3z**~**12z**: 2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl derivative of **3**~**12**)

KOH (2.2 equiv), **8z** provided **4z**, 9,11-di-*O*-methyl derivative (**11z**), and **12z**. The derivatives **4z**, **6z**, **7z**, and **9z**~**12z** were converted into the corresponding dimethylamino compounds **4**, **6**, **7**, and **9**~**12**, respectively. 11-*O*-Ethyl- and 11-*O*-propylerythromycin A (**13** and **14**) were prepared by selective 11-*O*-alkylation of **1** with the corresponding alkyl halide and NaH. As mentioned above, *O*-methylation of bis-protected **2** takes place at first at the C-6 and C-11 positions individually, and then proceeds stepwise by the following two main pathways: (A) C-11→C-11, C-9 (and C-11, C-4″)→C-9, C-11, C-4″; (B) C-6→C-6, C-11→C-6, C-11, C-4″→C-6, C-11, C-12, C-4″. The higher reactivity of the C-6 and C-11 hydroxyl groups for *O*-alkylation may be associated with the structure of **2**, having the steric neighboring oxygens at C-1, C-6, C-9, and C-11, which is deduced from X-ray analysis of **1**.[4] The details of reaction selectivity of the C-6 and C-11 hydroxyl groups will be described elsewhere.

The preliminary elucidation of the structures of **3** and **4** was previously reported.[2]

The structure of other *O*-alkyl erythromycin A derivatives has been well elucidated on the basis of ^{13}C NMR spectra as shown in Table 1. The ^{13}C NMR spectrum of tetra-*O*-methyl **7** showed methoxy signals derived from the tertiary C-6 and C-12 hydroxyl groups at 50.4 and 52.9 ppm, and those from the secondary C-11 and C-4″ hydroxyl groups at 60.6 and 62.1 ppm, respectively.

The chemical shifts of methoxy groups in other *O*-methyl compounds were similar to the corresponding those in **7**. In addition, the signal at about 48 ppm in **11** and **12** was assigned to the 9-OCH_3 group derived from the C-6/C-9 hemiacetal. As shown in Table 1, the following downfield shifts of α-carbon, compared to those of **1**, were caused by *O*-methylation: C-6 (δ_Δ 3.7~4.5), C-9 (δ_Δ 1.7~1.9), C-11 (δ_Δ 9.6~11.6), C-12 (δ_Δ 4.5~6.1), and C-4″ (δ_Δ 10.5~11.1).

The ^1H NMR spectrum of **8** showed several pairs of signals attributed to 11-OCH_3 (δ 3.48 and 3.37), 3″-OCH_3 (δ 3.35 and 3.30), and 3′-$N(CH_3)_2$ (δ 2.32 and 2.30), indicating that **8** existed as a mixture of C-9 keto and C-6/C-9 hemiacetal tautomers[5] with a ratio of *ca.* 1 : 1 in $CDCl_3$. Crystallization of **8** from acetone gave the C-9 keto tautomer whose structure has been confirmed by solid state ^{13}C NMR spectroscopy (unpublished data).

The ^1H and ^{13}C NMR spectra of **9**, **13**, and **14** in $CDCl_3$ showed the pattern similar to those of **8**, indicating the ratio of C-9 keto to C-6/C-9 hemiacetal to be *ca.* 1 : 1. Further, the mass spectra (FAB-MS or SI-MS) of *O*-alkyl erythromycin A derivatives exhibited the characteristic protonated molecular ion

Table 1. ^{13}C NMR chemical shifts for representative *O*-alkyl derivatives.

Carbon	Chemical shift (δ, ppm)[a]								
	1	**3**	**4**	**5**	**6**	**7**	**10**	**11**	**12**
1	175.8	175.9	175.6	175.8	175.8	175.1	176.9	177.2	177.2
2	44.8	45.1	44.9	45.0	44.8	44.8	44.7	47.7	48.0
3	79.9	78.5	78.6	78.4	78.7	78.8	81.0	80.8	80.9
4	39.3	39.3	38.2	39.0	38.2	38.4	43.6	41.4	41.2
5	83.5	80.8	79.8	80.5	79.2	79.1	88.7	89.3	89.2
6	74.8	78.5	79.0	78.7	79.1	79.3	75.4	84.2	84.0
7	38.4	39.4	37.7	36.9	37.7	37.3	39.6	39.8	39.9
8	45.0	45.3	45.5	43.9	45.6	43.9	44.6	30.3	30.0
9	221.6	221.1	217.4	218.7	217.5	217.8	216.8	109.9	109.7
10	37.9	37.3	37.9	38.7	37.9	40.3	43.6	44.8	45.0
11	68.7	69.1	77.7	70.9	77.7	80.3	78.4	94.9	95.1
12	74.7	74.3	76.0	79.2	76.0	80.8	79.4	73.5	73.5
13	76.8	76.7	77.5	74.8	77.4	76.2	77.1	80.0	80.1
14	21.0	21.1	21.5	21.2	21.5	21.9	24.5	23.9	24.0
15	10.6	10.6	10.6	10.5	10.5	10.5	11.9	11.7	11.7
2-CH_3	15.9	16.0	16.1	15.9	16.0	15.9	15.6	15.7	15.9
4-CH_3	9.0	9.1	9.2	9.0	9.2	9.3	9.8	10.3	10.2
6-CH_3	26.7	19.8	20.4	19.9	20.3	20.5	25.3	26.3	26.4
8-CH_3	18.3	18.0	19.4	18.3	19.4	19.6	21.1	17.1	17.2
10-CH_3	12.0	12.3	12.8	11.1	12.8	12.3	12.2	14.8	14.6
12-CH_3	16.1	16.0	17.4	17.1	17.4	16.4	14.6	25.1	25.2
6-OCH_3	—	50.7	50.6	50.9	50.6	50.4	—	—	—
9-OCH_3	—	—	—	—	—	—	—	47.7	47.6
11-OCH_3	—	—	60.8	—	60.8	60.6	61.9	58.7	59.0
12-OCH_3	—	—	—	53.2	—	52.9	49.5	—	—
1′	103.2	102.9	102.6	102.8	102.2	102.1	100.5	105.3	105.4
2′	70.9	71.0	71.0	71.4	71.2	71.2	71.5	70.6	70.6
3′	65.4	65.6	65.6	65.6	64.9	64.8	64.1	64.6	64.1
4′	28.6	28.6	28.5	28.5	28.5	28.7	29.7	29.9	29.8
5′	68.8	68.8	68.7	68.7	68.1	68.0	68.6	69.2	68.9
5′-CH_3	21.3	21.5	21.5	21.4	21.6	21.6	21.2	21.4	21.1
3′-$N(CH_3)_2$	40.2	40.3	40.3	40.2	40.2	40.2	40.4	40.5	40.4
1″	96.2	96.1	96.3	96.2	96.5	96.6	94.9	97.9	98.2
2″	34.9	34.9	35.0	34.9	35.5	35.5	35.1	34.9	35.5
3″	72.5	72.7	72.7	72.6	73.4	73.4	73.6	72.5	73.3
4″	77.9	78.0	77.9	77.9	89.0	89.0	88.9	77.6	88.4
5″	65.5	65.8	65.9	65.5	64.8	64.9	65.4	65.9	65.4
3″-CH_3	21.4	21.5	21.5	21.4	21.3	21.3	22.0	21.0	21.1
5″-CH_3	18.6	18.7	18.8	18.6	19.0	18.9	18.3	17.7	17.7
3″-OCH_3	49.4	49.5	49.5	49.4	49.7	49.6	49.3	49.3	49.6
4″-OCH_3	—	—	—	—	62.1	62.1	61.9	—	61.9

[a] Chemical shifts are in ppm downfield of TMS. ^{13}C NMR spectra were taken in $CDCl_3$ at 100.4 MHz.

peaks with the increasing mass units due to the additional alkyl groups.

Acid Stability

It has been reported[2] that compounds **3** and **4** are more acid-resistant than **1** and the 95% of their antibacterial activities against *Staphylococcus aureus* 209P-JC are retained after 30 minutes in the pH 2 solution. Several *O*-methyl compounds (**3**, **4**, **7**, and **8**) were exposed to dilute HCl (pH 2) at 20°C. The amounts of remaining substances were measured by HPLC. The $t_{1/2}$ values for **3**, **4**, and **7** were 25.1, 16.7, and 58.3 hours, respectively, whereas those of **1** and **8** were 0.03 and 0.02 hour, respectively. The

Table 2. *In vitro* antibacterial activity of *O*-alkyl erythromycin A derivatives.

Organisms	MIC (μg/ml)					
	1	**3**	**4**	**5**	**6**	**7**
Staphylococcus aureus 209P-JC	0.10	0.10	0.39	0.10	0.78	1.56
S. aureus Smith 4	0.20	0.10	0.39	0.20	1.56	6.25
S. aureus Terajima	0.10	0.10	0.39	0.10	1.56	3.13
S. aureus BB	0.10	0.10	0.39	0.10	1.56	3.13
S. aureus J-109	>100	>100	>100	>100	>100	>100
S. aureus B1	>100	>100	>100	>100	>100	>100
S. aureus C1	3.13	3.13	25	3.13	25	50
S. epidermidis IID 866	0.20	0.10	0.39	NT	1.56	3.13
S. epidermidis sp-al-1	0.39	0.20	0.39	0.20	1.56	6.25
Bacillus subtilis ATCC 6633	0.10	0.05	0.20	0.05	1.56	1.56
Micrococcus luteus ATCC 9341	0.025	0.012	0.025	0.025	0.05	0.20
Escherichia coli NIHJ JC-2	100	100	>100	>100	>100	>100
E. coli K-12	25	12.5	25	25	100	100
Klebsiella pneumoniae IFO 3317	100	100	>100	50	>100	>100

Organisms	MIC (μg/ml)						
	8	**9**	**10**	**11**	**12**	**13**	**14**
Staphylococcus aureus 209P-JC	0.20	0.78	3.13	3.13	6.25	0.39	0.78
S. aureus Smith 4	0.39	1.56	12.5	6.25	50	0.78	1.56
S. aureus Terajima	0.39	1.56	6.25	6.25	25	0.78	1.56
S. aureus BB	0.39	1.56	12.5	6.25	50	0.78	3.13
S. aureus J-109	>100	>100	>100	>100	>100	>100	>100
S. aureus B1	>100	>100	>100	>100	>100	>100	>100
S. aureus C1	6.25	25	>100	100	>100	6.25	25
S. epidermidis IID 866	0.20	0.78	6.25	3.13	25	0.39	0.78
S. epidermidis sp-al-1	0.39	1.56	6.25	3.13	25	0.78	1.56
Bacillus subtilis ATCC 6633	0.39	0.78	3.13	3.13	12.5	0.39	0.78
Micrococcus luteus ATCC 9341	0.05	0.10	0.39	0.39	1.56	0.10	0.20
Escherichia coli NIHJ JC-2	>100	>100	>100	>100	>100	>100	>100
E. coli K-12	50	>100	>100	>100	>100	50	100
Klebsiella pneumoniae IFO 3317	100	>100	>100	>100	>100	>100	>100

Medium: Sensitivity Test Agar (Eiken), inoculum size 10^6 cfu/ml.
NT: Not tested.

introduction of the methoxy group at the C-4″ position, in addition to the C-6 position, resulted in a further increase of acid-stability.

In Vitro Antibacterial Activity

The *in vitro* antibacterial activities of *O*-alkyl erythromycin A derivatives are shown in Table 2. Compounds **3** and **5** exhibited excellent activities and were equal to or 2-fold more potent than **1**. The methylation of the tertiary hydroxyl groups at C-6 or C-6 and C-12 resulted in no change or an increase of *in vitro* activity. The methylation of the secondary

Table 3. *In vivo* antibacterial activity of *O*-methyl erythromycin A derivatives after po administration against systemic infections in mice induced by *Staphylococcus aureus* Smith 4.

Compound	ED_{50} (mg/mouse)	
	(Test-1; n=20[a])	(Test-2; n=15[a])
1	0.977 (0.760~1.22)[b]	0.652 (0.519~0.851)[b]
3	0.273 (0.222~0.333)	0.086 (0.058~0.111)
4	0.446 (0.370~0.534)	
5		0.216 (0.161~0.285)
8	0.738 (0.554~0.914)	

[a] Number of test animals.
[b] Numbers within parentheses indicate 95% confidence limits.

Table 4. Lung and plasma levels of 6-*O*-methyl and 6,12-di-*O*-methyl erythromycin A (**3** and **5**) in rats after a single po administration of 20 mg/kg.

Compound	Lung level (μg/g fresh tissue)		Plasma level (μg/ml)	
	2 hours	4 hours	2 hours	4 hours
3 (n=3[a])	73.2±10.8[b]	47.9±6.9[b]	0.9±0.1[b]	0.5±0.1[b]
5 (n=3)	112.9±23.0	72.3±1.6	1.1±0.2	0.7±0.1
1 (n=4)	0.6±0.1	NZ	0.1±0.0	NZ

[a] Number of test animals.
[b] Mean±SE.
NZ: No measurable zone of inhibition in microbiological assay.

hydroxyl groups at C-11 or C-4″ resulted in a decrease of the *in vitro* activity as reported[6] for the esterification of **1**. The activities of *O*-methyl derivatives decreased with increasing the number or the chain length of the alkyl groups introduced. The activities of **11** and **12**, both having the 9-*O*-methyl-6,9-hemiacetal structure, were 32- to 500-fold less than that of **1**.

In Vivo Antibacterial Activity

The representative *O*-methyl compounds (**3**, **4**, **5**, and **8**), possessing potent *in vitro* activity against *S. aureus* Smith 4, were advanced to *in vivo* trials against a systemic infection of the same organism in mice. As shown in Table 3, compounds **3**, **4**, and **5** were 3.6~7.6, 2.2, and 3.0 times more effective than **1**, respectively. The efficacy of **8** was comparable to that of **1**. The improved therapeutic efficacy provided by 6-*O*-methyl derivatives may be related to the better absorption of these compounds after po administration.

Lung and Plasma Levels

The lung and plasma levels of compounds **3** and **5** were compared to those of **1** after po administration (20 mg/kg) in rats. The concentrations of **3** and **5** were 9 to 11 times higher than that of **1** in plasma and 122 to 188 times higher in lung at 2 hours after administration (Table 4). These concentrations remained at fairly high levels after 4 hours, while those of **1** were under the limit of detection. It has been reported[7] that affinities of *O*-alkyl derivatives to the lung tissues are higher in order of **7**>**6**>**3**=**4**>**8**>**1**. These results indicate that methylation of the tertiary hydroxyl groups at C-6 and/or C-12 of **1** contribute to the penetration of drugs to the lung.

Experimental

MP's were taken using a Yanagimoto micro-melting points apparatus and are uncorrected. IR spectra were recorded on a Jasco DS-701G IR spectrometer. FD-MS, HREI-MS, and SI-MS were recorded on a Hitachi M-80A mass spectrometer, and FAB-MS were on a Jeol JMS-SX 102 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Varian XL-200 or Jeol JNM-GX 400 spectrometers. The ^1H and ^{13}C assignments were determined by INEPT and ^1H-^1H or ^1H-^{13}C shift correlation 2D NMR spectra. Optical rotations were determined with a Jasco DIP-360 digital polarimeter. TLC was performed on E. Merck plates of Silica gel 60 using solvent systems A (EtOAc-hexane, 2:1) or B ($CHCl_3$-MeOH-NH_4OH, 8:2:0.01). Spots were visualized by spraying with 2% ceric sulfate-2 N H_2SO_4 solution followed by heating at 110°C. Silica gel column chromatography was performed with Silica gel 60 or Silica gel 60 silanised (70~230 mesh, E. Merck).

In Vitro and *In Vivo* Antibacterial Activities

Antibiotic susceptibility was determined by an agar dilution method using Sensitivity Test Agar

(Eiken). Mouse protection experiments were conducted by use of male ICR mice infected intraperitoneally with *S. aureus* Smith 4. The antibiotics in a 5% gum arabic were administered orally 1 hour after the infection. Mortality of the animals was recorded daily over a period of 7 days and ED_{50} was calculated.

Plasma and Tissue Levels

The antibiotics in a 5% gum arabic were administered orally to rats with the dosing of 20 mg/kg. The rats were sacrificed at 2 and 4 hours after administration. The lung was homogenized with 0.02 M phosphate buffer (pH 7.4) - MeOH (1 : 4). The concentrations of the antibiotics in lung and plasma were measured by a microbiological assay using *Micrococcus luteus* ATCC 9341.

General Procedure 1 for *O*-Alkylation of Erythromycin Derivatives

To a well stirred solution of the substrate in a solvent (5~20 ml/g substrate) at 0~5°C, were added successively the alkyl halide (1.2~8 equiv) and the base (1~6 equiv). The mixture was stirred for 0.5~2 hours at 0~5°C. At the end of the reaction, Et_3N (excess to alkyl halide) was added to quench the reaction. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed several times with brine, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to give the crude product which was purified by silica gel column chromatography. *O*-Alkylation was carried out using the following reaction solvents and bases: DMF, DMSO - THF (1 : 1) or DMSO - 1,2-dimethoxyethane (1 : 1); NaH (50 or 60%, coated with mineral oil), KOH (85%, freshly powdered).

General Procedure 2 for Deprotection and Reductive *N*-Methylation

A mixture of the substrate (0.01 mol) and 0.5 g of palladium black (or 1.0 g of 10% Pd-C) in a mixture of EtOH (150 ml) and water (17.5 ml) containing 0.78 g of AcOH and 2.5 g of AcONa was stirred vigorously for 7 hours at ambient temperature under atmospheric pressure of hydrogen. After the complete absence of the substrate, 35% aqueous HCHO (10 ml) was added, and the hydrogenation was continued for an additional 7 hours. The catalyst was filtered off and the filtrate was poured into water (750 ml). The pH of the mixture was adjusted to 10~10.5 with 2 N NaOH to afford the corresponding 3′-dimethylamino derivative as a precipitate which was then collected by filtration or extracted with organic solvent. The crude product was purified by silica gel column chromatography or by recrystallization.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6-*O*-methylerythromycin A (**3z**)

Compound **2**[3] (120 g, 0.12 mol) was treated with methyl iodide (45.6 g, 0.32 mol) and 85% KOH (7.52 g, 0.11 mol) in 800 ml of DMSO - 1,2-dimethoxyethane (1 : 1) according to the procedure 1. The resulting crude product was purified by silica gel column chromatography (EtOAc - hexane, 1 : 2) to give 47.4 g (39%) of **3z** (purity 94% by HPLC) and 50.5 g (42%) of **8z** and 18.0 g (15%) of the starting **2**. One g of **3z** was further purified by silica gel column chromatography ($CHCl_3$ - MeOH, 100 : 1) and crystallized from EtOH - water (2 : 1), affording 0.78 g of pure **3z**: MP 110~111°C; TLC (system A) Rf 0.72; IR (KBr) cm^{-1} 1730, 1685; FD-MS *m/z* 1,024 $(M+Na)^+$; $[\alpha]_D^{24}$ −91.4° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 3.00 (3H, 6-OCH_3), 2.86, 2.82 (3H, NCH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 220.9 (C-9), 175.8, 175.7 (C-1), 78.2 (C-6), 50.4 (6-OCH_3), 49.5, 49.1 (3″-OCH_3), 28.8 (N-CH_3), 19.8 (6-CH_3); *Anal* Calcd for $C_{53}H_{79}NO_{17}$: C 63.52, H 7.95, N 1.40. Found: C 63.24, H 7.55, N 1.41.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*-methylerythromycin A (**8z**)

O-Methylation of **2** (19.8 g, 0.02 mol) with methyl iodide (19.2 g, 0.135 mol) and 50% NaH (1.2 g, 0.025 mol) in DMF (80 ml) by the procedure 1 gave 14.2 g (71%) of **8z** as a colorless foam. Crystallization from ether - petroleum ether gave 11.7 g as crystals: MP 154.5~156°C; TLC (system A) Rf 0.58; IR (KBr) cm^{-1} 1755, 1732, 1703, 1685; $[\alpha]_D^{24}$ −94.5° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 3.47 (3H, 11-OCH_3), 2.88, 2.84 (3H, NCH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 217.1, 108.8 (C-9), 177.1, 176.0 (C-1); *Anal* Calcd for $C_{53}H_{79}NO_{17}$: C 63.52, H 7.95, N 1.40. Found: C 63.37, H 7.99, N 1.41.

6-*O*-Methylerythromycin A (**3**) and 6,12-Di-*O*-methylerythromycin A (**5**)

O-Methylation of **2** (30 g, 0.03 mol) with methyl iodide (17.1 g, 0.12 mol) and 85% KOH (2.38 g, 0.036 mol) in 600 ml of DMSO - 1,2-dimethoxyethane (1 : 1) by the procedure 1 gave 13.7 g (45% yield) of

3z (purity 70% by HPLC). Deprotection and reductive *N*-methylation of this substance (10 g) were carried out according to the procedure 2. The resulting product was suspended in ether (500 ml) and stirred for 5 hours. The ether layer was separated by filtration and evaporated. The residue was crystallized from EtOH to give 4.6 g (28% from **2**) of **3** as colorless needles: MP 225~227°C (literature[2] 222~225°C).

The mother liquor was evaporated to dryness to give a foam (1.6 g). One g of this foam was chromatographed over reversed phase silica gel column with 0.1 M phosphate buffer (pH 7.0) - MeOH (2 : 3). The eluate showing Rf 0.26 by TLC (E. Merck plates of silanised Silica gel 60; 0.1 M pH 7.4, phosphate buffer - MeOH, 2 : 3) was evaporated to remove the most of MeOH. The resultant mixture was basified (pH 9) using Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over $MgSO_4$, and evaporated *in vacuo* to afford 0.12 g (1.1% from **2**) of **5** which was crystallized from EtOH to give colorless needles: MP 126~128 and 193~195°C; TLC (system B) Rf 0.39; IR (KBr) cm^{-1} 1735, 1695; SI-MS *m/z* 762 (M+H); ^1H NMR (200 MHz, $CDCl_3$) δ 3.46 (s, 12-OCH_3), 3.33 (s, 3″-OCH_3), 3.09 (s, 6-OCH_3), 2.28 (s, $N(CH_3)_2$), 1.41 (s, 6-CH_3). ^{13}C NMR: See Table 1; *Anal* Calcd for $C_{39}H_{71}NO_{13}$: C 61.47, H 9.39, N 1.84. Found: C 61.39, H 9.53, N 1.74.

6,11-Di-*O*-methylerythromycin A (**4**) and 6,11,4″-Tri-*O*-methylerythromycin A (**6**)

A solution of **3z** (6 g, 6 mmol) in 120 ml of DMSO - 1,2-dimethoxyethane (1 : 1) was treated with methyl iodide (4.56 g, 32 mmol) and 85% KOH (0.95 g, 14 mmol) according to the procedure 1. The product was purified by silica gel column chromatography (EtOAc - hexane, 1 : 1) to give 3.27 g (54%) of **4z** (Rf 0.72, system A) and 1.67 g (27%) of **6z** (Rf 0.78, system A).

Deprotection and *N*-methylation of **4z** (1 g) and **6z** (1.6 g) by the procedure 2 gave **4** (0.64 g, 85%) and **6** (0.63 g, 52%), respectively. **4** was crystallized from CH_2Cl_2 - ether: MP 252~257°C (literature[2] 252~256°C). **6** was crystallized from $CHCl_3$ - hexane: MP 229~230°C; TLC (system B) Rf 0.49; IR (KBr) cm^{-1} 1730, 1700; FAB-MS *m/z* 776 $(M+H)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 3.59 (s, 11-OCH_3), 3.54 (s, 4″-OCH_3), 3.32 (s, 3″-OCH_3), 3.11 (s, 6-OCH_3), 2.26 (s, $N(CH_3)_2$), 1.41 (s, 6-CH_3); ^{13}C NMR: See Table 1.

6,11,12,4″-Tetra-*O*-methylerythromycin A (**7**) and 11,12,4″-Tri-*O*-methylerythromycin A (**10**)

O-Methylation of **2** (10 g, 10 mmol) with methyl iodide (13.7 g, 96 mmol) and 50% NaH (2.88 g, 60 mmol) in DMF (80 ml) by the procedure 1 and silica gel column chromatography (EtOAc - hexane, 1 : 1~1 : 2) of the product gave 2.95 g (*ca.* 28%) of a mixture of **7z** and **10z** (Rf 0.80, system A) and 5.7 g (55%) of **12z** (Rf 0.83, system A). Deprotection and *N*-methylation of the mixture by the procedure 2 gave 1.13 g (14% from **2**) of **7** which was crystallized from $CHCl_3$ - petroleum ether: MP 238.5~240°C (dec); TLC (system B) Rf 0.49; IR (KBr) cm^{-1} 1727, 1710; FAB-MS *m/z* 790 $(M+H)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 3.54 (s, 4″-OCH_3), 3.49 (s, 11-OCH_3), 3.34 (s, 12-OCH_3), 3.33 (s, 3″-OCH_3), 3.13 (s, 6-OCH_3), 2.30 (s, $N(CH_3)_2$), 1.41 (s, 6-CH_3); ^{13}C NMR: See Table 1; *Anal* Calcd for $C_{41}H_{75}NO_{13}$: C 62.33, H 9.57, N 1.77. Found: C 61.92, H 9.62, N 1.76.

After the filtration of **7**, the mother liquor was evaporated to dryness. The residue was chromatographed on a silica gel column with MeOH - $CHCl_3$ (1 : 49~1 : 9) to give further 0.42 g (5% from **2**) of **7** and 0.3 g (4% from **2**) of **10** as a colorless foam. For compound **10**: MP 105.5~108.5°C; TLC (system B) Rf 0.34; IR (KBr) cm^{-1} 1730, 1708; HREI-MS *m/z* 775.5052 (M^+, calcd for $C_{40}H_{73}NO_{13}$: *m/z* 775.5082); ^1H NMR (200 MHz, $CDCl_3$) δ 3.49 (s, 4″-OCH_3), 3.44 (s, 11-OCH_3), 3.32 (s, 3″-OCH_3), 3.08 (s, 12-OCH_3), 2.37 (s, $N(CH_3)_2$). ^{13}C NMR: See Table 1.

11-*O*-Methylerythromycin A (**8**)

Deprotection and successive *N*-methylation of **8z** (6.0 g, 6 mmol) were carried out according to the procedure 2 to afford 4.0 g (90%) of **8** as a colorless foam which was crystallized from acetone at ambient temperature to give 3.73 g (83%) of the pure C-9 keto tautomer of **8** as colorless crystals: MP 186~188°C; TLC (system B) Rf 0.36; IR (KBr) cm^{-1} 1738, 1732, 1705; SI-MS *m/z* 748 $(M+H)^+$; UV $\lambda_{max}^{CHCl_3}$ nm 292 (ε 20.0); $[\alpha]_D^{24}$ −79.6° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 3.48, 3.37 (3H, 11-OCH_3), 3.35, 3.30 (3H, 3″-OCH_3) 2.32, 2.30 (6H, $N(CH_3)_2$), 1.45, 1.40 (6-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 217.4, 108.0 (C-9), 177.2, 176.1 (C-1), 105.1, 102.6 (C-1′), 97.2, 95.8 (C-1″), 60.9, 58.1 (11-OCH_3), 49.4 (3″-OCH_3), 40.5, 40.3 ($N(CH_3)_2$), 27.3, 25.4 (6-CH_3); *Anal* Calcd for $C_{38}H_{69}NO_{13}$: C 61.02, H 9.30, N 1.87. Found: C 61.09, H 9.46, N 1.82.

11,4″-Di-*O*-methylerythromycin A (**9**)

O-Methylation of **2** (2 g, 2 mmol) with dimethyl sulfate (1 ml, 10.5 mmol) and 50% NaH (0.2 g, 4.2 mmol) in DMF (20 ml) was carried out according to the procedure 1. Purification of the product by silica gel column chromatography (EtOAc - hexane, 1 : 1) gave 0.3 g (15%) of **8z** and 0.63 g (31%) of **9z** (Rf 0.67, system A). Debenzylation and *N*-methylation of **9z** (0.6 g, 0.6 mmol) by the procedure 2 gave 0.23 g (51%) of **9** as a colorless foam: MP 98~99°C; TLC (system B) Rf 0.46; IR (KBr) cm^{-1} 1730; FAB-MS *m/z* 762 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 3.55 (3H, 4″-OCH_3), 3.49, 3.38 (3H, 11-OCH_3), 3.34, 3.30 (3H, 3″-OCH_3), 2.33, 2.29 (6H, $N(CH_3)_2$), 1.45, 1.41 (3H, 6-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 217.8, 108.0 (C-9), 177.3, 176.2 (C-1), 105.0, 102.3 (C-1′), 97.7, 96.2 (C-1″), 88.9, 88.7 (C-4″), 62.1, 62.0 (4″-OCH_3), 60.9, 58.4 (11-OCH_3), 49.7 (3″-OCH_3), 40.4, 40.2 ($N(CH_3)_2$).

9-*O*-Deoxo-6-deoxy-6,9-epoxy-9-methoxy-11-*O*-methylerythromycin A (**11**)

Compound **8z** (3.0 g, 3 mmol) was further methylated with methyl iodide (2.28 g, 16 mmol) and 85% KOH (0.44 g, 6.7 mmol) in 30 ml of DMSO - 1,2-dimethoxyethane (1 : 1) according to the procedure 1. Purification of the product by silica gel column chromatography (EtOAc - hexane, 1 : 3~1 : 2) gave 0.35 g (12%) of **4z**, 1.22 g (40%) of **11z**, and 0.88 g (29%) of **12z**. Deprotection and *N*-methylation of **11z** (1.2 g) by the procedure 2 gave 0.49 g (55%) of **11** as crystals from CH_2Cl_2 - hexane: MP 170~172°C; TLC (system B) Rf 0.40; IR (KBr) cm^{-1} 1730; FAB-MS *m/z* 762 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 3.38 (s, 11-OCH_3), 3.29 (s, 3″-OCH_3), 3.21 (s, 9-OCH_3), 2.37 (s, $N(CH_3)_2$), 1.37 (s, 6-CH_3); ^{13}C NMR: See Table 1.

9-Deoxo-6-deoxy-6,9-epoxy-9-methoxy-11,4″-di-*O*-methylerythromycin A (**12**)

Compound **12z** (5.7 g, 5.5 mmol), obtained in the preparation of **7**, was hydrogenated according to the procedure 2. Purification of the resulting product by silica gel column chromatography (MeOH - $CHCl_3$, 1 : 19~1 : 9) afforded 2.9 g (68%) of **12** as a colorless foam: MP 107~114°C; TLC (system B) Rf 0.53; IR (KBr) cm^{-1} 1730; HREI-MS *m/z* 775.5076 (M^+, calcd for $C_{40}H_{73}NO_{13}$: *m/z* 775.5082); ^1H NMR (200 MHz, $CDCl_3$) δ 3.53 (s, 4″-OCH_3), 3.39 (s, 11-OCH_3), 3.28 (s, 3″-OCH_3), 3.21 (s, 9-OCH_3), 2.35 (s, $N(CH_3)_2$), 1.38 (s, 6-CH_3); ^{13}C NMR: See Table 1; *Anal* Calcd for $C_{40}H_{73}NO_{13}$: C 61.91, H 9.48, N 1.81. Found: C 61.69, H 9.42, N 1.76.

11-*O*-Ethylerythromycin A (**13**)

The *O*-alkylation of **1** (7.3 g, 9.9 mmol) with ethyl iodide (7.8 g, 50 mmol) and 50% NaH (0.59 g, 12 mmol) in DMF (40 ml) was carried out according to the procedure 1. Purification of the product by silica gel column chromatography ($CHCl_3$ - MeOH, 9 : 1) and crystallization from acetone - petroleum ether gave 3.1 g (41%) of **13**: MP 123.5~126.5°C; TLC (system B) Rf 0.41; IR (KBr) cm^{-1} 1735; FAB-MS *m/z* 762 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 3.38, 3.33 (3H, OCH_3), 2.38, 2.33 (6H, $N(CH_3)_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 217.5, 108.3 (C-9), 177.2, 176.2 (C-1), 105.0, 102.6 (C-1′), 97.2, 95.8 (C-1″), 68.5, 65.3 (OCH_2-), 49.42, 49.36 (OCH_3), 40.5, 40.3 ($N(CH_3)_2$); *Anal* Calcd for $C_{39}H_{71}NO_{13}$: C 61.47, H 9.39, N 1.84. Found: C 61.13, H 9.29, N 1.83.

11-*O*-*n*-Propylerythromycin A (**14**)

O-Alkylation of compound **1** (11 g, 15 mmol) with *n*-propyl iodide (16 g, 94 mmol) and 50% NaH (1.31 g, 27 mmol) in DMF (60 ml) was carried out according to the procedure 1. The product was crystallized from acetone to give 4.7 g (40%) of **14**: MP 121.5~125.5°C; TLC (system B) Rf 0.37; IR (KBr) cm^{-1} 1730; FAB-MS *m/z* 776 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 3.35, 3.31 (3H, OCH_3), 2.33, 2.31 (6H, $N(CH_3)_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 217.2, 108.6 (C-9), 177.1, 176.2 (C-1), 104.6, 102.5 (C-1′), 97.0, 95.7 (C-1″), 74.3, 71.0 (OCH_2-), 49.4 (OCH_3), 40.4, 40.3 ($N(CH_3)_2$).

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