SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF *O*-METHYLAZITHROMYCIN DERIVATIVES

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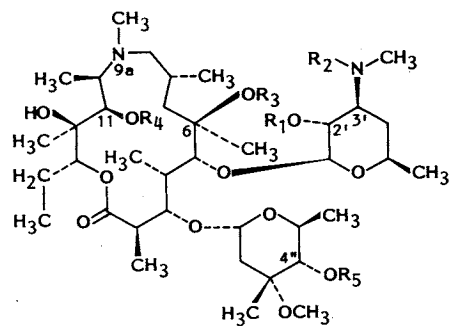
A series of *O*-methylazithromycin derivatives have been synthesized and their antibacterial activities were compared with those of azithromycin (**1**). *O*-Methylation of **1** proceeded stepwise by the two main pathways beginning at the C-6 and C-11 hydroxyl groups, individually. Among *O*-methyl derivatives, 6-*O*-methylazithromycin A (**11**) was slightly less active than **1**. The methylation of the secondary hydroxyl group at the C-11 position resulted surprisingly in an increase of their *in vitro* activity. The antibacterial activities of novel azalides decreased with increasing the number of the methyl groups introduced.

Several new macrolide antibiotics with improved pharmacokinetics and greater and broader activity than erythromycin A have appeared in recent years.[1,2] Azithromycin (**1**),[3,4] the prototype azalide antibiotic, differs structurally from erythromycin A by the insertion of a 9a-methyl substituted nitrogen in the aglycone moiety, as well as expansion of the ring to a 15-membered azalactone.[5] This modification produces an enhanced spectrum and potency against Gram-negative bacteria including *Haemophilus influenzae*, *Neisseria gonorrhoeae and Branhamella catarrhalis*[6] and superior stability in an acid environment.[7] When compared to erythromycin A, azithromycin also has greater oral bioavailability, much longer elimination half-life, and much higher tissue concentrations.[8~10]



Another successful strategy for inhibiting decomposition of erythromycin A under acidic condition is the synthesis of *O*-alkyl derivatives of erythromycins.[11~13] Among them, 6-*O*-methylerythromycin A, clarithromycin, has greater antibacterial activity against Gram-positive bacteria and *Mycoplasma* sp. than parent antibiotic.[14] Consequently, we were interested to examine the influence of similar chemical modifications on the antibacterial properties of 15-membered azalides

1	$R_1=R_3=R_4=R_5=H$	$R_2=CH_3$
2	$R_1=R_2=CO_2CH_2C_6H_5$	$R_3=R_4=R_5=H$
3	$R_1=R_2=CO_2CH_2C_6H_5$	$R_3=CH_3$ $R_4=R_5=H$
4	$R_1=R_2=CO_2CH_2C_6H_5$	$R_3=R_4=CH_3$ $R_5=H$
5	$R_1=R_2=CO_2CH_2C_6H_5$	$R_3=R_5=H$ $R_4=CH_3$
6	$R_1=R_2=CO_2CH_2C_6H_5$	$R_3=R_4=R_5=CH_3$
7	$R_1=R_2=R_4=R_5=H$	$R_3=CH_3$
8	$R_1=R_2=R_5=H$	$R_3=R_4=CH_3$
9	$R_1=R_2=R_3=R_5=H$	$R_4=CH_3$
10	$R_1=R_2=H$	$R_3=R_4=R_5=CH_3$
11	$R_1=R_4=R_5=H$	$R_2=R_3=CH_3$
12	$R_1=R_5=H$	$R_2=R_3=R_4=CH_3$
13	$R_1=R_3=R_5=H$	$R_2=R_4=CH_3$
14	$R_1=H$	$R_2=R_3=R_4=R_5=CH_3$

and report here results related to azithromycin.

Chemistry

Reaction of **1** with benzyl chloroformate and $NaHCO_3$ afforded 2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-*N*-demethylazithromycin (**2**) in 78% yield. *O*-Methylation of **2** with methyl iodide (8 equiv) and NaH (8 equiv) in dimethyl sulfoxide (DMSO)-tetrahydrofuran (THF) (1:1) at 0~5°C gave 6-*O*-methylated compound (**3**, 58%), 6,11-di-*O*-methylated compound (**4**, 5%) and 11-*O*-methylated compound (**5**, 26%). *O*-Alkylation of **2** with 18 equiv methyl iodide at room temperature afforded 6,11-di-*O*-methylated derivative **4** as the major product (42%), compounds **3** and **5** and the new 6,11,4″-tri-*O*-methylated derivative (**6**) as the minor product (7.3%). More vigorous reaction conditions in *N*,*N*-dimethylformamide (DMF) as the solvent gave 6,11,4″-tri-*O*-methylazithromycin **6** as the only product (73%). The mixtures, thus obtained, were subjected to silica gel column chromatography to yield TLC (CH_2Cl_2-CH_3OH-conc NH_4OH, 90:9:0.5) homogenous compounds **3**~**6** with Rf values 0.661, 0.811, 0.843 and 0.881, respectively.

After usual deprotection[15] with Pd-C in EtOH containing sodium acetate-acetic acid buffer (pH 5.0) and reductive *N*-methylation of 3′-*N*-demethyl derivatives **7**~**10** with aqueous formaldehyde and formic acid in $CHCl_3$, the corresponding *O*-methyl compounds **11**~**14** were isolated and identified as 6-*O*-methyl- (**11**), 6,11-di-*O*-methyl- (**12**), 11-*O*-methyl- (**13**) and 6,11,4″-tri-*O*-methylazithromycin (**14**). The novel azithromycin derivatives were purified by column chromatography as well as crystallization whenever possible.

The molecular formula of **11** was determined as $C_{39}H_{74}N_2O_{12}$ from elemental analysis, mass and NMR spectra, indicating the introduction of one methyl group to **1**. EI-MS at 20 eV of **11** showed the molecular ion at *m/z* 762. In addition, prominent ions at *m/z* 604 (10.1%) and 603 (24.4%) due to the sequential elimination of desosamine (*m/z* 158, 10.0%) and cladinose (*m/z* 159, 1.4%) were present in the

Table 1. ^1H NMR chemical shifts (δ)[a] of **11** and **13** in comparison with **1**.

Proton No.	1	11	13	Proton No.	1	11	13
2	2.73	2.74	2.74	9a-NCH_3	2.32	2.30	2.25
3	4.27	4.33	4.44	10-CH_3	1.09	1.16	1.05
4	2.00	2.06	2.00	11-OCH_3	—	—	3.59
5	3.64	3.65	3.65	12-CH_3	1.10	1.10	1.11
7ax	1.81	1.71	1.76	1′	4.44	4.44	4.46
7eq	1.25	1.39	1.30	2′	3.24	3.26	3.31
8	2.03	2.02	2.02	3′	2.44	2.51	2.46
9ax	2.55	2.33	2.53	3′-$N(CH_3)_2$	2.29	2.32	2.29
9eq	2.05	2.11	2.06	4′eq	1.68	1.67	1.67
10	2.69	2.71	2.68	4′ax	1.23	1.30	1.29
11	3.69	3.69	3.44	5′	3.51	3.53	3.52
13	4.70	5.40	4.69	5′-CH_3	1.24	1.23	1.22
14eq	1.89	1.73	1.91	1″	5.19	5.02	5.16
14ax	1.46	1.52	1.45	2″eq	2.37	2.35	2.37
15	0.89	0.94	0.89	2″ax	1.62	1.57	1.58
2-CH_3	1.20	1.23	1.18	3″-CH_3	1.25	1.24	1.24
4-CH_3	1.06	1.07	1.03	3″-OCH_3	3.35	3.33	3.35
6-CH_3	1.32	1.30	1.29	4″	3.04	3.03	3.03
6-OCH_3	—	3.39	—	5″	4.09	4.07	4.09
8-CH_3	0.91	0.93	0.91	5″-CH_3	1.34	1.31	1.32

[a] δ Values in ppm from TMS, measured in $CDCl_3$ at 300 MHz, as determined from ^1H-^1H 2D homonuclear shift correlated experiments.

Table 2. ^{13}C NMR chemical shifts of *O*-methylazithromycin derivatives **11**~**14**.

Carbon No.	Chemical shift (δ^a, ppm)					Carbon No.	Chemical shift (δ^a, ppm)				
	1	**11**	**12**	**13**	**14**		**1**	**11**	**12**	**13**	**14**
C-1	178.5	177.6	177.7	177.9	177.9	12-CH_3	14.3	16.7	16.7	17.1	15.8
C-2	45.1	45.1	45.2	45.6	45.1	9a-CH_3	36.4	36.8	36.8	35.9	36.8
C-3	78.1	77.9	78.3	78.0	76.8	6-OCH_3	—	52.9	5.28	—	51.0
C-4	41.7	41.9	41.7	42.8	41.7	11-OCH_3	—	—	62.0	62.1	62.0
C-5	83.6	83.7	83.2	83.7	78.8	C-1′	102.9	103.1	102.6	103.0	102.9
C-6	74.3	79.2	79.1	74.4	80.7	C-2′	70.9	70.9	71.0	70.91	70.9
C-7	42.4	42.9	42.8	42.8	42.8	C-3′	65.7	65.7	64.7	65.7	65.1
C-8	26.7	26.8	27.2	26.7	27.6	3′-$N(CH_3)_2$	40.4	40.4	40.3	40.4	40.3
C-9	70.1	68.8	68.9	70.9	68.6	C-4′	28.8	28.8	28.7	28.9	28.9
C-10	62.2	61.7	61.6	62.6	62.0	C-5′	68.6	68.8	68.4	68.6	68.4
C-11	74.2	76.6	89.0	85.0	89.0	5′-CH_3	21.4	21.4	21.4	21.7	21.4
C-12	73.6	73.8	73.9	73.2	73.8	C-1″	94.8	95.2	95.5	94.6	96.1
C-13	77.4	74.6	77.3	77.7	76.0	C-2″	34.8	34.9	35.1	34.8	35.7
C-14	21.2	21.9	21.9	21.7	22.7	C-3″	72.9	72.9	73.7	73.0	73.8
C-15	11.3	11.03	11.0	11.3	11.2	3″-CH_3	21.6	21.6	21.4	21.4	21.4
2-CH_3	15.0	14.6	14.9	14.8	14.3	3″-OCH_3	49.4	49.5	49.7	49.5	49.7
4-CH_3	9.2	9.6	9.2	7.2	8.2	C-4″	78.1	78.1	78.1	78.2	87.3
6-CH_3	27.6	27.6	26.8	27.7	26.8	4″-OCH_3	—	—	—	—	61.3
8-CH_3	22.0	22.0	22.0	22.2	21.9	C-5″	65.5	65.6	65.1	65.4	64.6
10-CH_3	7.5	9.4	9.6	9.2	9.6	5″-CH_3	18.3	18.1	18.4	18.1	18.2

[a] Chemical shifts are in ppm downfield of TMS. ^{13}C NMR spectra were taken in $CDCl_3$ at 300 MHz, as determined from ^1H-^{13}C 2D heteronuclear shift correlated experiments.

mass spectrum, suggesting that the new methoxy group in **11** was introduced at azithronolide moiety. The ^1H and ^{13}C NMR spectra of **11** were directly compared with those of **1**.[16] The ^1H NMR spectrum of **11** is similar to that of **1** except for the new *O*-methyl signal at 3.39 ppm (Table 1). In the one-dimensional ^{13}C NMR spectrum of **11** revealed by DEPT technique, a typical downfield chemical shift of C-6 (+4.9 ppm) singlet was observed together with the new methoxy signal at 52.9 ppm compared to **1** (Table 2). By the application of the reported considerations with regard to *O*-methylation shift,[12,17] it was assigned that the methoxy group is attached at C-6 position. An unambiguous NMR assignments of **11** was made by means of homonuclear ^1H-^1H and heteronuclear ^1H-^{13}C 2D NMR spectroscopy.

Compound **13** exhibited the new *O*-methyl signal due to 11-OCH_3 at 3.59 ppm. In the corresponding ^{13}C NMR spectrum, C-11 (85.0 ppm) doublet of **13** was 8.4 ppm further downfield than that of **11**. As expected with the published substituent effect in erythromycins,[12,14] the new methoxy signal was observed at 62.1 ppm. The ^1H and ^{13}C chemical shifts of methoxy groups in other *O*-methyl compounds were similar to the corresponding those in **11** and **13**. In addition, the peak at 3.48 ppm in **14** was assigned to the 4″-OCH_3 group derived from the secondary C-4″ hydroxyl group of **1**. As shown in Table 2, the significant downfield shift of α-carbon (C-4″, +9.2 ppm) together with the new *O*-methyl ^{13}C chemical signal at 61.3 ppm, compared to **1**, was also observed. The connectivities between proton resonances at 3.48 (4″-OCH_3) and 3.52 ppm (11-OCH_3) and their directly attached carbon atoms at 61.3 and 62.0 ppm, respectively, was established using 2D ^1H-^{13}C COSY experiment.

In Vitro Antibacterial Activity

The *in vitro* antibacterial activities of *O*-methylazithromycins **11**~**14** are shown in Table 3. Compounds **11** and **13** exhibited excellent activities against a variety of standard strains. 11-*O*-Methylazithromicin (**13**)

Table 3. Antibacterial *in vitro* activity of novel *O*-methylazithromycins **11**~**14**.

Organisms	MIC (μg/ml)				
	1	**11**	**12**	**13**	**14**
Micrococcus flavus ATCC 6538 P	1.56	0.39	1.56	0.2	3.125
Corynebacterium xerosis NCTC 9755	6.25	12.5	12.5	1.56	25.0
Staphylococcus aureus ATCC 10240	0.39	0.79	0.78	0.1	3.125
S. epidermidis ATCC 12228	0.1	0.1	1.56	0.1	3.125
Streptococcus faecalis ATCC 8043	0.05	0.05	0.78	0.05	0.78
Bacillus subtilis NCTC 8236	0.39	0.2	0.78	0.1	3.125
B. pumilus NCTC 8241	0.2	0.2	0.78	0.05	3.125
B. cereus NCTC 10320	0.39	0.78	1.56	0.1	3.125
Micrococcus luteus ATCC 9341	0.05	0.0125	0.05	0.0125	0.05
Pseudomonas aeruginosa NCTC 10490	100.0	100.0	100.0	25.0	200.0
Escherichia coli ATCC 10536	0.78	3.125	6.25	0.78	6.25

Method: Determined by tube dilution method using brain heart infusion medium.
Incubation: 24~48 hours, 37°C.
Inculum size: 10^{-5}~10^{-6} cfu/ml.

is equal to or several times more potent than **1**. However, 6-*O*-methylazithromycin (**11**) was equal to or slightly less effective than **1** and **13**. The activities of *O*-methyl derivatives decreased with increasing the number of the alkyl groups introduced as reported previously for the methylation of erythromycins A and B.[14]

Experimental

MP's were taken using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 G spectrometer. Electron impact mass spectra were recorded on a Shimadzu GCMS-QP 1000 mass spectrometer at 20 eV and ion source temperature of 250°C. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Jeol FX-100 or GEM-300 spectrometers. TLC was performed on E. Merck plates of Silica gel 60 using solvent system A (CH_2Cl_2-CH_3OH-conc NH_4OH, 90:9:0.5) or B (EtOAc-*n*-hexane-Et_2NH, 100:100:20). Spots were visualized by spraying with 5% H_2SO_4-EtOH solution followed by heating at 110°C. Silica gel column chromatography was performed with Silica gel 60 (70~230 mesh, E. Merck).

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylazithromycin (**2**)

To a stirred solution of azithromycin dihydrate (90 g) and $NaHCO_3$ (172 g) in 420 ml of benzene, carbobenzoxy chloride (225 ml) was added successively at 55~60°C for 1 hour. The mixture was stirred for 3 hours at the same temperature and then left to stand overnight at room temperature. The suspension was extracted with 0.25 N HCl, the benzene soln dried ($CaCl_2$) and evaporated under reduced pressure. Precipitation of the residue from diethyl ether-petroleum ether (60 ml/2.8 liters) afforded **2** (89.1 g, 77.5%) as colorless needles; MP 148~154°C; TLC, system A, Rf 0.704; IR ($CHCl_3$) cm^{-1} 3510, 3350, 2960, 1740, 1690, 1450, 1376, 1330, 1255, 1160, 1115, 1050, 995; ^{1}H NMR (100 MHz, $CDCl_3$) δ 2.30 (3H, 9a-NCH_3), 2.84, 2.80 (3H, 3′-NCH_3), 3.40 (3H, 3″-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3 (C-1), 100.1 (C-1′), 95.2 (C-1″), 75.0 (C-6), 74.6 (C-12), 69.4 (C-9), 64.6 (C-10), 49.6, 49.0 (3″-OCH_3), 37.0 (9a-NCH_3), 26.0 (C-8); EI-MS *m/z* 1,002 (M^+); *Anal* Calcd for $C_{53}H_{82}N_2O_{16}$: C 63.45, H 8.24, N 2.79. Found: C 63.02, H 8.57, N 2.90.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6-*O*-methylazithromycin (**3**) and 2′-O,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*-methylazithromycin (**5**)

To a stirred solution of **2** (6 g) in DMSO-TMF (1:1, 60 ml) at 0~5°C, were added succesively methyl iodide (3 ml, 8 equiv) and 55~60% NaH dispersion (2.1 g, 8 equiv) for 2 hours. The above mixture was

stirred for 1 hour, the suspension was poured into satd NaCl soln, and extracted with EtOAc. The organic layer was washed with satd NaCl soln, dried over K_2CO_3 and evaporated under reduced pressure. The crude product was chromatographed on a silica gel column (CH_2Cl_2 - CH_3OH - conc NH_4OH, 90 : 9 : 0.5) to afford **3** (3.5 g, 58%), **4** (0.31 g, 5%) and **5** (1.58 g, 26%).

For compound **3**: TLC, system A, Rf 0.661; IR ($CHCl_3$) cm^{-1} 3570, 3500, 2960, 2920, 1740, 1690, 1450, 1380, 1325, 1290, 1225, 1200, 1160, 1120, 1050; ^1H NMR (100 MHz, $CDCl_3$) δ 2.29 (3H, 9a-NCH_3), 2.881, 2.85 (3H, 3'-NCH_3), 3.38 (6H, 6-OCH_3 and 3''-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7 (C-1), 69.9 (C-9), 34.9 (9a-NCH_3), 78.1 (C-6), 74.7 (C-11), 73.9 (C-12) and 52.8 (6-OCH_3); EI-MS *m/z* 1,016 (M^+); *Anal* Calcd for $C_{54}H_{84}N_2O_{16}$: C 63.75, H 8.32, N 2.75. Found: C. 62.53, H 8.64, N 2.52.

For compound **5**: TLC, system A, Rf 0.843; IR ($CHCl_3$) cm^{-1} 3510, 2975, 2940, 1730, 1460, 1380, 1335, 1295, 1255, 1160, 1090, 1005, 985; ^1H NMR (100 MHz, $CDCl_3$) δ 2.24 (3H, 9a-NCH_3), 2.81, 2.85 (3H, 3-NCH_3), 3.37 (3H, 3''-OCH_3), 3.57 (3H, 11-OCH_3); EI-MS *m/z* 1,016 (M^+); *Anal* Calcd for $C_{54}H_{84}N_2O_{16}$: C 63.75, H 8.32, N 275. Found: C 63.42, H. 8.56, N 2.62.

2'-*O*,3'-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6,11-di-*O*-methylazithromycin (**4**)

To a stirred soln of **2** (6 g) and methyl iodide (6.6 ml, 18 equiv) in DMSO - THF was added successively 55~60% NaH dispersion (2.1 g, 8 equiv) at ambient temperature for 2 hours and the reaction mixture was stirred for 4 hours at the same temperature. Purification as described above afforded **3** (1.2 g, 19.7%), **4** (2.58 g, 42%), **5** (1.43 g, 23.5%) and **6** (0.46%, 7.3%).

For compound **4**: TLC, system A, Rf 0.811; IR ($CHCl_3$) cm^{-1} 3570, 3490, 1740, 1690, 1455, 1380, 1330, 1295, 1260, 1200, 1160, 1120, 1095, 1055, 1005, 990, 980; ^1H NMR (100 MHz, $CDCl_3$) δ 2.29 (3H, 9a-NCH_3), 2.84, 2.80 (3H, 3'-NCH_3), 3.38 (6H, 6-OCH_3 and 3''-OCH_3) and 3.49 (3H, 11-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4 (C-1), 69.5 (C-9), 35.3 (9a-NCH_3), 49.2 (3''-OCH_3); EI-MS *m/z* 1,030 (M^+); *Anal* Calcd for $C_{55}H_{86}N_2O_{16}$: C 64.12, H. 8.42, N 2.72. Found: C 64.51, H 8.26, N 2.53.

2'-*O*,3'-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6,11,4''-tri-*O*-methylazithromycin (**6**)

To a stirred soln of **2** (6 g) and methyl iodide (6 ml, 16 equiv) in DMF (60 ml) was added successively 55~60% NaH dispersion (2.1 g, 8 equiv) at ambient temperature for 2 hours. The reaction mixture was stirred for 4 hours and left to stand overnight. Similar treatment and purification as described above afforded **5** (1.29 g, 20.9%) and **6** (4.56 g, 73.1%).

For compound **6**: TLC, system A, Rf 0.881; IR ($CHCl_3$) cm^{-1} 3510, 2955, 1735, 1690, 1455, 1375, 1290, 1260, 1200, 1155, 1055; ^1H NMR (100 MHz, $CDCl_3$) δ 2.25 (3H, 9a-NCH_3), 2.83, 2.80 (3H, 3'-NCH_3), 3.37 (3H, 3''-OCH_3), 3.31 (3H, 6-OCH_3), 3.47 (3H, 4''-OCH_3), 3.49 (3H, 11-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0 (C-1), 69.9 (C-9), 36.0 (9a-NCH_3), 79.1 (C-6), 52.8 (6-OCH_3), 49.2 and 49.5 (3''-OCH_3), 36.5 (3'-NCH_3); *Anal* Calcd for $C_{56}H_{88}N_2O_{16}$: C 64.41, H 8.49, N 2.68. Found: C 64.75, H 8.32, N 2.56.

N-Demethyl-6-*O*-methylazithromycin (**7**)

To a soln of **3** (2.0 g) in EtOH (30 ml) and water (10 ml) containing 0.19 ml AcOH and 0.30 g AcONa was added Pd - C 10% (0.7 g) and the mixture was stirred under hydrogen atmosphere for 10 hours at ambient temperature. The catalyst was filtered off, and the filtrate evaporated under reduced pressure. To the obtained residue water (30 ml) and $CHCl_3$ (30 ml) were added, and the product isolated by pH gradient extraction (pH 5.0 and 9.0). The organic layer at pH 9.0 was dried (K_2CO_3) and evaporated to give 1.03 g (70%) of **7**; MP 114~118°C; TLC, system A, Rf 0.182; IR ($CHCl_3$) cm^{-1} 3670, 3500, 2960, 2920, 1725, 1460, 1375, 1345, 1320, 1280, 1260, 1165, 1120, 1085, 1045, 1010, 995, 900; ^1H NMR (300 MHz, $CDCl_3$) δ 2.28 (3H, 9a-NCH_3), 2.41 (3H, 3'-NCH_3), 3.31 (3H, 3''-OCH_3), 3.38 (3H, 6-OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.2 (C-1), 79.2 (C-6), 52.7 (6-OCH_3), 27.0 (C-8), 73.7 (C-12), 102.7 (C-1'), 74.5 (C-2'), 60.5 (C-3'), 33.1 (3'-NCH_3), 95.7 (C-1''), 35.0 (C-2''), 73.0 (C-3''), 65.7 (C-5''); EI-MS *m/z* 748 (M^+); *Anal* Calcd for $C_{38}H_{72}N_2O_{12}$: C 60.95, H 9.69, N 3.74. Found: C 60.51, H 9.86, N 3.64.

N-Demethyl-6,11-di-*O*-methylazithromycin (**8**)

Deprotection of **4** (165 mg) in EtOH and AcOH - AcONa buffer (pH 5) in the hydrogen atmosphere using Pd - C 10% as catalyst according to the procedure as described for **7** afforded **8** (93 mg, 76.2%); MP 95~98°C; TLC, system A, Rf 0.331; IR ($CHCl_3$) cm^{-1} 3560, 3500, 2980, 2940, 1740, 1465, 1385, 1325,

1260, 1170, 1130, 1100, 960, 810; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (3H, 9a-NCH_3), 2.42 (3H, 3'-NCH_3), 3.31 (3H, 3''-OCH_3), 3.37 (3H, 6-OCH_3), 3.52 (3H, 11-OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.7 (C-1), 65.9 (C-9), 36.8 (9a-NCH_3), 79.3 (C-6), 88.9 (C-11), 52.7 (6-OCH_3), 62.0 (11-OCH_3), 33.1 (3'-NCH_3) and 49.7 (3''-OCH_3); EI-MS *m/z* 762 (M^+); *Anal* Calcd for $C_{39}H_{74}N_2O_{12}$: C 61.39, H 9.78, N 3.69. Found: C 61.38, H 9.63, N 3.48.

N-Demethyl-11-*O*-methylazithromycin (**9**)

Deprotection of **5** (250 mg) in EtOH and AcOH - AcONa buffer (pH 5.0) under hydrogen atmosphere using Pd - C 10% as catalyst according to the procedure as described for **7** gave 168 mg (89.5%) of **9** as colorless foam; MP 110~113°C; TLC, system A, Rf 0.244; IR ($CHCl_3$) cm^{-1} 3500, 2970, 2940, 1735, 1460, 1380, 1165; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (3H, 9a-NCH_3), 2.46 (3H, 3'-NCH_3), 3.34 (3H, 3''-OCH_3), 3.59 (3H, 11-OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.6 (C-1), 70.7 (C-9), 35.8 (9a-NCH_3), 74.4 (C-6), 85.0 (C-11), 62.7 (11-OCH_3), 36.7 (3'-NCH_3), 49.4 (3''-OCH_3); EI-MS *m/z* 748 (M^+); *Anal* Calcd for $C_{38}H_{72}N_2O_{12}$: C 60.95, H 9.69, N 3.74. Found: C 60.51, H 9.86, N 3.64.

N-Demethyl-6,11,4''-tri-*O*-methylazithromycin (**10**)

Deprotection of **6** (3.35 g) in EtOH and AcOH - AcONa buffer (pH 5.0) according to the procedure as described for **7** afforded 1.41 g (56.7%) of colorless foam which was purified by silica gel column chromatography (CH_2Cl_2 - CH_3OH - conc NH_4OH, 90 : 9 : 0.5) to give the pure **10**; MP 93~97°C; TLC, system A, Rf 0.263; IR ($CHCl_3$) cm^{-1} 3500, 2975, 2940, 1740, 1465, 1385, 1260, 1160, 1105, 1015, 755; ^{1}H NMR ($CDCl_3$) δ 2.26 (3H, 9a-NCH_3), 2.39 (3H, 3'-NCH_3), 3.31 (6H, 3''-OCH_3, 6-OCH_3), 3.48 (4''-OCH_3), 3.52 (11-OCH_3); ^{13}C NMR ($CDCl_3$) δ 175.0 (C-1), 64.8 (C-9), 79.8 (C-6), 50.6 (6-OCH_3), 86.1 (C-11), 59.1 (11-OCH_3), 87.7 (C-4''), 60.9 (4''-OCH_3); EI-MS *m/z* 776 (M^+); *Anal* Calcd for $C_{40}H_{76}N_2O_{12}$: C 61.81, H 9.86, N 3.60. Found: C 61.56, H 9.71, N 3.15.

6-*O*-Methylazithromycin (**11**)

To a soln of **7** (0.5 g) in $CHCl_3$ (30 ml) were added 37% aqueous HCOH (0.128 ml) and 98% HCOOH (0.118 ml), and the reaction mixture was stirred vigorously for 8 hours at reflux temperature. After the complete absence of **7** (TLC, system A), the soln was poured into water (20 ml), the pH of the mixture adjusted with 1 N HCl to 5.0, the aqueous layer separated and washed with $CHCl_3$. To the aqueous layer was added $CHCl_3$ (20 ml), the pH of the soln was adjusted with 2 N NaOH to 9.0, the layers separated, and the aqueous layer extracted with $CHCl_3$. The combined organic extracts at pH 9.0 were dried (K_2CO_3) and evaporated *in vacuo* to give 0.46 g (90.1%) of **11** as a colorless foam; MP 103~109°C; TLC, system A, Rf 0.346; IR (KBr) cm^{-1} 3500, 2980, 2940, 1740, 1462, 1385, 1330, 1280, 1260, 1170, 1112, 1059, 1018, 1055; ^{1}H NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C NMR (300 MHz, $CDCl_3$): Table 2; EI-MS *m/z* 762; *Anal* Calcd for $C_{39}H_{74}N_2O_{12}$: C 61.39, H 9.78, N 3.69. Found: C 60.97, H 9.70, N 3.46.

6,11-Di-*O*-methylazithromycin (**12**)

Reductive *N*-methylation of **8** (0.49 g) was carried out according to the procedure described for **11** to afford 0.46 g (92.3%) of the pure **12** as colorless foam; MP 100~103°C; TLC, system A, Rf 0.391; IR ($CHCl_3$) cm^{-1} 3500, 2980, 2940, 1735, 1465, 1385, 1165, 1105, 760; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, 9a-NCH_3), 2.32 (6H, 3'-$N(CH_3)_2$), 3.32 (3H, 3''-OCH_3), 3.38 (3H, 6-OCH_3), 3.52 (3H, 11-OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$): Table 2; EI-MS *m/z* 776 (M^+); *Anal* Calcd for $C_{40}H_{76}N_2O_{11}$: C 61.82, H 9.86, N 3.6. Found: C 61.48, H 9.36, N 3.72.

11-*O*-Methylazithromycin (**13**)

Reductive *N*-methylation of **9** (0.59 g) with 37% aqueous HCOH (0.151 ml) and 98% HCOOH (0.139 ml) in $CHCl_3$ (30 ml) was carried out according to the procedure described for **11** (81.5%). Compound **13** was obtained as colorless glass, which was crystallized from ether - *n*-hexane; MP 102~104°C; TLC, system A, Rf 0.428; IR (KBr) cm^{-1} 3510, 2975, 2940, 1738, 1460, 1350, 1165, 1054; ^{1}H NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C NMR (300 MHz, $CDCl_3$): Table 2; EI-MS *m/z* 762 (M^+); *Anal* Calcd for $C_{39}H_{74}N_2O_{12}$: C 61.39, H 9.78, N 3.69. Found: C 61.56, H 9.70, N 3.42.

6,11,4″-Tri-*O*-methylazithromycin (**14**)

Reductive *N*-methylation of **10** (1.2 g) was carried out with 37% HCOH soln (0.131 ml) and 98% HCOOH soln in $CHCl_3$ (30 ml) according to the procedure described for **11**. Purification of the product by silica gel column chromatography (CH_2Cl_2 - CH_3OH - conc NH_4OH, 90 : 9 : 0.5) afforded 0.75 g (64.4%) of **14** as colorless foam; MP 112~116°C; TLC, system A, Rf 0.456; IR ($CHCl_3$) cm^{-1} 3500, 2980, 2940, 1740, 1465, 1385, 1165, 1100, 1015; ^1H NMR (300 MHz, $CDCl_3$) δ 2.22 (3H, 9a-NCH_3), 2.31 (6H, 3′-$N(CH_3)_2$), 3.32 (3H, 3″-OCH_3), 3.30 (6-OCH_3), 3.48 (4″-OCH_3), 3.52 (11-OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$): Table 2; EI-MS *m/z* 790 (M^+); *Anal* Calcd for $C_{41}H_{78}N_2O_{12}$: C 62.25, H 9.94, N 3.53. Found: C 61.86, H 9.43, N 3.26.

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