VOL. 54 NO. 8, AUG. 2001 THE JOURNAL OF ANTIBIOTICS pp. 664 — 678

Synthesis and Antibacterial Activity of the Tricyclic Ketolides TE-802 and Its Analogs

MASATO KASHIMURA*, TOSHIFUMI ASAKA, YOKO MISAWA,
KEITA MATSUMOTO and SHIGEO MORIMOTO

Research Center, Taisho Pharmaceutical Co., Ltd.,
1-403 Yoshino-cho, Omiya-shi, Saitama 330-8530, Japan

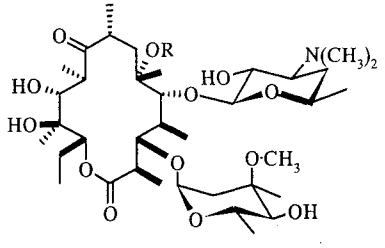
(Received for publication February 19, 2001)

The novel 6-*O*-methyl tricyclic ketolides TE-802 and its analogs were synthesized by two successive cyclization reactions, 11,12-cyclic carbamate formation by intramolecular Michael addition and 9,11-diazaheptene ring construction by intramolecular dehydration reaction. These new tricyclic ketolides exhibited good *in vitro* antibacterial activity against not only erythromycin-susceptible strains but also erythromycin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*, which are problematic pathogens of nosocomial and community-acquired respiratory tract infections, respectively.

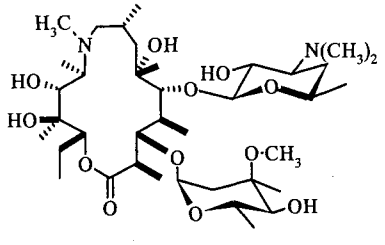
Macrolide antibiotics have been used for almost a half-century against many infectious diseases, especially respiratory tract infections caused by Gram-positive bacteria and *Mycoplasma* bacteria, *Mycoplasma*, *Clamydia* and *Legionella*. Erythromycin (erythromycin A, EM-A, **1**) is one of the most important macrolide antibiotics. A number of EM-A derivatives have been prepared in order to improve the poor bioavailability of EM-A, which results from its acid-instability[1]. These efforts contributed to successful development of so-called the second-generation macrolide antibiotics such as clarithromycin (CAM, **2**)[2], roxithromycin and azithromycin (AZM, **3**)[3] in the early 1990 s.

These macrolide antibiotics have increased acid stability and exhibit some pharmacological profiles superior to those of EM-A. Recently, however, the increase in frequency of erythromycin-resistant bacteria has become a serious therapeutic problem[4]. Thus, new macrolides having expanded antibacterial spectra including erythromycin-resistant organisms have been expected. In the course of our study on chemical modification of EM-A, we found a unique synthetic method for the construction of a 9,11; 11,12-cyclic system. Herein, we report the synthesis and evaluation of novel ketolides with a unique tricyclic structure in their aglycon moiety.

Fig. 1.



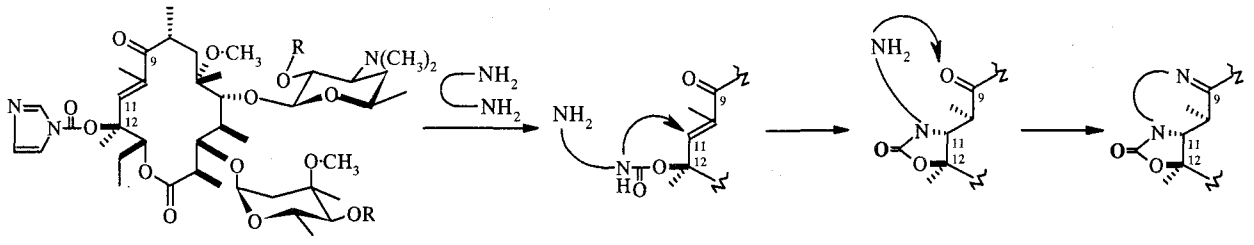
1 : R = H Erythromycin A
2 : R = CH_3 Clarithromycin



3 : Azithromycin

* Corresponding author: m.kashimura@po.rd.taisho.co.jp

Fig. 2.



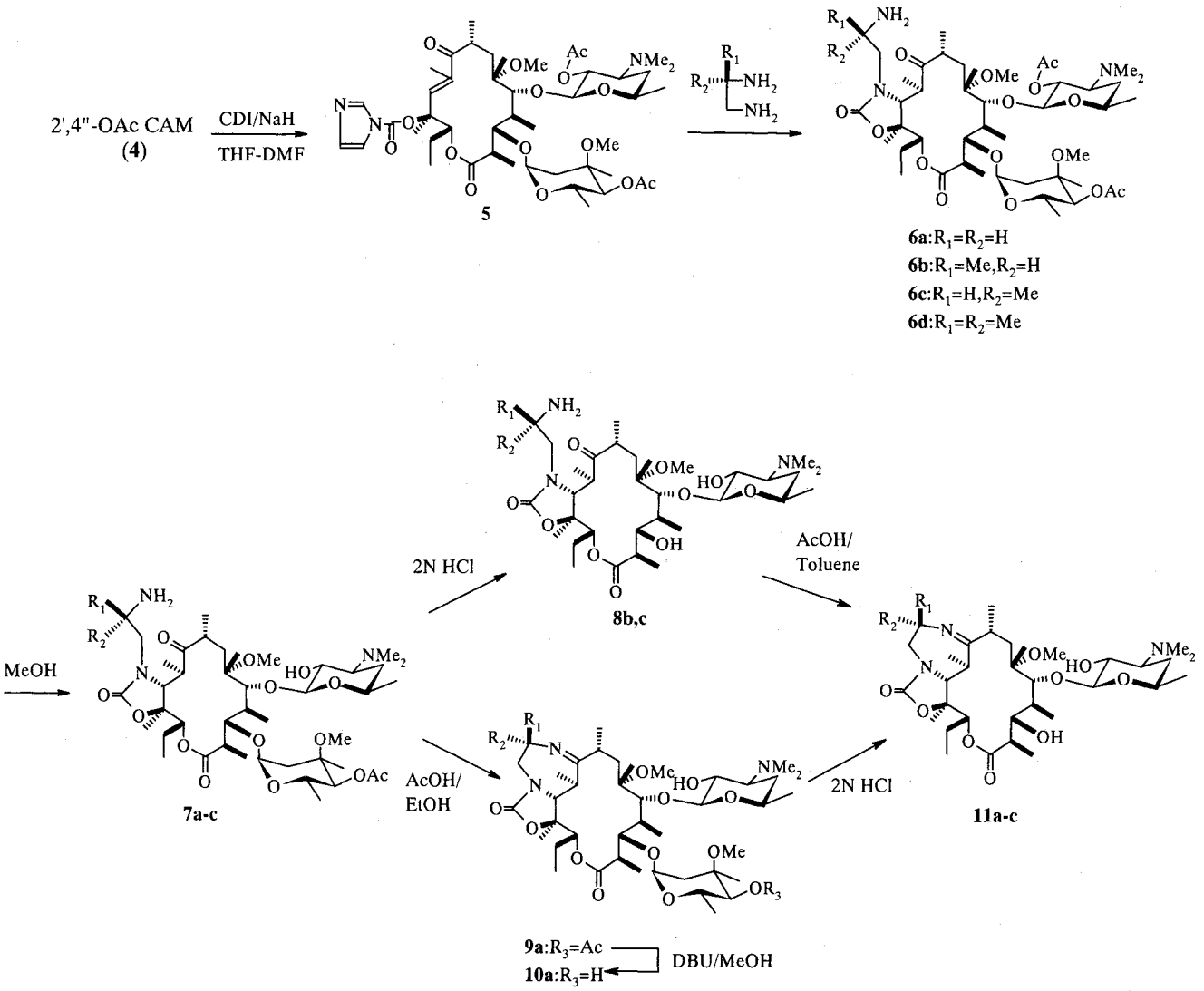
Chemistry

It has been reported[5] that 10,11-anhydro-12-*O*-acyl imidazolide could be converted into 11,12-cyclic carbamate by treatment with various amines. On the other hand, the derivation of 9-keto moiety to an imino group has also been known as 9-hydrazine[6] and 9-oxime[7] derivatives. We have applied these independent findings to a design of a new macrolide antibiotic with a unique tricyclic aglycone nucleus. Our synthetic strategy is shown in Figure 2. We planned to treat 12-*O*-acyl imidazolide with alkyldiamine to form 11,12-cyclic carbamate followed by cyclization between 9-keto with the remaining amino group on the alkyl side chain on the 11,12-carbamate ring by intramolecular dehydration reaction.

According to our plan, compound **5** was treated with 10 equivalents of ethylenediamine in acetonitrile (CH_3CN) at ambient temperature. With disappearance of starting material **5**, 11,12-cyclic carbamate compound **6a** newly appeared. After deprotection of the acetyl group at the 2′-position of **6a**, the structure of **7a** was determined by NMR spectroscopic analysis. In the ^1H NMR spectrum of **7a**, the olefin proton at the 11-position (6.66 ppm) of **5** disappeared and a new methine proton (3.66 ppm) of **7a** was observed. In the ^{13}C NMR spectrum of **7a**, C-11 (137.8 ppm) in **5** was shifted upfield to 60.2 ppm in **7a**. In addition, in the ^1H-Detected Multiple-bond Heteronuclear Multiple Quantum Coherence (HMBC) spectrum of **7a**, correlation between 11-H and the carbonyl carbon of carbamate was observed. These spectral data indicated formation of the desired 5-membered carbamate ring. Interestingly, we predominantly obtained compound **6a**, which had natural 10-*R* stereochemistry. We will discuss the stereochemistry at the 10-position in detail later. Next, we investigated the intramolecular dehydration reaction of **7a**~**c** to obtain a 9,11-ring. This diazaheptene ring construction process was the key step in obtaining the tricyclic compound. In our initial investigations, the free amine **7a** was heated in alcohol (EtOH or MeOH) for a few hours to obtain a 9-imino compound. However, we detected only a trace amount of the desired 9-imino derivative on TLC analysis.

We therefore investigated reaction conditions for ring construction at the 9-position. It became apparent that the addition of a small excess of organic acids (1.5~2.5 equiv) such as formic acid and acetic acid was effective for this reaction. Compound **7a** was heated at 60°C with glacial acetic acid (1.9 equiv) in EtOH for 4 hours to obtain the corresponding 9-imino compound **9a** in good yield. In further study, it was revealed that toluene was also a good solvent for intramoleculer dehydration reaction. In the ^{13}C NMR spectrum of **9a**, the 9-keto signal disappeared (216.1 ppm in **7a**) and the 9-imine signal was newly observed at 181.5 ppm. Formation of a diazaheptene ring was confirmed by 2D NMR studies (^1H-^1H COSY, ^1H-^{13}C COSY and HMBC) of **9a**. As typical evidence, correlation between the proton of 11-NCH_2– and each carbon of C-11 and carbonyl of carbamate, and between the proton of 9=NCH_2– and carbon of C-9 have been observed in HMBC spectrum of **9a**. Then compound **9a** was treated with 5 equiv of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in MeOH to obtain compound **10a**. Since we could obtain the desired tricyclic macrolide, we attempted to synthesize 9,11-diazaoctene and diazanonene ring compounds. For the above purpose, 11-*N*-aminopropyl and 11-*N*-aminobutyl 11,12-cyclic carbamates, synthesized from **5** using 1,3-diaminopropane and 1,4-diaminobutane according to the synthesis of **6a**, were subjected to the above ring construction conditions, but we could not obtain the expected diazaoctene and diazanonene derivatives. Perhaps, the aminoethyl group would be a favorable chain length for intramolecular ring construction at 9-position, while the

Scheme 1.

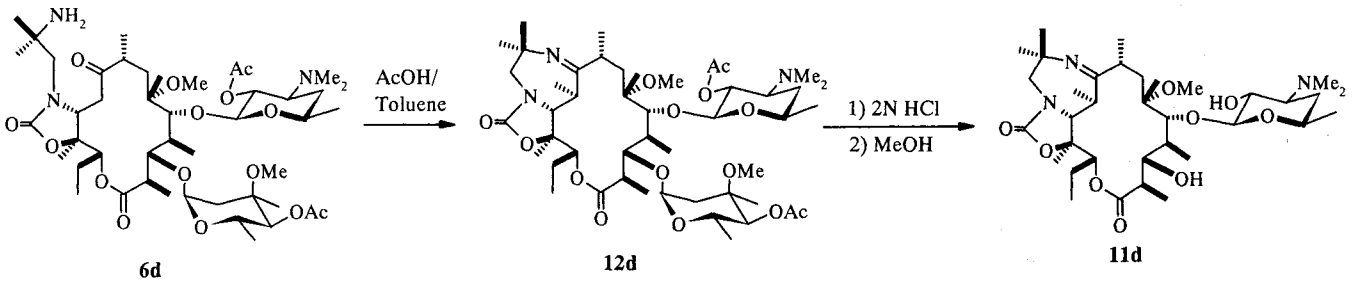


aminopropyl and aminobutyl groups may be difficult to approach to the carbonyl group at 9-position due to its long chain length.

On the other hand, we found 3-keto derivatives exhibiting good *in vitro*[8] and *in vivo* antibacterial activities in our previous studies of modification at the 3-position of erythromycins. We therefore determined the conversion of tricyclic macrolides to 3-keto derivatives. Then **9a** was treated with 2 N hydrochloric acid to obtain decladinosyl compound **11a** in high yield. Compound **11d** was also synthesized from **6d** in a manner similar to that of **11a** (Scheme 2). In this step, we confirmed that removal of cladinose could be carried out prior to 9,11-cyclization. We have synthesized compounds **11b** and **11c** from compounds **7b** and **7c**, derived from **5** with 1,2-diaminopropane, by the latter method. Compound **8b** and its epimer **8c** were separated in this step using silica gel column chromatography. After protection of the 2′-hydroxy group of tricyclic compounds **11a**~**d** by acetic anhydride, obtained **12a**~**d** were subjected to modified Pfitzner-Moffat oxidation[9] (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl), DMSO and pyridinium trifluoroacetate in dichloromethane (CH_2Cl_2)) to obtain 3-keto compounds **15a**~**d**.

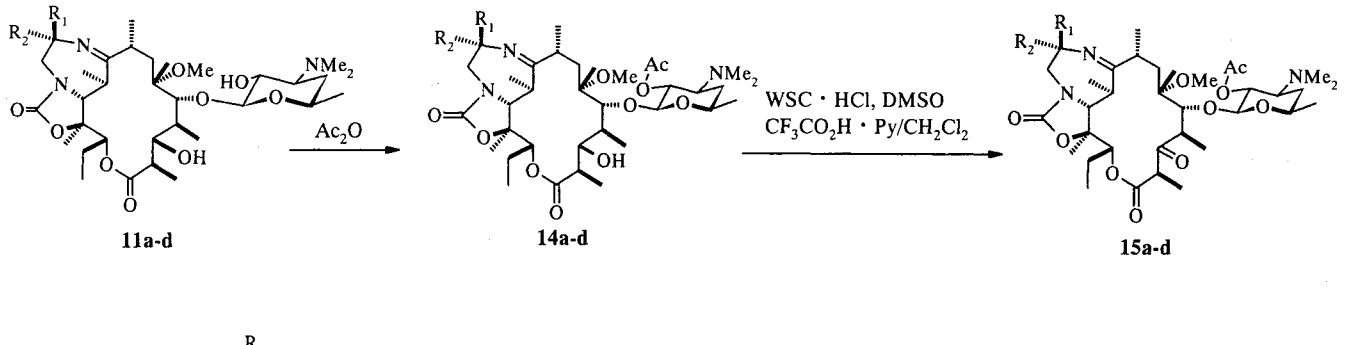
Finally, desired tricyclic ketolides **16a**~**d** were obtained by methanolysis of **15a**~**d** in good yield. Compounds **16b** and **16c** are epimers with respect to the 9,11-*N*-(2-methyl)nitriloethano moiety in the diazaheptene ring. We

Scheme 2.

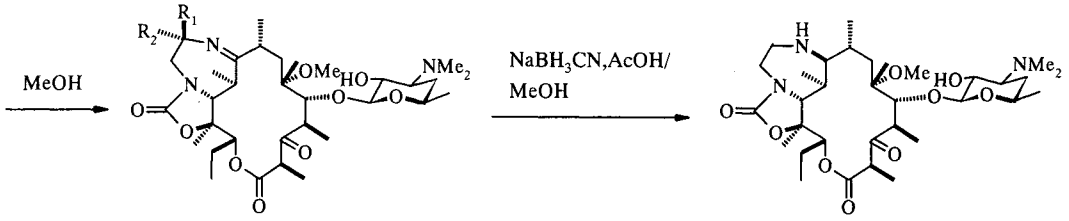


6d 12d 11d

Scheme 3.



11a-d 14a-d 15a-d



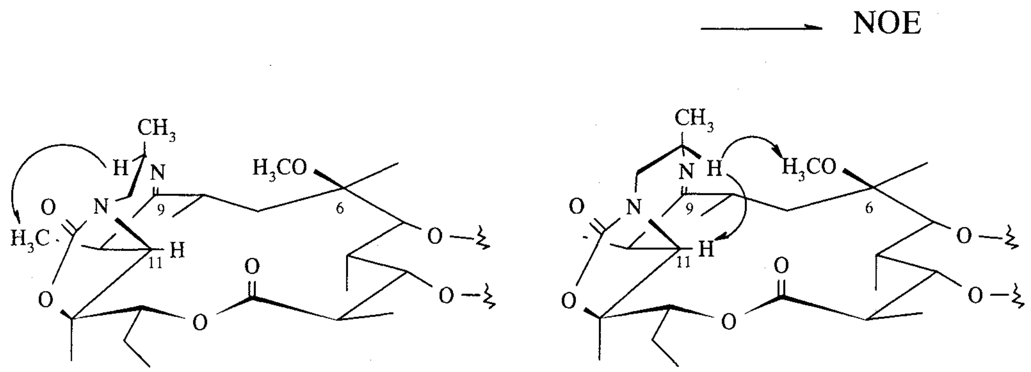
16a:R_1=R_2=H (**TE-802**)
16b:R_1=Me,R_2=H
16c:R_1=H,R_2=Me
16d:R_1=R_2=Me

17

have therefore studied the stereochemistry at the diazaheptene ring by NOESY experiment. The NOESY spectrum of **16b** revealed NOE enhancement between the methine proton on 9,11-*N*-(2-methyl)nitriloethano moiety and the proton of 10-CH_3. On the other hand, the NOESY spectrum of **16c** revealed NOE enhancements between the methine proton on 9,11-*N*-(2-methyl)nitriloethano moiety and each proton of 6-OCH_3 and 11-H. These results revealed that the stereochemistry of 9,11-*N*-(2-methyl)-nitriloethano moiety to be *R*-configuration for **16b** and *S*-configuration for **16c**. The stereochemistry and molecular structure of **16c** were determined by X-ray crystallographic analysis.

9-Amino compound **17** was synthesized from **16a** using $NaBH_3CN$ and acetic acid in methanol. In the ^{13}C NMR spectrum of **17**, upfield shift of C-9 (70.5 ppm (doublet) in **17**) from 181.0 ppm (singlet) in **16a** was observed. The stereochemistry of the 9-position of **17** was determined by NOESY experiment. The NOESY spectrum of **17** revealed NOE enhancements between the proton of 9-H and each proton of 8-CH_3 and 10-CH_3. Thus, the stereochemistry of C-9 in **17** was determined as *S*-configuration.

Fig. 3. Stereochemistries of diazaheptene moiety in **16b** and **16c**.



16b **16c**

Fig. 4. Structure of **16c**.

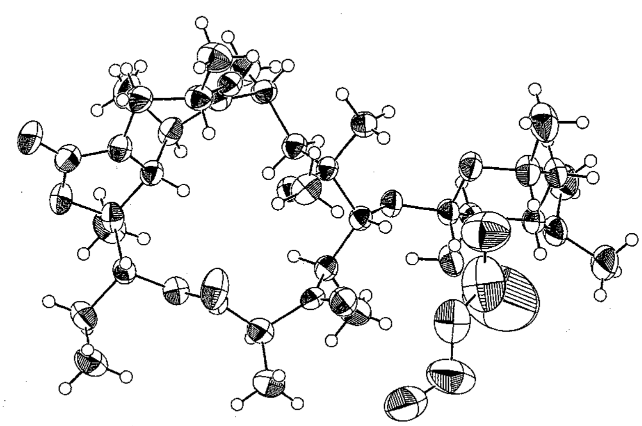
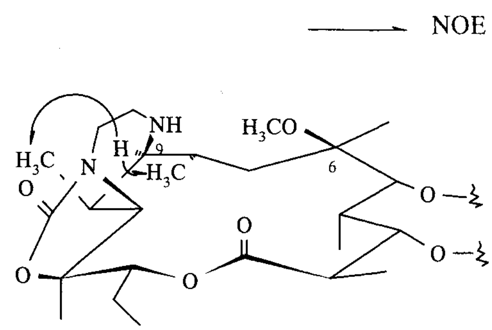


Fig. 5. Stereochemistry at 9-position in **17**.



In Vitro Antibacterial Activity

In vitro activities (MICs) were measured using standard agar dilution methods. First, we studied structure-activity relationships for a variety of 3-positions of tricyclic macrolides (Table 3). Compound **10a**, a tricyclic compound, was 2 to 4 times more active than CAM (**2**) against erythromycin-susceptible organisms, whereas **10a** was inactive against erythromycin-resistant organisms, similar to CAM (**2**). Compound **11a**, decladinosyl **10a**, exhibited weak *in vitro* activity against most organisms (MICs: 3.13~>100 μg/ml). Compared with **10a** and **11a**, TE-802 (**16a**), a 3-keto compound, exhibited excellent activity against erythromycin-susceptible and -resistant strains, except for highly resistant *S. pneumoniae* 221. Although it had been thought that the cladinose at the 3-position was essential for antibacterial activity, antibacterial activity could be revived by oxidation of the 3-hydroxy group. The above results revealed that a combination of tricyclic aglycon and 3-keto structure yielded strong antibacterial activity.

Next, *in vitro* activities of tricyclic ketolides **16a~d** and **17** were compared with those of CAM (**2**) and AZM (**3**) against both standard strains and erythromycin-resistant organisms (Tables 4 and 5). As shown in Table 4, the potency of 9-amino compound **17** was equal to that of AZM (**3**), and 2 or 4 times less active than those of CAM (**2**) and 9-imino compounds **16a~d**. The potencies of **16a~d** were similar to that of CAM (**2**) against most standard strains, but 4 to 32 times stronger than that of CAM (**2**) against Enterococci.

In addition, tricyclic ketolides exhibited greatly improved

Table 1. ^{1}H NMR chemical shifts for tricyclic macrolides.

Position	16a	16b	16c	16d	17	10a	11a
2	3.82	3.82	3.79	3.79	3.85	2.86	2.70
3	--	--	--	--	--	3.82	3.58
4	3.08	3.08	3.04	3.06	3.14	1.90	2.03
5	4.21	4.19	4.16	4.18	4.27	3.64	3.68
7_A	1.50	1.48	1.51	1.51	1.25	1.60	1.48
7_B	1.71	1.69	1.71	1.69	1.51	1.60	1.70
8	2.70	2.69	2.69	3.14	2.17	2.71	2.73
9	--	--	--	--	2.47	--	--
10	2.73	2.71	2.72	2.75	1.99	2.75	2.80
11	3.73	3.74	3.66	3.50	3.72	3.67	3.68
13	4.95	4.92	4.94	4.86	4.96	4.98	5.09
14_A	1.55	1.55	1.57	1.55	1.57	1.53	1.55
14_B	1.91	1.90	1.94	1.92	1.94	1.87	1.91
9-NCH_A	3.78	3.91	3.99	--	2.77	3.78	3.76
9-NCH_B	3.78	--	--	--	3.15	3.78	3.83
9-$NC(CH_3)_A$	--	1.27	1.34	1.29	--	--	--
9-$NC(CH_3)_B$	--	--	--	1.33	--	--	--
11-NCH_A	2.95	2.67	3.38	2.92	2.82	3.01	3.00
11-NCH_B	3.99	3.85	3.47	3.78	4.01	3.99	4.01
2-CH_3	1.39	1.39	1.41	1.41	1.37	1.22	1.27
4-CH_3	1.29	1.28	1.27	1.27	1.29	1.08	1.12
6-CH_3	1.36	1.37	1.39	1.35	1.33	1.42	1.41
8-CH_3	1.05	1.04	1.06	1.03	0.94	1.04	1.05
10-CH_3	1.22	1.22	1.18	1.23	1.09	1.20	1.22
12-CH_3	1.48	1.47	1.47	1.48	1.47	1.42	1.46
14-CH_3	0.86	0.86	0.88	0.87	0.86	0.85	0.84
6-OCH_3	2.74	2.71	2.67	2.70	2.90	3.09	3.02
1'	4.30	4.30	4.28	4.28	4.31	4.43	4.37
2'	3.19	3.20	3.19	3.19	3.19	3.19	3.25
2'-OH	3.49	3.48	3.47	--	--	3.41	3.36
3'	2.44	2.45	2.44	2.44	2.45	2.42	2.48
3'-$N(CH_3)_2$	2.26	2.27	2.26	2.27	2.27	2.28	2.26
$4'_A$	1.23	1.22	1.22	1.23	1.23	1.20	1.24
$4'_B$	1.67	1.67	1.66	1.66	1.67	1.66	1.68
5'	3.54	3.54	3.53	3.53	3.54	3.48	3.54
5'-CH_3	1.24	1.24	1.24	1.23	1.25	1.25	1.27
1"						4.92	
$2"_A$						1.57	
$2"_B$						2.37	
4"						3.03	
5"						4.02	
3"-CH_3						1.26	
5"-CH_3						1.31	
3"-OCH_3						3.33	

All spectra were taken in $CDCl_3$ at 500 MHz and chemical shifts are reported in ppm relative to TMS.

Table 2. ^{13}C NMR chemical shifts for tricyclic macrolides.

Position	16a	16b	16c	16d	17	10a	11a
1	169.5	169.5	169.4	169.5	169.8	176.7	175.6
2	51.2	51.2	51.2	51.2	51.5	45.5	44.7
3	204.2	204.2	203.8	204.0	204.0	77.9	78.7
4	48.1	48.2	48.6	48.6	47.4	39.8	36.3
5	79.2	79.4	80.4	79.7	78.1	80.0	88.1
6	78.5	78.6	78.6	78.6	79.0	79.2	78.8
7	38.6	38.6	39.9	39.5	35.7	38.1	38.3
8	42.8	42.9	43.5	44.4	33.5	43.2	43.2
9	181.0	178.1	176.5	174.9	70.5	181.6	181.1
10	36.3	36.2	35.3	35.9	34.7	36.5	36.6
11	59.9	59.5	61.8	61.7	61.1	59.7	60.4
12	81.5	81.5	81.0	81.4	82.1	81.9	82.0
13	76.5	76.3	78.0	77.4	76.7	75.3	75.3
14	22.1	22.0	22.1	21.8	22.2	21.8	22.0
9-NC	49.6	53.4	54.4	58.5	47.3	49.5	49.2
9-NC$(CH_3)_A$	--	22.9	20.9	25.2	--	--	--
9-NC$(CH_3)_B$	--	--	--	32.8	--	--	--
11-NCH_2	42.3	48.0	48.4	53.4	46.4	42.4	42.5
11-NCOO-12	156.0	156.1	156.5	156.6	156.3	156.3	156.3
2-CH_3	16.4	16.6	17.1	17.1	15.6	15.9	15.2
4-CH_3	14.4	14.4	14.4	14.5	14.7	9.0	8.3
6-CH_3	19.6	19.7	19.4	19.5	20.0	20.0	18.7
8-CH_3	19.1	19.1	19.7	19.6	23.0	19.6	19.5
10-CH_3	10.9	11.1	16.8	16.1	19.4	10.9	11.0
12-CH_3	12.8	12.7	13.9	13.3	13.1	12.5	12.7
14-CH_3	10.4	10.4	10.4	10.4	10.4	10.3	10.1
6-OCH_3	49.1	49.0	49.5	49.2	49.0	49.9	49.1
1'	103.9	103.9	104.1	104.1	103.8	102.9	106.9
2'	70.3	70.3	70.3	70.3	70.3	70.9	70.7
3'	65.9	65.9	65.9	65.9	65.8	65.5	65.7
4'	28.1	28.1	28.1	28.2	28.1	28.6	28.0
5'	69.5	69.5	69.5	69.6	69.5	68.8	70.4
3'-$N(CH_3)_2$	40.2	40.2	40.2	40.3	40.2	40.2	40.2
5'-CH_3	21.2	21.1	21.1	21.2	21.1	21.4	21.2
1"						95.9	
2"						34.7	
3"						72.6	
4"						77.9	
5"						65.6	
3"-CH_3						21.5	
5"-CH_3						18.6	
3"-OCH_3						49.4	

All spectra were taken in $CDCl_3$ at 125 MHz and chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm).

Table 3. Antibacterial activities of tricyclic compounds.

Strain	MIC(μg/ml)			
	TE-802 (16a)	**10a**	**11a**	**CAM (2)**
Erythromycin-susceptible				
Staphylococcus aureus 209P-JC	0.10	0.05	100	0.10
Enterococcus faecalis ATCC19433	0.10	0.20	25	0.78
E. faecium ATCC19434	0.10	0.78	3.13	3.13
Moraxella catarrhalis ATCC25238	0.39	0.20	25	0.20
Streptococcus pneumoniae IID 553	0.05	0.05	3.13	0.10
Escherichia coli TM36	3.13	3.13	50	6.25
Erythromycin-resistant				
S. aureus B1	0.39	>100	>100	>100
S. aureus T166	0.39	>100	>100	>100
S. pneumoniae 210	0.10	0.39	6.25	0.78
S. pneumoniae 221	>100	>100	>100	>100

Inoculumn size : 10^6 cfu/ml.

Table 4. Antibacterial activities of tricyclic ketolides **16a~d** and **17** against standard strains.

Strain	MIC(μg/ml)						
	TE-802 (16a)	**16b**	**16c**	**16d**	**17**	**CAM (2)**	**AZM (3)**
Staphylococcus aureus 209P-JC	0.10	0.10	0.20	0.20	0.39	0.10	0.39
S.epidermidis IID866	0.10	0.10	0.10	0.20	0.39	0.10	0.39
Streptococcus pneumoniae IID 553	0.05	0.05	0.05	0.05	0.10	0.05	0.20
S.pyogenes IID 689	0.05	0.05	0.05	0.05	0.20	0.05	0.10
Enterococcus faecalis ATCC29212	0.20	0.20	0.20	0.20	0.39	0.78	12.5
E.faecium ATCC19434	0.025	0.05	0.025	0.05	0.10	0.78	3.13
Haemophilus influenzae ATCC43095	6.25	6.25	3.13	6.25	12.5	6.25	1.56
Moraxella catarrhalis ATCC25238	0.20	0.39	0.20	0.20	0.78	0.20	0.10
Klebsiella pneumoniae IFO3317	6.25	12.5	6.25	12.5	25	50	3.13
Escherichia coli NIHJ JC-2	100	>100	50	100	>100	100	12.5

Inoculumn size : 10^6 cfu/ml.

activities (MICs : 0.20~1.56 μg/ml) against erythromycin-resistant *S. aureus* (inducible type), although CAM (**2**) and AZM (**3**) were inactive (MICs : >100 μg/ml) against these strains. Furthermore, tricyclic ketolides were 4 to 32 times more active than CAM (**2**) against intermediate resistant *S. pneumoniae*. Surprisingly, tricyclic ketolides exhibited strong activity (MICs : 1.56~6.25 μg/ml) against highly resistant *S. pneumoniae* 225, against which CAM (**2**) and AZM (**3**) were entirely inactive, as well.

Discussion

In the intramoleculer Michael addition of 10,11-anhydro-12-carbamate compound, we considered two possibilities concerning cyclization mode because we used alkyldiamine as an amine source.

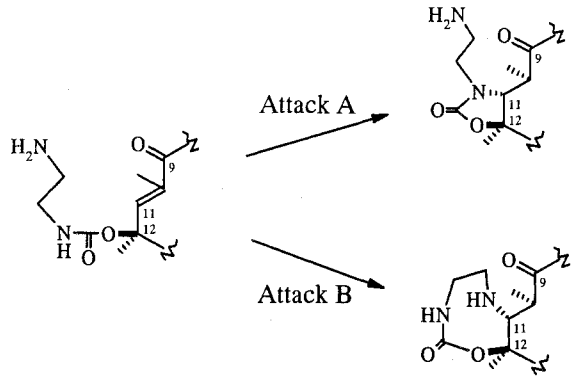
The first possibility was the formation of the desired 5-membered cyclic carbamate by attack of carbamate nitrogen atom on enone (attack A). The second possibility was the formation of 8-membered cyclic carbamate by attack of primary amine of alkyl side chain on enone

Table 5. Antibacterial activities of tricyclic ketolides **16a**~**d** and **17** against resistant strains.

Strain	MIC(μg/ml)						
	TE-802 (16a)	**16b**	**16c**	**16d**	**17**	**CAM (2)**	**AZM (3)**
Inducible type							
Staphylococcus aureus B1	0.20	0.20	0.20	0.20	0.78	>100	>100
S. aureus C1	0.39	0.20	0.39	0.39	1.56	>100	>100
S. aureus 166	1.56	0.39	1.56	1.56	1.56	>100	>100
Constitutive type							
S. aureus K-2	>100	>100	>100	>100	>100	>100	>100
Intermediate resistant							
Streptococcus pneumoniae 217	0.10	0.10	0.10	0.05	0.10	1.56	1.56
S. pneumoniae 224	0.10	0.10	0.10	0.10	0.20	0.78	1.56
S. pneumoniae 210*	0.10	0.10	0.10	0.10	0.20	0.78	0.78
Highly resistant							
S. pneumoniae 225*	6.25	1.56	3.13	3.13	>100	>100	>100
S. pneumoniae 229	>100	>100	>100	>100	>100	>100	>100

Inoculumn size : 10^6 cfu/ml.
* Penicillin-resistant strain.

Fig. 6.



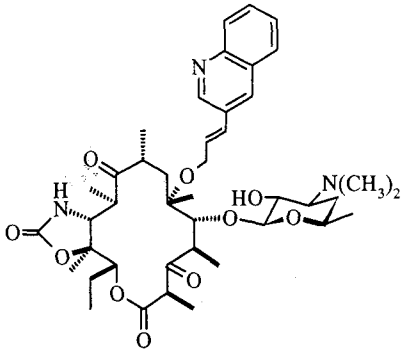
(attack B). In our case, the desired 5-membered cyclic carbamate could be predominantly obtained. We speculate that the carbamate nitrogen atom would be spatially close to the end carbon (11-C) of enone in the Michael addition for attack on A. Furthermore, the 5-membered product would be more stable than the 8-menbered product thermodynamically.

It has been reported[10] by GRIESGRABER *et al.* that the reaction of 3-keto derivative of **5** with hydrazine yielded the kinetically unnatural 10-*S* 11,12-cyclic carbazate, and that the stereochemistry at the 10-position could be thermodynamically converted to natural 10-*R*. In our case, we obtained compound **6a** with natural 10-*R* stereochemistry with high selectivity (10*R* : 10*S*=92 : 8) with use of a large excess of ethylenediamine (10 equiv) even at room temperature. We speculate that the basicity of excess ethylenediamine is preferable for obtaining the natural 10-*R* configuration. The ratio of 10*R*/10*S* became 80/20 when 4.0 equiv of ethylenediamine was used, and the ratio of 10*R*/10*S* ultimately reversed to 40/60 with use of 2.5 equiv of diamine.

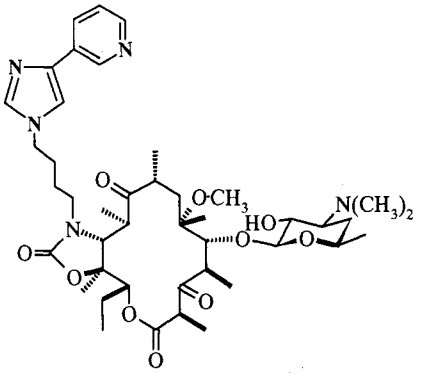
As a new ketolide series, ABT-773[11] and telithromycin[12] have been reported by Abbott Laboratories and Aventis Pharma. These compounds were also effective against erythromycin-resistant organisms similar to TE-802. The structural feature of these compounds is their possession of one or two aromatic heterocycles on a side chain at 6- (in ABT-773) or 11- (in telithromycin) position.

It appears that these aromatic heterocycles play a significant role in binding to ribosomes of erythromycin-resistant bacteria[13]. Among recent ketolides, TE-802 is conspicuously unique in having the heterocycle introduced not on a side chain but inlaid into the aglycon moiety itself as a diazaheptene ring.

Fig. 7.



ABT-773



Telithromycin

Tricyclic ketolides with strong antibacterial activities are expected to be promising next-generation macrolide antibiotics.

Experimental

Mp's are uncorrected. IR spectra were recorded with a Paragon 1000 PC FT-IR (Perkin-Elmer). NMR spectra were recorded with a Jeol alpha-500 and Jeol lambda-500 spectrometers. Assignments for proton and carbon signals were based on ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC experiments. Mass spectra were measured on Platform-LC (Micromass) spectrometer by Electro Spray Ionization Mass Spectrometry (ESI-MS). HRFAB-MS was recorded on a JMS-SX102 (JEOL) mass spectrometer.

2′,4″-Di-*O*-acetyl-6-*O*-methylerythromycin A (**4**)

To a stirred solution of **2** (500 g, 0.668 mol) and 4-dimethylaminopyridine (32.7 g, 0.267 mol, 0.4 equiv) in CH_2Cl_2 (1000 ml) was added acetic anhydride (221 ml, 2.34 mol, 3.5 equiv) in one portion, and the mixture was stirred for 2 days at ambient temperature. The reaction mixture was poured into 0.2 N NaOH soln, and extracted. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evapolated under reduced pressure. The crude product was crystallized from EtOAc to afford **4** (485 g, 87%) as colorless prisms, mp 239~242°C: IR (KBr) cm^{-1} 3487, 1756, 1689; ESI-MS *m/z* 854.3 $(M+Na)^+$; HRFAB-MS *m/z* 832.5058 ($M+H^+$, calcd for $C_{42}H_{73}NO_{15}$: *m/z* 832.5058); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05 (3H, s, 2′-$OCOCH_3$), 2.10 (3H, s, 4″-$OCOCH_3$), 2.28 (6H, s, 3′-$N(CH_3)_2$), 3.01 (3H, s, 6-OCH_3), 3.20 (1H, s, 12-OH), 3.35 (3H, s, 3″-OCH_3), 3.98 (1H, s, 11-OH), 4.67 (1H, d, J=9.5 Hz, 4″-H), 4.76 (1H, dd, J=7.5 and 10.5 Hz, 2′-H), 5.07 (1H, dd, J=1.5 and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9 (4″-$OCOCH_3$), 21.1 (2′-$OCOCH_3$), 40.7 (3′-$N(CH_3)_2$), 49.3 (3″-OCH_3), 50.5 (6-OCH_3), 72.0 (C-2′), 78.6 (C-4″), 95.8 (C-1″), 99.9 (C-1′), 170.0 (2′-$OCOCH_3$), 170.4 (4″-$OCOCH_3$), 175.5 (C-1), 221.1 (C-9).

10,11-Anhydro-2′,4″-di-*O*-acetyl-12-*O*-imidazolylcarbonyl-6-*O*-methylerythromycin A (**5**)

To a solution of **4** (150 g, 0.180 mol) and 1,1′-carbonyldiimidazole (73.1 g, 0.451 mol, 2.5 equiv) in DMF-THF (2 : 3, 600 ml) was added 60% sodium hydride (9.37 g, 0.234 mol, 1.3 equiv) with ice-cooling. After the reaction mixture was stirred for 3.5 hours at ambient temperature, H_2O (1000 ml) was added at 0°C. The above mixture was extracted with EtOAc and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was crystallized from isopropanol to yield **5** (102 g, 62%) as colorless prisms, mp 144~146°C: IR (KBr) cm^{-1} 2979, 1760, 1740, 1674; ESI-MS *m/z* 930.6 $(M+Na)^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (3H, s, 2′-$OCOCH_3$), 2.12 (3H, s, 4″-$OCOCH_3$), 2.27 (6H, s, 3′-$N(CH_3)_2$), 3.14 (3H, s, 6-OCH_3), 3.34 (3H, s, 3″-OCH_3), 6.66 (1H, s, 11-H), 7.07 (1H, m, imidazole-H), 7.36 (1H, m, imidazole-H), 8.08 (1H, m, imidazole-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.7 (3′-$N(CH_3)_2$), 49.5 (3″-OCH_3), 50.8 (6-OCH_3), 117.0, 130.9, 137.0 (imidazole), 137.8 (C-11), 169.9 (2′-$OCOCH_3$),

170.5 (4″-OCOCH$_3$), 174.5 (C-1), 204.7 (C-9). *Anal* Calcd for $C_{46}H_{73}N_3O_{15}$: C 60.84, H 8.10, N 4.63. Found: C 60.51, H 8.28, N 4.46.

4″-*O*-Acetyl-11-amino-11-*N*-aminoethyl-11-deoxy-6-*O*-methylerythromycin A 11,12-Cyclic Carbamate (**7a**)

Ethylenediamine (1.40 ml, 20.9 mmol, 10.0 equiv) was added to a solution of **5** (1.90 g, 2.09 mmol) in CH_3CN (6 ml). The reaction mixture was stirred for 20 hours at room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure to yield **6a** (1.80 g) as a colorless foam. Crude **6a** (1.80 g) was dissolved in MeOH (20 ml) and stirred for 20 hours. The reaction mixture was concentrated under reduced pressure to yield **7a** (1.64 g, 91%) as a colorless foam, IR (KBr) cm^{-1} 3470, 2984, 1736; ESI-MS *m/z* 880.4 $(M+Na)^+$; HRFAB-MS *m/z* 858.5326 ($M+H^+$, calcd for $C_{43}H_{75}N_3O_{14}$: *m/z* 858.5327); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.11 (3H, s, 4″-OCOCH$_3$), 2.30 (6H, s, 3′-N(CH$_3$)$_2$), 3.05 (3H, s, 6-OCH$_3$), 3.32 (3H, s, 3″-OCH$_3$), 3.66 (1H, s, 11-H), 4.56 (1H, d, *J*=7.5 Hz, 1′-H), 4.68 (1H, d, *J*=10.0 Hz, 4″-H), 4.97 (1H, d, *J*=4.5 Hz, 1″-H), 5.10 (1H, dd, *J*=2.0 and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 39.4 (11-NCH$_2$–), 40.2 (3′-N(CH$_3$)$_2$), 46.9 (9-NCH$_2$–), 49.5 (3″-OCH$_3$), 50.6 (6-OCH$_3$), 60.2 (C-11), 78.6 (C-4″), 96.1 (C-1″), 102.2 (C-1′), 158.0 (11-NCOO–), 170.4 (4″-OCOCH$_3$), 176.4 (C-1), 216.1 (C-9).

4″-*O*-Acetyl-11-amino-9-deoxo-11-deoxy-9,11-*N*-nitriloethano-6-*O*-methylerythromycin A 11,12-Cyclic Carbamate (**9a**)

To a stirred solution of **7a** (5.00 g, 5.91 mmol) in EtOH (60 ml) was added glacial acetic acid (0.630 ml, 11.0 mmol, 1.9 equiv). The reaction mixture was stirred at 60°C for 4 hours and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure followed by the addition of $CHCl_3$ and 2 N NaOH soln. The two layers were separated and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure. Silica gel column chromatography ($CHCl_3$-MeOH-NH_4OH (30 : 1 : 0.1)) of the crude product yielded **9a** (3.20 g, 65%) as a white powder. Crystallization of **9a** from diisopropyl ether afforded colorless needles, mp 249~251°C: IR (KBr) cm^{-1} 2976, 1760, 1744; ESI-MS *m/z* 862.4 $(M+Na)^+$; HRFAB-MS *m/z* 840.5230 ($M+H^+$, calcd for $C_{43}H_{73}N_3O_{13}$: *m/z* 840.5222); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.11 (3H, s, 4″-OCOCH$_3$), 2.30 (6H, s, 3′-N(CH$_3$)$_2$), 3.01 (1H, m, 11-NCH_AH$_B$–), 3.09 (3H, s, 6-OCH$_3$), 3.19 (1H, dd, *J*=7.0 and 10.5 Hz, 2′-H), 3.32 (3H, s, 3″-OCH$_3$), 3.43 (1H, br s, 2′-OH), 3.67 (1H, d, *J*=1 Hz, 11-H), 3.73~3.82 (2H, m, 9=NCH$_2$–), 3.99 (1H, m, 11-NCH$_A$H$_B$–), 4.68 (1H, d, *J*=10.0 Hz, 4″-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9 (4″-OCOCH$_3$), 40.2 (3′-N(CH$_3$)$_2$), 42.5 (11-CH$_2$–), 49.5 (9-NCH$_2$– and 3″-OCH$_3$), 50.0 (6-OCH$_3$), 59.7 (C-11), 71.1 (C-2′), 78.6 (C-4″), 79.1 (C-6), 81.9 (C-12), 95.8 (C-1″), 102.2 (C-1′), 156.3 (11-NCOO–), 170.5 (4″-OCOCH$_3$), 176.6 (C-1), 181.5 (C-9).

11-Amino-9-deoxo-11-deoxy-9,11-*N*-nitriloethano-6-*O*-methylerythromycin A 11,12-Cyclic Carbamate (**10a**)

The solution of **9a** (7.00 g, 8.34 mmol) and DBU (6.2 ml, 41.5 mmol, 5.0 equiv) in MeOH (70 ml) was refluxed for 4 hours and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure to yield crude **10a** as a colorless foam. Crystallization from AcOEt-CH_2Cl_2 of crude **10a** yielded 3.2 g (48%) of compound **10a**, mp 253~255°C: IR (KBr) cm^{-1} 3503, 2970, 1764, 1656; ESI-MS *m/z* 820.4 $(M+Na)^+$; *Anal* Calcd for $C_{41}H_{71}N_3O_{12}$: C 61.71, H 8.97, N 5.27. Found: C 61.52, H 9.10, N 5.31.

11-Amino-9-deoxo-11-deoxy-9,11-*N*-nitriloethano-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**11a**)

Compound **9a** (3.20 g, 3.86 mmol) was dissolved in EtOH-2 N HCl (1 : 1, 30 ml) and stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure followed by the addition of EtOAc and 2 N NaOH soln. The two layers were separated and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure to give **11a** (2.20 g, 89%) as a white foam, IR (KBr) cm^{-1} 3472, 2980, 1754; ESI-MS *m/z* 662.3 $(M+Na)^+$; HRFAB-MS *m/z* 640.4171 ($M+H^+$, calcd for $C_{33}H_{57}N_3O_9$: *m/z* 640.4173); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, t, *J*=7.5 Hz, 14-CH$_3$), 2.26 (6H, s, 3′-N(CH$_3$)$_2$), 3.01 (3H, s, 6-OCH$_3$), 4.36 (1H, d, *J*=7.5 Hz, 1′-H), 5.09 (1H, dd, *J*=2.5 and 10.5 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.2 (3′-N(CH$_3$)$_2$), 42.6 (9-NCH$_2$–), 49.1 (6-OCH$_3$), 49.3 (11-NCH$_2$–), 107.0 (C-1′), 156.4 (carbamate), 175.6 (C-1), 181.7 (C-9).

2′-*O*-Acetyl-11-amino-9-deoxo-11-deoxy-9,11-*N*-nitriloethano-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**14a**)

A solution of **11a** (2.00 g, 3.13 mmol) in acetone (20 ml) was treated with acetic anhydride (0.540 ml, 5.71 mmol, 1.8 equiv) at room temperature for 3 hours. The reaction was quenched by addition of 0.2 N NaOH soln and extracted

into EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure to yield **14a** (1.71 g, 80%) as a white foam. Crystallization of **14a** from diisopropyl ether afforded colorless needles, mp 194~196°C; IR (KBr) cm^{-1} 3450, 2972, 1740, 1656; ESI-MS *m/z* 704.3 $(M+Na)^+$; HRFAB-MS *m/z* 682.4277 ($M+H^+$, calcd for $C_{35}H_{59}N_3O_{10}$: *m/z* 682.4279); ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, t, J=7.5 Hz, 14-CH_3), 2.06 (3H, s, 2′-$OCOCH_3$), 2.26 (6H, s, 3′-$N(CH_3)_2$), 2.99 (3H, s, 6-OCH_3), 4.58 (1H, d, J=8.0 Hz, 1′-H), 4.76 (1H, dd, J=8.0 and 10.5 Hz, 2′-H), 5.08 (1H, dd, J=1.5 and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.6 (3′-$N(CH_3)_2$), 42.6 (9-NCH_2–), 49.2 (6-OCH_3), 49.4 (11-NCH_2–), 99.9 (C-1′), 156.4 (11-NCOO–), 169.8 (2′-$OCOCH_3$), 175.3 (C-1), 181.4 (C-9).

11-Amino-9-deoxo-3,11-dideoxy-9,11-*N*-nitriloethano-3-oxo-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**16a**)

The solution of **14a** (1.71 g, 2.51 mmol), DMSO (3.56 ml, 50.2 mmol, 20.0 equiv), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.88 g, 15.0 mmol, 6.0 equiv) in CH_2Cl_2 (17 ml) and pyridine hydrochloride (2.90 g, 15.0 mmol, 6.0 equiv) was stirred at room temperature for 3.5 hours. The reaction mixture was concentrated under reduced pressure followed by the addition of chloroform ($CHCl_3$) and 2 N NaOH soln. The two layers were separated and the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and evaporated under reduced pressure to give crude **15a** (1.53 g). The crude **15a** (1.53 g) was dissolved in MeOH (15 ml) and stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$ - MeOH - NH_4OH (25 : 1 : 0.1)) and crystallized from EtOAc - CH_2Cl_2 gave **16a** (0.957 g, 60%), mp: 243~245°C: IR (KBr) cm^{-1} 2940, 1760, 1650; ESI-MS *m/z* 660.5 $(M+Na)^+$; *Anal* Calcd for $C_{33}H_{55}N_3O_9$: C 62.14, H 8.69, N 6.59. Found: C 61.87, H 8.61, N 6.51.

4″-*O*-Acetyl-11-amino-11-*N*-(2-amino)propyl-11-deoxy-6-*O*-methylerythromycin A 11,12-Cyclic Carbamate (**7b** and **7c**)

These compounds were prepared from **5** (6.00 g, 6.61 mmol) and 1,2-diaminopropane (2.82 ml, 33.1 mmol, 5.0 equiv) in a manner similar to that described for the preparation of **7a**. The mixture of **7b** and **7c** (5.7 g) was obtained.

11-Amino-11-*N*-(2-amino)propyl-11-deoxy-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**8b** and **8c**)

The mixture of **7b** and **7c** (280 g, 0.373 mol) was dissolved in 2 N HCl (900 ml) and stirred at 60°C for 3 hours. To the reaction mixture was added 2 N NaOH soln (1200 ml), and the resulting precipitates were filtered, washed with H_2O and dried to afford a mixture of **8b** and **8c** (144 g). The above filtrate (aqueous layer) was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diethyl ether to yield a mixture of **8b** and **8c** (28.2 g). The combined mixture of **8b** and **8c** (172 g) was charged to silica gel column chromatography ($CHCl_3$ - MeOH - NH_4OH (15 : 1 : 0.1)) for separation of single isomer **8b** (58.2 g, 24% from **5**) and another isomer **8c** (65.8 g, 28% from **5**).

8b: Mp 221~223°C (crystallized from EtOAc); IR (KBr) cm^{-1} 3364, 2978, 1756; ESI-MS *m/z* 694.4 $(M+Na)^+$; HRFAB-MS *m/z* 672.4447 ($M+H^+$, calcd for $C_{34}H_{61}N_3O_{10}$: *m/z* 672.4435); ^1H NMR (500 MHz, $CDCl_3$) δ 0.85 (3H, t, J=7.5 Hz, 14-CH_3), 1.04 (3H, d, J=7.0 Hz, 10-CH_3), 1.12 (3H, d, J=6.5 Hz, 11-$NCH_2CH(CH_3)NH_2$), 1.37 (3H, s, 6-CH_3), 1.46 (3H, s, 12-CH_3), 2.26 (6H, s, 3′-$N(CH_3)_2$), 2.97 (3H, s, 6-OCH_3), 3.06 (1H, m, 11-$NCH_2CH(CH_3)NH_2$), 3.40 (1H, dd, J=10.0 and 14.0 Hz, 11-$NCH_AH_BCH(CH_3)NH_2$), 3.64 (1H, dd, J=3.0 and 14.0 Hz, 11-$NCH_AH_BCH(CH_3)NH_2$), 3.76 (1H, s, 11-H), 5.31 (1H, dd, J=2.5 and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.5 (11-$NCH_2CH(CH_3)NH_2$), 40.5 (3′-$N(CH_3)_2$), 44.9 (11-$NCH_2CH(CH_3)NH_2$), 49.7 (6-OCH_3), 51.6 (11-$NCH_2CH(CH_3)NH_2$), 60.6 (C-11), 106.8 (C-1′), 158.5 (11-NCOO–), 176.0 (C-1), 215.7 (C-9).

8c: Mp 231~223°C (crystallized from EtOAc-CH_2Cl_2); IR (KBr) cm^{-1} 3353, 2972, 1756; ESI-MS *m/z* 694.5 $(M+Na)^+$; HRFAB-MS *m/z* 672.4446 ($M+H^+$, calcd for $C_{34}H_{61}N_3O_{10}$: *m/z* 672.4435); ^1H NMR (500 MHz, $CDCl_3$) δ 0.85 (3H, t, J=7.5 Hz, 14-CH_3), 1.03 (3H, d, J=7.0 Hz, 10-CH_3), 1.13 (3H, d, J=3.0 Hz, 11-$NCH_2CH(CH_3)NH_2$), 1.37 (3H, s, 6-CH_3), 1.45 (3H, s, 12-CH_3), 2.26 (6H, s, 3′-$N(CH_3)_2$), 3.02 (3H, s, 6-OCH_3), 3.24 (1H, m, 11-$NCH_2CH(CH_3)NH_2$), 3.45 (2H, m, 11-$NCH_2CH(CH_3)NH_2$), 3.84 (1H, s, 11-H), 5.19 (1H, dd, J=3.0 and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6 (11-$NCH_2CH(CH_3)NH_2$), 40.2 (3′-$N(CH_3)_2$), 45.2 (11-$NCH_2CH(CH_3)NH_2$), 49.4 (6-OCH_3), 52.3 (11-$NCH_2CH(CH_3)NH_2$), 62.0 (C-11), 106.7 (C-1′), 157.4 (11-NCOO–), 175.6 (C-1), 215.4 (C-9).

11-Amino-9-deoxo-11-deoxy-9,11-*N*-(2*R*-methyl)-nitriloethano-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**11b**)

This compound was prepared from **8b** (86.0 g, 0.128 mol) in a manner similar to that described for the preparation of **9a** using toluene instead of EtOH as a solvent. Obtained crude product was crystallized from diethyl ether to afford **11b** (62.4 g, 75%) as colorless prisms, mp 145~147°C: IR (KBr) cm^{-1} 3424, 2942, 1736; ESI-MS *m/z* 676.5 $(M+Na)^+$; HRFAB-MS *m/z* 654.4328 ($M+H^+$, calcd for $C_{34}H_{59}N_3O_9$: *m/z* 654.4330); ^1H NMR (500 MHz, $CDCl_3$) δ 0.83 (3H, t, J=7.5 Hz, 14-CH_3), 1.40 (3H, s, 6-CH_3), 1.45 (3H, s, 12-CH_3), 2.26 (6H, s, 3′-$N(CH_3)_2$), 2.72 and 3.85 (each 1H, m, 11-$NCH_2CH(CH_3)$-N–), 3.01 (3H, s, 6-OCH_3), 3.74 (1H, s, 11-H), 3.96 (1H, m, 11-$NCH_2CH(CH_3)$N–), 5.07 (1H, dd, J=2.0 and 11.0 Hz, 13-H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.7 (11-$NCH_2CH(CH_3)$N–), 40.2 (3′-$N(CH_3)_2$), 48.3 (11-$NCH_2CH(CH_3)$N=9), 49.0 (6-OCH_3), 53.2 (11-$NCH_2CH(CH_3)$N–), 60.0 (C-11), 106.8 (C-1′), 156.5 (11-NCOO-12), 175.6 (C-1), 178.8(C-9).

11-Amino-9-deoxo-3,11-dideoxy-9,11-*N*-(2*R*-methyl)-nitriloethano-3-oxo-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**16b**)

This compound was prepared from **11b** (60.0 g, 91.8 mmol) in a manner similar to that described for the preparation of **16a** (2′-*O*-acetylation, oxidation of 3-OH and 2′-deacetylation). Obtained crude product was crystallized from EtOAc-CH_2Cl_2 to yield **16b** (33.8 g, 57%) as colorless prisms, mp 179~181°C: IR (KBr) cm^{-1} 3430, 2972, 1766, 1650; ESI-MS *m/z* 674.4 $(M+Na)^+$; *Anal* Calcd for $C_{34}H_{57}N_3O_9$: C 62.65, H 8.81, N 6.45. Found: C 62.32, H 8.83, N 6.17.

11-Amino-9-deoxo-11-deoxy-9,11-*N*-(2*S*-methyl)-nitriloethano-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**11c**)

This compound was prepared from **8c** (70.0 g, 0.104 mol) in a manner similar to that described for the preparation of **9a** using toluene instead of EtOH as a solvent. Obtained crude product was crystallized from diethyl ether to yield **11c** (52.0 g, 76%) as colorless prisms, mp 136~137°C: IR (KBr) cm^{-1} 3406, 2972, 1770, 1651; ESI-MS *m/z* 676.4 $(M+Na)^+$; HRFAB-MS *m/z* 654.4332 ($M+H^+$, calcd for $C_{34}H_{59}N_3O_9$: *m/z* 654.4330); ^1H NMR (500 MHz, $CDCl_3$) δ 0.85 (3H, t, J=7.5 Hz, 14-CH_3), 1.34 (3H, d, J=6.5 Hz, 11-$NCH_2CH(CH_3)$N–), 1.40 (3H, s, 6-CH_3), 1.46 (3H, s, 12-CH_3), 2.25 (6H, s, 3′-$N(CH_3)_2$), 3.01 (3H, s, 6-OCH_3), 3.23 and 3.78 (each 1H, m, 11-$NCH_2CH(CH_3)$N–), 3.61 (1H, d, J=1.0 Hz, 11-H), 4.13 (1H, m, 11-$NCH_2CH(CH_3)$N=9), 5.04 (1H, dd, J=2.0 and 11.0 Hz, 13-H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.0 (11-$NCH_2CH(CH_3)$N–), 40.2 (3′-$N(CH_3)_2$), 47.9 (11-$NCH_2CH(CH_3)$N–), 49.3 (6-OCH_3), 55.7 (11-$NCH_2CH(CH_3)$N–), 78.7 (C-6), 81.8 (C-12), 156.8 (11-NCOO–), 175.6 (C-1), 178.0 (C-9).

11-Amino-9-deoxo-3,11-dideoxy-9,11-*N*-(2*S*-methyl) nitriloethano-3-oxo-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**16c**)

This compound was prepared from **11c** (52.0 g, 79.5 mmol) in a manner similar to that described for the preparation of **16a** (2′-*O*-acetylation, oxidation and 2′-deacetylation). Crude product was crystallized from EtOAc to yield **16c** (33.9 g, 65%) as colorless prisms, mp 200~201°C: IR (KBr) cm^{-1} 3406, 2990, 1777, 1652; ESI-MS *m/z* 674.5 $(M+Na)^+$; *Anal* Calcd for $C_{34}H_{57}N_3O_9$: C 62.65, H 8.81, N 6.45. Found: C 62.63, H 8.80, N 6.52.

2′,4″-Di-*O*-acetyl-11-amino-9-deoxo-11-deoxy-9,11-*N*-(2,2-dimethyl)nitriloethano-6-*O*-methylerythromycin A 11,12-Cyclic Carbamate (**12d**)

12-*O*-Imidazolylcarbonyl compound **5** (163 g, 0.180 mol) was treated with 1,2-diamino-2-methylpropane (74.8 ml, 0.721 mol, 4.0 equiv) in THF in a manner similar to that described for the preparation of **6a**. Then obtained **6d** was converted to **12d** (57.4 g, 35%) in a manner similar to that described for the preparation of **9a** using toluene instead of EtOH as a solvent, mp 155~158°C (crystallized from Me_2CO-*n*-hexane): IR (KBr) cm^{-1} 2968, 1782, 1651; ESI-MS *m/z* 932.7 $(M+Na)^+$; HRFAB-MS *m/z* 910.5630 ($M+H^+$, calcd for $C_{47}H_{79}N_3O_{14}$: *m/z* 910.5640); ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, t, J=7.5 Hz, 14-CH_3), 1.30 (3H, s, 11-$NCH_2C(CH_3)_A(CH_3)_B$N–), 1.33 (3H, s, 11-$NCH_2C(CH_3)_A(CH_3)_B$N–), 1.36 (3H, s, 6-CH_3), 1.42 (3H, s, 12-CH_3), 2.05 (3H, s, 2′-$OCOCH_3$), 2.11 (3H, s, 4″-$OCOCH_3$), 2.28 (6H, s, 3′-$N(CH_3)_2$), 2.97 (1H, d, J=15.0 Hz, 11-$NCH_AH_BC(CH_3)_2$N–), 3.06 (3H, s, 6-OCH_3), 3.36 (3H, s, 3″-OCH_3), 3.50 (1H, d, J=2.0 Hz, 11-H), 3.79 (1H, d, J=15.0 Hz, 11-$NCH_AH_BC(CH_3)_2$N–), 4.66 (1H, d, J=7.8 Hz, 1′-H), 4.68 (1H, d, J=8.5 Hz, 4″-H), 4.76 (1H, dd, J=7.8 and 10.5 Hz, 2′-H), 4.92 (1H, dd, J=2.3 and 10.8 Hz, 13-H), 4.97 (1H, d, J=5.0 Hz, 1″-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.1 (11-$NCH_2C(CH_3)_A(CH_3)_B$N–), 32.8 (11-$NCH_2C(CH_3)_A(CH_3)_B$N–), 40.7 (3′-$N(CH_3)_2$), 49.3 (3″-OCH_3), 49.9 (6-OCH_3), 53.2 (11-NCH_2–), 58.5 (9=$NC(CH_3)_2$–), 61.2 (C-11), 71.9 (C-2′), 78.5 (C-4″), 79.0 (C-6), 81.6 (C-12), 95.6 (C-1″), 100.1 (C-1′), 156.6 (11-NCOO–), 169.8 (2′-$OCOCH_3$), 170.4 (4″-$OCOCH_3$), 175.3 (C-9), 176.5 (C-1).

11-Amino-9-deoxo-11-deoxy-9,11-*N*-(2,2-dimethyl)-nitriloethano-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**11d**)

Cyclic compound **12d** (54.0 g, 59.3 mmol) was treated with 2 N HCl soln (200 ml) in a manner similar to that described for the preparation of **11a** to obtain crude **13d**. Then crude **13d** was treated with MeOH to remove the acetyl group at 2′-position followed by crystallization from CH_3CN to yield **11d** (28.0 g, 71%) as colorless prisms, mp 148~150°C: IR (KBr) cm^{-1} 3554, 2972, 1760, 1651; ESI-MS *m/z* 690.3 $(M+Na)^+$; HRFAB-MS *m/z* 668.4487 ($M+H^+$, calcd for $C_{35}H_{61}N_3O_9$: *m/z* 668.4486); ^1H NMR (500 MHz, $CDCl_3$) δ 1.32 (3H, s, 9-NC(CH_3)$_A$(CH_3)$_B$$CH_2$–), 1.33 (3H, s, 9-NC($CH_3$)$_A$($CH_3$)$_B$$CH_2$–), 2.25 (6H, s, 3′-N($CH_3$)$_2$), 2.97 (1H, d, *J*=15.0 Hz, 9-NC(CH_3)$_2$$CH_A$$H_B$N–), 3.01 (3H, s, 6-$OCH_3$), 3.57 (1H, s, 11-H), 3.82 (1H, d, *J*=15.0 Hz, 9-NC(CH_3)$_2$$CH_A$$H_B$N–), 4.35 (1H, d, *J*=7.5 Hz, 1′-H), 5.00 (1H, dd, *J*=2.0 and 11.0 Hz, 13-H); ^{13}C NMR (75 MHz, $CDCl_3$) 24.9 (9-NC(CH_3)$_A$(CH_3)$_B$$CH_2$–), 32.9 (9-NC($CH_3$)$_A$($CH_3$)$_B$$CH_2$–), 40.2 (3′-N($CH_3$)$_2$), 49.2 (6-$OCH_3$), 53.3 (11-$NCH_2$–), 58.6 (9-N*C*($CH_3$)$_2$–), 61.7 (C-11), 78.8 (C-6), 81.8 (C-12), 106.9 (C-1′), 156.6 (11-NCOO–), 175.1 (C-9), 175.7 (C-1).

11-Amino-9-deoxo-3,11-dideoxy-9,11-*N*-(2,2-dimethyl)-nitriloethano-3-oxo-5-*O*-desosaminyl-6-*O*-methylerythronolide A 11,12-Cyclic Carbamate (**16d**)

This compound was prepared from **11d** (52.7 g 78.9 mmol) in a manner similar to that described for the preparation of **16a** (2′-*O*-acetylation, oxidation and 2′-deacetylation). Obtained crude product was crystallized from CH_3CN to yield **16d** (29.5 g, 56%) as colorless prisms, mp 158~160°C: IR (KBr) cm^{-1} 3456, 2971, 1767, 1651; ESI-MS *m/z* 688.4 $(M+Na)^+$; *Anal* Calcd for $C_{35}H_{59}N_3O_9$: C 63.13, H 8.93, N 6.31. Found: C 62.96, H 8.89, N 6.31.

11-Amino-9-deoxo-3,11-dideoxy-9-*N*,11-*N*-ethano-3-oxo-5-*O*-desosaminyl-6-*O*-methylerythronolide A 9-Amine 11,12-Cyclic Carbamate (**17**)

To a stirred solution of **16a** (0.760 g, 1.19 mmol) and glacial acetic acid (1.36 ml, 2.38 mmol, 2.0 equiv) in EtOH (10 ml) was added $NaBH_3CN$ (0.300 g, 4.77 mmol, 4.0 equiv) at room temperature, and the reaction mixture was stirred for 15 hours. The reaction mixture was poured into sat. $NaHCO_3$ soln and extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated under reduced pressure. Crystallization of crude product from EtOAc yielded **17** (0.374 g, 49%) as colorless prisms, mp 231~233°C: IR (KBr) cm^{-1} 3351, 2968, 1765; ESI-MS *m/z* 662.5 $(M+Na)^+$; *Anal* Calcd for $C_{33}H_{57}N_3O_9$: C 61.95, H 8.98, N 6.57. Found: C 61.81, H 8.94, N 6.61.

In Vitro Antibacterial Activity

Erythromycin-resistant organisms (Tables 3 and 5) are all clinical isolates. *Streptococcus pneumoniae* 217, 224 and 210 are efflux-resistant strains encoded by *mefA* gene. *Streptococcus pneumoniae* 229 and 221 are MLS_B-resistant strains encoded by *ermB* gene. *Streptococcus pneumoniae* 225 carries both *mefA* and *ermB* genes.

Acknowledgments

The authors would like to thank Mr. T. ONO and Mrs. K. SUZUKI for microbiological examination. We also acknowledge their help and support of our colleagues in the structure analysis group. The authors are also indebted to Dr. T. ADACHI for valuable suggestion and discussion.

References

1) KURATH, P.; P. H. JONES, R. S. EGAN & T. J. PERUN: Acid degradation of erythromycin A and erythromycin B. Experientia 27: 362, 1971
2) MORIMOTO, S.; Y. TAKAHASHI, Y. WATANABE & S. OMURA: Chemical modification of erythromycins. I. Synthesis and antibacterial activity of 6-*O*-methylerythromycin A. J. Antibiotics 37: 187~189, 1984
3) BRIGHT, G. M.; A. A. NAGEL, J. BORDNER, K. A. DESAI, J. N. DIBRINO, J. NOWAKOWSKA, L. VINCENT, R. M. WALTROUS, F. C. SCIAVOLINO, A. R. ENGLISH, J. A. RETSEMA, M. A. ANDERSON, L. A. BRENNAN, R. J. BOROVOY, C. R. CIMOCHOWSKI, J. A. FAIELLA, A. E. GIRARD, D. GIRARD, C. HERBERT, M. MANOUSOS & R. MASON: Synthesis, *in vitro* and *in vivo* activity of novel 9-deoxo-9a-aza-9a-homoerythromycin A derivatives; a new class of macrolide antibiotics, the azalides. J. Antibiotics 41: 1029~1047, 1988
4) (a) NEU, H. C.: The crisis in antibiotic resistance. Science 257: 1064~1073, 1992; (b) APPELBAUM, P. C.: Antimicrobial resistance in *Streptococcus pneumoniae*. An overview. Clin. Infect. Dis. 15: 77~83, 1992; (c) FRIEDLAND, I. R.; G. H. MCCRACKEN: Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. 331: 377~382, 1994
5) BAKER, W. R.; J. D. CLARK, R. L. STEPHENS & K. H. KIM: Modification of macrolide antibiotics. Synthesis of 11-deoxy-11-(carboxyamino)-6-*O*-methylerythromycin A 11,12-(cyclic esters) *via* an intramolecular Michael reaction of *O*-carbamates with an α, β-unsaturated ketone. J. Org. Chem. 53: 2340~2345, 1988
6) WATANABE, Y.; S. MORIMOTO, T. ADACHI, M. KASHIMURA & T. ASAKA: Chemical modification of erythromycins. IX. Selective methylation at the C-6 hydroxyl group of erythromycin A oxime derivatives and preparation of clarithromycin. J. Antibiotics 46: 647~660, 1993

7) SIGAL Jr., M. V.; P. F. WILEY, K. GERZON, E. H. FLYNN, U. C. QUARCK & O. WEAVER: Erythromycin VI. Degradation studies. J. Am. Chem. Soc. 78: 388~395, 1956
8) ASAKA. T.; M. KASHIMURA, Y. MISAWA. S. MORIMOTO & K. HATAYAMA: 5-*O*-Desosaminylerythronolide A derivative. USP5591837, January 7, 1997
9) PFITZNER, K. E. & J. G. MOFFATT: Sulfoxide-carbodiimide reactions. I. A facile oxidation of alcohols. J. Am. Chem. Soc. 87: 5661~5670, 1965
10) GRIESGRABER, G.; Y. S. OR, D. T. W. CHU, A. M. NILIUS, P. M. JOHNSON, R. K. FLAMM, R. F. HENRY & J. J. PLATTNER: 3-Keto-11,12-carbazate derivatives of 6-*O*-methylerythromycin A. Synthesis and *in vitro* activity. J. Antibiotics 49: 465~477, 1996
11) OR, Y. S.; R. F. CLARK, S. WANG, D. T. W. CHU, A. M. NILUS, R. K. FLAMM, M. MITTEN, P. EWING, J. ALDER & Z. MA: Design, synthesis, and antimicrobial activity of 6-*O*-substituted ketolides active against resistant respiratory tract pathogens. J. Med. Chem. 43: 1045~1049, 2000
12) ALEXIS, D.; C. AFOURIDAS, J.-M. AUGER, Y. BENEDETTI, A. VONNEFOY, F. BRETIN, J.-F. CHANTOT, A. DUSSARAT, C. FROMENTIN, S. G. D'AMBRIERES, S. LACHAUD, P. LACHAUD, O. L. MARTRET, V. LOYAU, N. TESSOT, J.-M. PEJAC & S. PERRON: Synthesis and antibacterial activity of HMR 3647. A new ketolide highly potent against erythromycin-resistant and susceptible pathogens. Bioorg. Med. Chem. Lett. 9: 3075~3080, 1999
13) FERNANDES, P. B.; W. R. BAKER, L. A. FREIVERG, D. J. HARDY & E. J. MCDONALD: New macrolides active against *Streptococcus pyogenes* with inducible or constitutive type of macrolide-lincosamide-streptogramin B resistance. Antimicrob. Agents Chemother. 33: 78~81, 1989