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SIXTEEN-MEMBERED MACROLIDES: CHEMICAL MODIFICATIONS AND FUTURE APPLICATIONS

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Abstract – To produce a novel macrolide antibiotic, which is biologically stable and effective against erythromycin-resistant *Streptococcus pneumoniae* constitutively expressing the *erm* gene, we designed and synthesized a variety of novel macrolides starting from 16-membered macrolides. Initially, metabolically stable 16-membered macrolides were produced by constructing a 4-*O*-alkylated cladinose moiety as a stable neutral sugar. Then, an arylalkyl group was introduced to the lactone ring to improve the antibacterial activities against resistant *S. pneumoniae*. Although the novel analogues, which possess an arylalkyl group at the C-3 position or the western hemisphere improved antibacterial activities against inducible resistant and efflux type *S. pneumoniae*, they did not have sufficient activities against constitutive *erm*-resistant *S. pneumoniae*. Further, exploration of a novel macrolactone led to identification of a unique 11-azalide framework. We systematically synthesized 14- to 16-membered azalides and azalactams, and a 16-membered azalide (azalactone) was selected as a template for further medicinal chemistry. Finally, we optimized the connecting position of an arylalkyl group, structure of an arylalkyl moiety, and a neutral sugar moiety, and we synthesized a novel 15-β-substituted 16-membered 11-azalide, which was biologically stable and effective against constitutive *erm*-resistant *S. pneumoniae*.

CONTENTS

INTRODUCTION

In 1957, Woodward[1] defined a series of glycosides with a large lactone ring as "macrolides", and the term "macrolide" was used to denote a new class of natural products. In the field of medicinal chemistry, macrolides are typically classified as antibacterial macrolide antibiotics, antifungal polyene antibiotics, and other macrolides like avermectin, however, recently some large-ring lactones without a carbohydrate moiety are known as macrolides, for example FK-506. In this account, the term "macrolide" indicated an antibacterial macrolide antibiotic.[2] Clinically important macrolides such as clarithromycin[3] (CAM) and azithromycin[4] (AZM) consist of derivatives possessing a 14- to 16-membered lactone (Figure 1). Starting materials of these derivatives are 14-membered erythromycin (EM) or 16-membered leucomycins (LMs) and midecamycin A_1 (MDM). CAM and AZM are known as "blockbuster drugs" in the worldwide market.

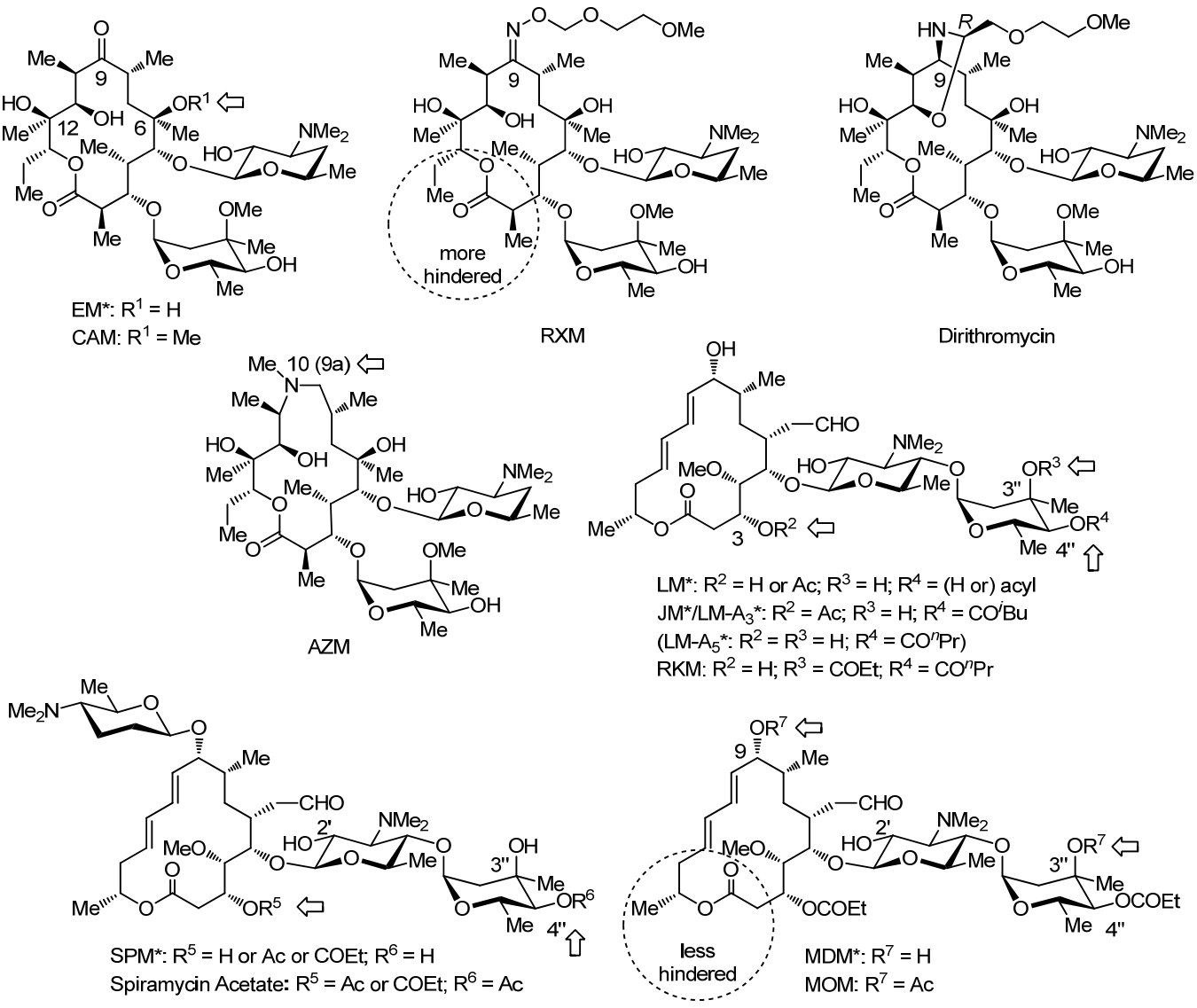


Figure 1. Structures of a Variety of Clinically Used Macrolide Antibiotics (*Natural Products).

Macrolides are effective against respiratory infections, and they are administered to pediatric patients because of their ease of oral administration and established safety. Macrolides exhibit strong antibacterial activities, especially against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae* and thus they are clinically important therapeutic agents. The domestic sales of macrolides and quinolones in Japan, unlike those of cephalosporins, are relatively stable (Figure 2[5a]). CAM and AZM are major macrolides, and their domestic sales are still quite high (Figure 3[5b]), although their basic patents (original substrate patents) in Japan have expired in 2006 (CAM) and 2005 (AZM), respectively. Macrolide antibiotics inhibit protein synthesis[6] by binding to closely related sites on the 50S subunit of the 70S ribosome of bacteria.

Figure 2. Domestic Sales of Oral Antibacterial Agents in 2000-2012.

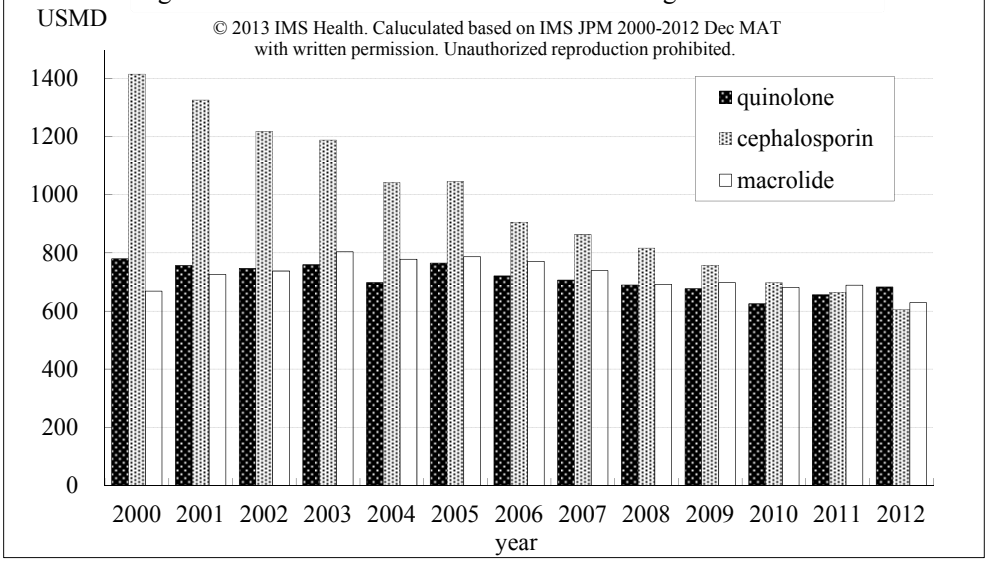
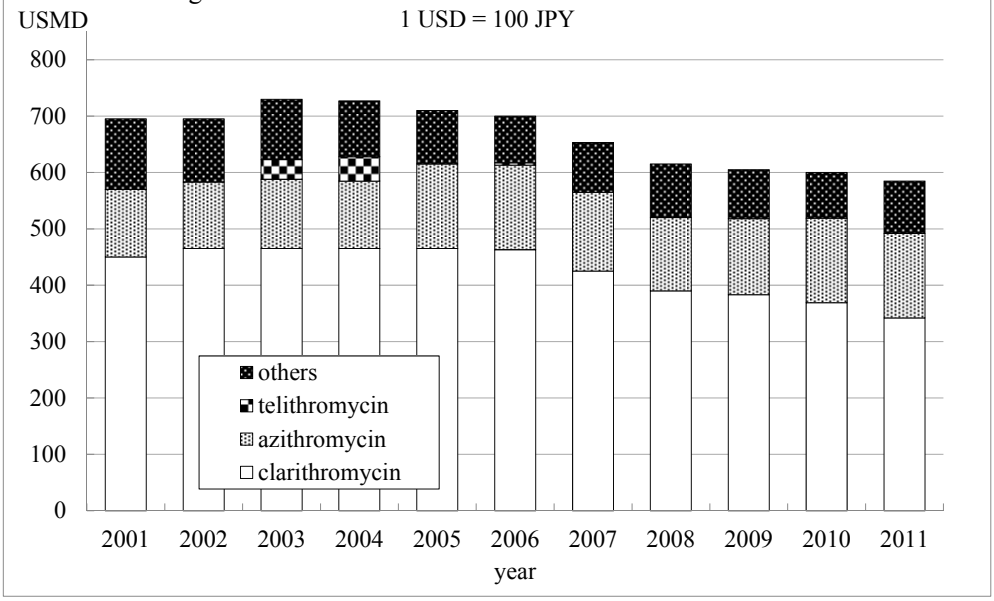


Figure 3. Domestic Sales of Oral Macrolides in 2001-2011.



A comprehensive study of 14-membered and 16-membered macrolides by Kirst and Sides[7] showed that the 14-membered macrolides have stronger antibacterial activities and higher plasma concentration than the 16-membered macrolides. On the other hand, the 16-membered macrolides are superior to 14-membered macrolides because of lower incidence of drug interactions, lesser gastrointestinal stimulation, and acceptable bitter taste. Moreover, natural 16-membered macrolides[8] and chemically modified 16-membered macrolides such as rokitamycin[9] (RKM) and miokamycin[10] (MOM) cannot become a substrate of the efflux pump by an *mef* gene, and thus they show remarkable antibacterial activities against a large variety of resistant bacteria, which possess the *mef* gene. Because of the advantages mentioned above, we used the 16-membered macrolides as our research target. The naturally occurring 14- and 16-membered macrolides are not always stable. Chemical instability under acidic conditions and metabolic instability under physiological conditions are the issues that need to be addressed in the 14- and 16-membered macrolides, respectively (cf. sections 1.1. and 1.2.).

Until the 1980s, improvement in the stability of macrolide antibiotics was an important issue, however, EM resistant *S. pneumoniae* (ERSP) with an *erm* gene that emerged in the 1990s caused clinical problems because this type of ERSP could not be inhibited by CAM, AZM, or 16-membered macrolides. We focused on improvement in the stability of 16-membered macrolides and found a practical solution at an early stage. Then, we tried to design and synthesize a novel 16-membered macrolide, which is effective against resistant *S. pneumoniae* with an *erm* gene, by using different chemical approaches. Finally, we synthesized a novel and metabolically stable 16-membered 11-azalide, which was effective against resistant *S. pneumoniae* and resistant *S. pyogenes* with an *erm* gene. In this account, we describe (i) the regulation of metabolism of the 16-membered macrolides, (ii) several approaches to synthesize 16-membered macrolide derivatives, which are active against resistant *S. pneumoniae*, (iii) discovery of 16-membered 11-azalide framework, and (iv) its final optimization by accurate medicinal chemistry.

A carbohydrate moiety cannot be easily introduced during the synthesis of antibacterial macrolides similar to that observed in the total synthesis of macrolides. For example, Tatsuta *et al.*[11] completed and reported introduction of a carbohydrate moiety to a protected lactone ring before the construction of an aglycone framework in the total synthesis of carbomycin B or oleandomycin. These findings indicate that efficient introduction of a carbohydrate moiety to a macrolactone is extremely difficult. Although initially we used glycosylation reactions to determine concept molecules (sections 1.3. and 1.5.), subsequently, we synthesized 16-membered macrolides without glycosylation reaction and focused on future development of process chemistry.

RESULTS AND DISCUSSION

1. Studies on the Metabolism of 16-Membered Macrolides and Generation of Metabolically Stable

16-Membered Macrolides[12]

1.1. Improvement in the Stability of EM under Acidic Conditions

Although EM has strong antibacterial activities, the spiroketal (Figure 4) formed through formation of an enol ether[13] between a hydroxyl group at the C-6 position and a carbonyl group at the C-9 position under acidic conditions and subsequent attack of a tertiary hydroxyl group at the C-12 position at the C-9 position does not have marked antibacterial activities. Morimoto *et al*.[3] performed regioselective 6-*O*-methylation to avoid intramolecular ketal formation and generated CAM (Figure 1) with significantly improved pharmacokinetics. The clinical efficacy of CAM is enhanced by (i) strong activity of a major metabolite against *H. influenzae* and (ii) a variety of non-antibacterial activities,[14] for example, immunomodulatory properties. D'Ambrieres *et al*.[15] converted a carbonyl group at the C-9 position of EM to an alkyloxy-imino ether to synthesize roxithromycin (RXM) (Figure 1), which showed improved pharmacokinetics. Djokić *et al*.[4] transformed a 14-membered lactone of EM to a 15-membered azalactone using Beckmann rearrangement and finally synthesized 15-membered azalide, AZM (Figure 1) with a nitrogen atom-containing lactone ring. AZM is more stable and shows stronger antibacterial activity than EM against Gram-negative bacteria, including *H. influenzae*. Although CAM required twice a day administration, a long plasma half-life enabled once a day administration of AZM.

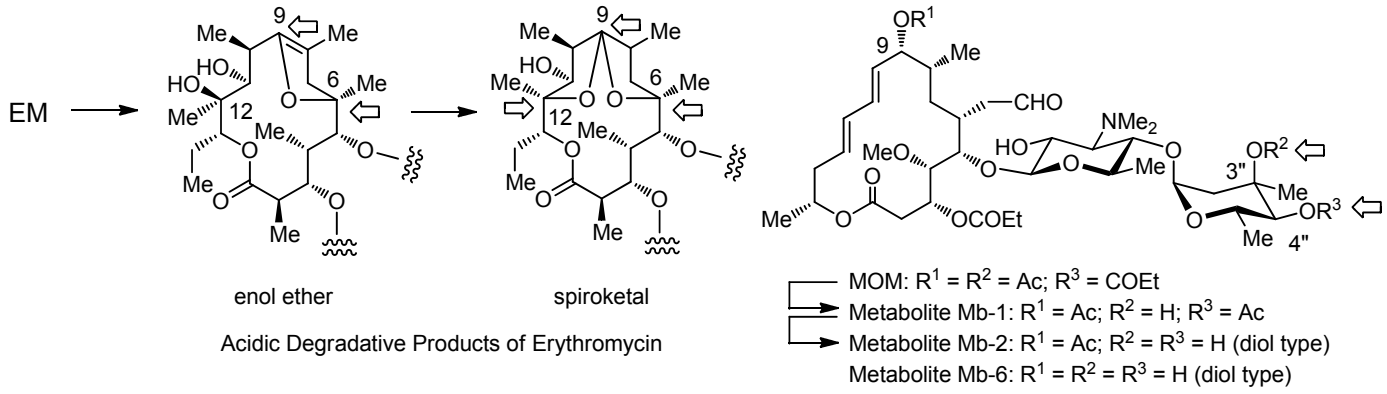


Figure 4. Chemical Instability of EM in Acidic Conditions and Metabolic Transformation of MOM.

1.2. Improvement of Metabolic Stability of 16-Membered Macrolides under Physiological Conditions

Typically, the C-3" position of the neutral sugar moiety in natural 16-membered macrolides is a free hydroxyl group and the C-4" position is an acyloxy group, except spiramycin (SPM) (Figure 1). The acyl groups at the C-4" position are mobile under physiological conditions, and natural 16-membered macrolides are metabolized to their 3", 4"-diol analogues with markedly low antibacterial activities. Ōmura *et al*.[9] introduced a propionyl group to the C-3" position of leucomycin A_5 (LM-A_5) to generate

RKM (Figure 1), and Omoto *et al*.[10] introduced two acetyl groups to the C-9 and C-3" positions of MDM to generate MOM (Figure 1). One of the major metabolic pathways of MOM in humans is hydrolysis of the propionyl group at the C-4" position[16] and simultaneous intramolecular migration of the 3"-acetyl group to the C-4" position to afford a metabolite Mb-1 (Figure 4), which shows acceptable antibacterial activities. In addition, the major metabolic pathways of RKM[17] include *in vivo* deacylation at the neutral sugar moiety. Compared to their parent natural products, RKM and MOM showed improved pharmacokinetics, and their antibacterial activities against inducible methylase-type resistant *S. pneumoniae* with an *erm* gene (inducible resistant *S. pneumoniae*) increased by introduction of an acyl group to the C-3" position. Moreover, these two derivatives showed stronger antibacterial activity than CAM or AZM against resistant *S. pneumoniae* with an *mef* gene (efflux pump type) (minimum inhibitory concentrations (MIC, μg/mL) of RKM, MOM, CAM, and AZM are 0.13. 0.25, 1, and 1, respectively). On the other hand, introduction of an acetyl group to the 9-hydroxyl group in MOM increased hydrophobicity of the entire molecule, and the bitter taste peculiar to macrolides could be partially masked. The modified MOM continued to show efficacy as an oral antibiotic agent, particularly in the case of pediatric patients. Under physiological conditions, the acetyl group at the C-4" position of Mb-1 is removed to form Mb-2 (Figure 4). Thus, even these molecules, which possess 2 acyl groups at the C-3" and C-4" positions, did not show significant metabolic stability[18] in clinical conditions. Improvement in pharmacokinetics may partially contribute to the desired properties of macrolide antibiotics proposed by Kirst.[19]

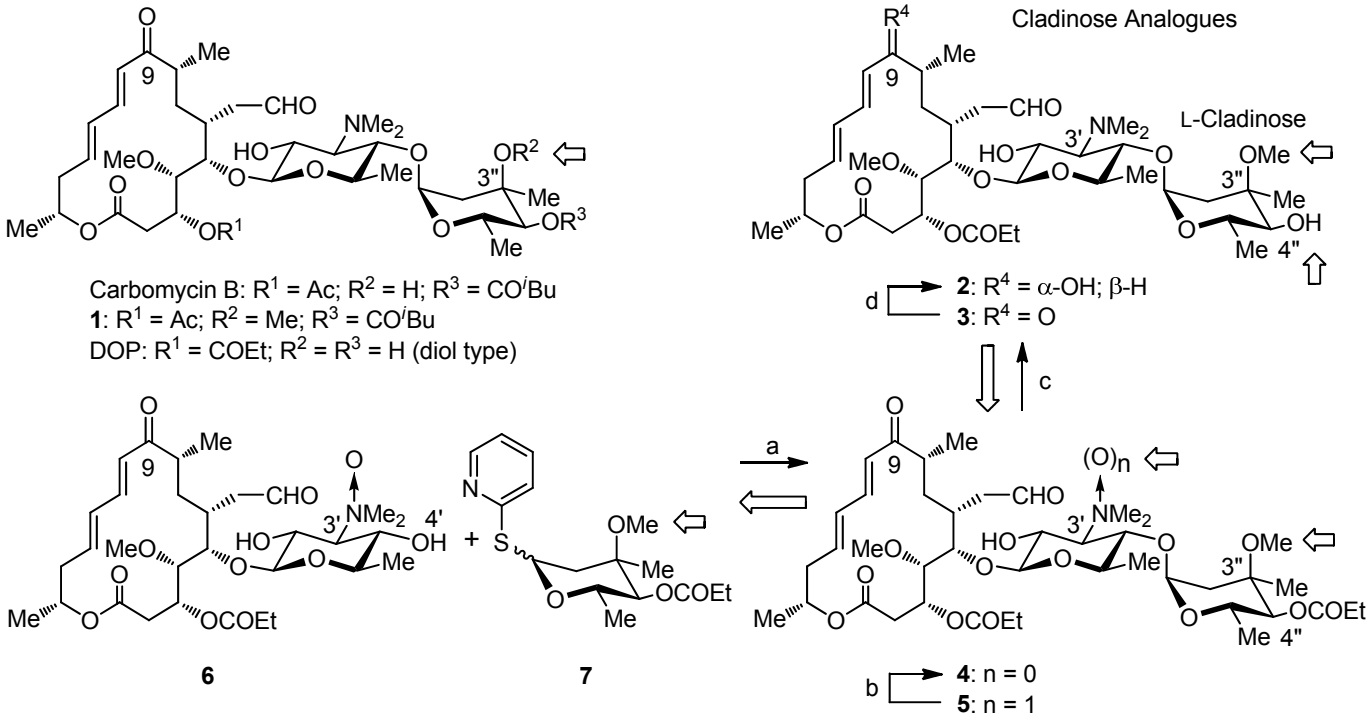
1.3. Design and Synthesis of Metabolically Programmed 16-Membered Macrolides

Part 1: Discovery of Cladinose Analogues in 16-Membered Macrolides

Meiji Seika Kaisha, Ltd., (now, Meiji Seika Pharma Co., Ltd.) has discontinued the studies on macrolides since the development of MOM further, it was very difficult to catch up with current studies on macrolides performed by other major research groups such as the Kitasato Institute, Taisho Pharmaceutical, Abbott, and Roussel (Hoechst Marion Roussel). Our group consists of not only general chemists but a carbohydrate chemist and a fermentation professional. Thus, we focused on glycosylation and biotransformation (bioconversion) as novel approaches for developing 16-membered macrolides. To date, the major chemical modifications in 16-membered macrolides include simple acylations because the 16-membered macrolides have many chemically sensitive moieties (cf. Figure 34). The findings from studies on synthetic chemistry, biotransformation, and metabolism enable the development of metabolically stable 16-membered macrolides (section 1.6.).

In 1977, Tatsuta *et al*.[20] reported synthesis of a cladinose analogue of carbomycin B, compound (**1**) (Scheme 1), as a pioneer work in the application of "glycal chemistry" and showed that **1** had stronger

antibacterial activity than carbomycin B against *Mycobacterium smegmatis*, but its activity was comparable to that of carbomycin B against other bacteria. Although **1** was the first example of a cladinose analogue of a 16-membered macrolide, no information was available about its structure-activity relationship (SAR) or metabolism. Thus, we hypothesized that compound **1** with "an acyl group at the C-4" is more stable under physiological conditions than the natural 16-membered macrolides, including carbomycin B, because of steric hindrance of the 3"-methoxy group" and "if an acyl group at the C-4" position was removed, a cladinose-type analogue (compound **2** or **3** in Scheme 1) might be still more potent than the diol-type metabolite (metabolite Mb-2 or Mb-6 in Figure 4)" because the 14-membered macrolides, which possess a cladinose-type neutral sugar, showed strong antibacterial activities.



[a]*Reagents and conditions:* (a) **7** (6.0 eq), $AgClO_4$ (10 eq), MS4A, MeCN, -15 °C, 2 h and rt, 18 h in a dark place, 38% plus 38% of β-anomer based on consumed **6**; (b) Ph_3P (18 eq), CH_2Cl_2, 32 °C, 72 h, 72%; (c) *Paecilomyces* sp. PF1108, 26 °C, 9 days, 43%; (d) *Streptomyces mycarofaciens* SF2772, 28 °C, 24 h, 45-60%.

Scheme 1. Design and Preliminary Preparation of Cladinose Analogues of 16-Membered Macrolide[a].

We studied cladinose analogues **2** and **3** as our target molecules. Because direct introduction of a methyl group into the 3"-hydroxyl group was not easy, we used a glycosylation method. We used 1-(2-pyridylthio) sugar for glycosylation[21] similar to that used by Woodward *et al.*[22] for successful asymmetric total synthesis of EM. A cladinose moiety was regioselectively introduced into the C-4' position of **6** *via* glycosylation in the presence of anhydrous silver perchlorate and pulverized molecular sieves in dry MeCN to afford the desired α-glycoside (**5**) together with a β-anomer. High

α-stereoselectivity could not be achieved because of the lack of neighboring group effects in this 2-deoxy donor (**7**). Few studies have reported high stereoselectivity in the preparation of 2-deoxyglycosides, except in the case of some strategies.[23] According to the 3"-methoxy group supposedly, a propionyl group at the C-4" position of **4** was stable as we expected, and our first hypothesis was qualitatively confirmed as a biotransformation level. Common microorganisms such as *Mucor spinescens* IAM 6071 are unable to cleave the 4"-*O*-propionyl group of compound **4**; this phenomenon is considered attributable to steric hindrance by the 3"-methoxy group. If steric hindrance is indeed the case of this phenomenon, then our first hypothesis would be valid. However, since this phenomenon was observed only during biotransformation, the validation would be conditional, not absolute. Therefore, we performed the desired deacylation using *Paecilomyces* sp. PF1108 as a special case to obtain a cladinose analogue (**3**). Finally, stereoselective reduction at the C-9 position of **3**[24] smoothly proceeded by biotransformation using *Streptomyces mycarofaciens* SF2772[25] to afford compound **2**. Our previous studies[26] on macrolides showed that the 16-membered macrolide derivatives, which possess an sp^3 carbon at the C-9 position, showed better pharmacokinetics than those possessing an sp^2 carbon. Another study showed[27] that chemical reduction of a carbonyl group at the C-9 position of antibiotics in the leucomycin family resulted in a mixture of diastereoisomers, α- and β-alcohol. Various other methods for reducing the C-9 carbonyl group of 16-membered macrolides to the corresponding alcohol using different synthetic[28] and biochemical[29] approaches have been reported.

Table 1. Antibacterial Activities of L-Cladinose Analogues (**2** and **3**) and Corresponding Diol Analogues (MIC, μg/mL).

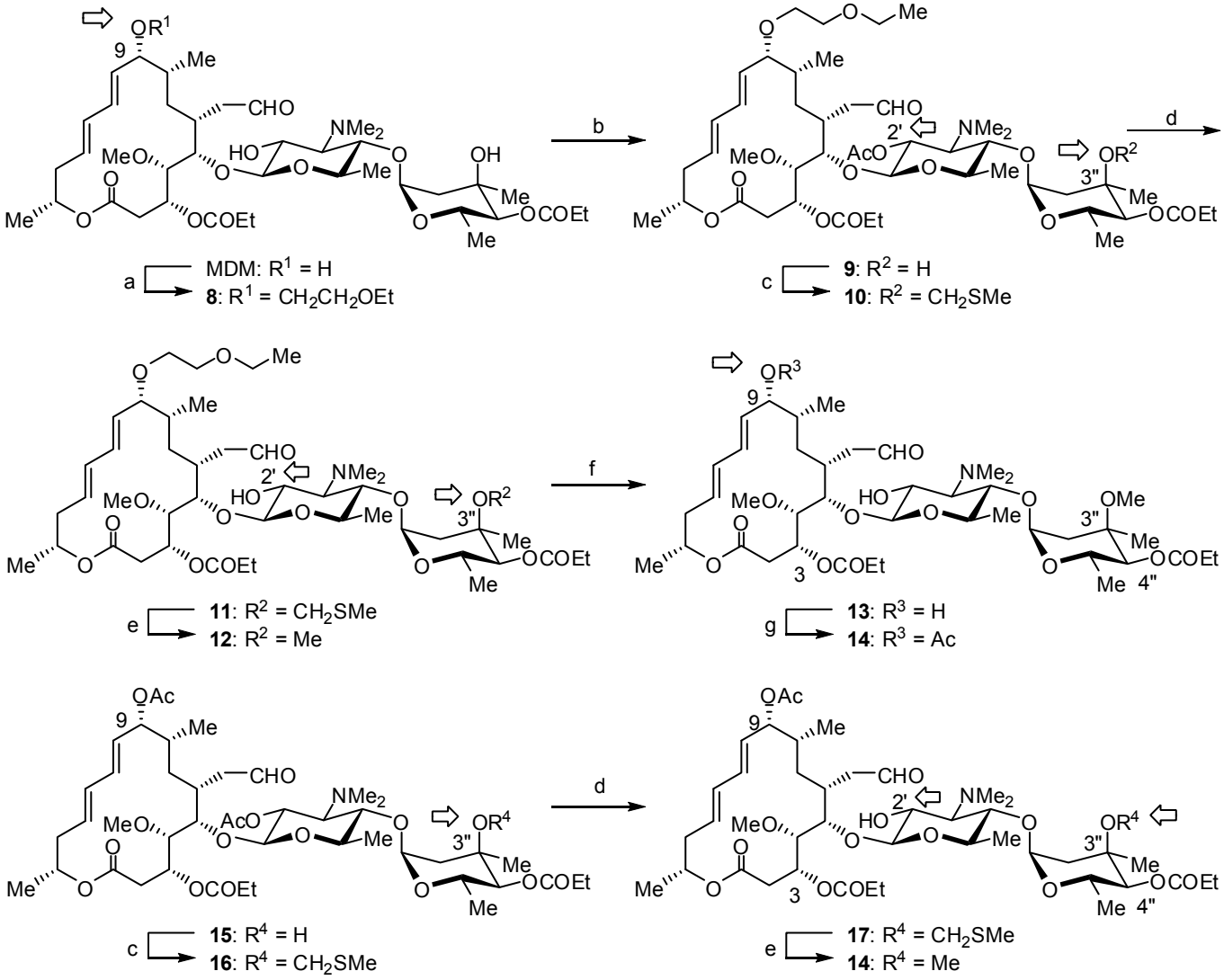
Test organisms	**2**	**3**	Mb-6	DOP
Enterococcus faecalis W-73	0.78	0.39	12.5	3.13
Streptococcus pneumoniae IP692	0.10	0.05	0.78	0.39
S. pneumoniae Type I	0.10	<0.025	0.78	0.20
Streptococcus pyogenes Cook	0.20	0.10	1.56	0.39
Moraxella catarrhalis W-0500	3.13	1.56	25	12.5
M. catarrhalis W-0506	3.13	0.78	25	12.5
Haemophilus influenzae 9334	12.5	1.56	100	50
H. influenzae Type b	50	12.5	>100	100

2 *vs*. Mb-6: 3-*O*-acyl derivatives; **3** *vs*. DOP: 3-*O*-acyl-9-dehydro derivatives.

The cladinose analogues **2** and **3**, especially the 9-dehydro derivative (**3**), showed very strong antibacterial activities. The antibacterial activities of cladinose analogues **2** and **3** against clinically

important pathogens in respiratory infections were around 8 times stronger (precisely 4 to 32 times stronger) than those of the corresponding diol analogues, Mb-6[30] (Figure 4) and DOP[31] (Scheme 1, Table 1[32]). This finding prompted us to exhaustively explore cladinose analogues of 16-membered macrolides. We hypothesized that "(i) an acyl group at the C-4" position is rather stable under physiological conditions and (ii) if the acyl group is removed by metabolism, the cladinose-type metabolite exhibits stronger antibacterial activities than the diol-type metabolite".

Because we obtained attractive and novel cladinose analogues using glycosylation and biotransformation, we decided to explore an alternative synthetic route for extensive preparation of cladinose analogues,



[a]*Reagents and conditions:* (a) $EtOCH=CH_2$ (9.3 eq), PPTS (1.5 eq), CH_2Cl_2, 25 °C, 16 h, 92%; (b) Ac_2O (2.1 eq), MeCN, 40 °C, 16 h, 99%; (c) Ac_2O (30 eq), DMSO, 33 °C, 64 h, 70% plus 18% of recovered starting material; (d) MeOH, 30 °C, 16 h, 92-96%; (e) deactivated Raney nickel, EtOH, 25 °C, 20 min., 56-61%; (f) AcOH, aq MeCN, 25 °C, 16 h, 91%; (g) AcCl (4.4 eq), pyridine (4.4 eq), Et_3N (3.7 eq), PhMe, rt, 1 h, 79%.

Scheme 2. Facile Synthesis of Cladinose Analogues[a].

which could also be used to scale-up production. We had some difficulties in direct methylation of a hydroxyl group at the C-3" position in the presence of a 4"-*O*-acyl group, an intact aldehyde, and an unmodified lactone ring; thus, we selected an indirect method to introduce a methyl group into a tertiary hydroxyl group at the C-3" position (Scheme 2). We used a methylthiomethyl (MTM) ether as a key intermediate to generate a 3"-OMe group. A 3"-MTM ether of MDM has already been reported[33] as a useful semisynthetic analogue of MDM. A study performed in the late 1960s showed that an MTM ether converted into a methyl ether *via* heterogeneous hydrogenolysis.[34] We selected an ethoxyethyl group as a protecting group at the C-9 position to isolate a 9-hydroxyl analogue (**13**), because the 9-*O*-acetyl group as a protecting group was not appropriately cleaved. A key compound **11**, however, possessed chemically sensitive functional groups, a diene and an aldehyde, which were labile under hydrogenolysis conditions. As we expected, the standard protocol of hydrogenolysis of **11** easily afforded an undesired perhydrogenated compound with a 3"-OMe analogue. However, well-controlled deactivated Raney nickel under optimized conditions converted **11** to the desired 3"-methyl ether (**12**) in a moderate yield.[35]

Before detailed evaluation of the selected cladinose analogue(s), we performed SAR studies. We used MDM and josamycin (JM) (LM-A_3) to obtain the SAR information at the C-3, C-9, and C-4" positions. The cladinose-type MDM derivatives (3-*O*-COEt and 4"-*O*-COEt) and the corresponding JM derivatives (3-*O*-Ac and 4"-*O*-CO^{i}Bu) showed almost similar antibacterial activities. Antibacterial activities of 9-*O*-COEt derivatives were weaker than those of the corresponding 9-*O*-Ac derivatives (data not shown). The *in vitro* activity of **13** was slightly stronger than that of **14** (Table 2), but we selected a 9-*O*-Ac-analogue (**14**) for further studies because we expected it to have better pharmacokinetics and tolerable bitter taste (see the last part of section 1.2). Compound **14** was efficiently prepared from **15** in 3 steps (Scheme 2).

Table 2. Antibacterial Activities of 4"-*O*-Acyl-L-Cladinose Analogues (MIC, μg/mL).

Test organisms	**13**	**14**	MDM	MOM
Enterococcus faecalis W-73	1.56	1.56	3.13	1.56
Streptococcus pneumoniae IP692	0.10	0.10	0.39	0.20
S. pneumoniae Type I	0.10	0.10	0.39	0.20
Streptococcus pyogenes Cook	0.10	0.20	0.20	0.20
Moraxella catarrhalis W-0500	0.78	1.56	3.13	1.56
M. catarrhalis W-0506	0.78	1.56	3.13	1.56
Haemophilus influenzae 9334	1.56	3.13	6.25	6.25
H. influenzae Type b	12.5	25	25	25

Figure 5. Time Course of Serum Concentration of **14** and MOM after Oral Dosing (200 mg/kg) in Mice (n = 4).

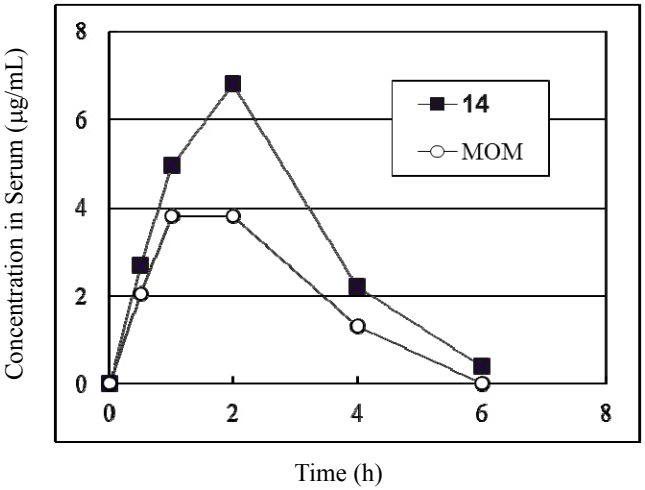
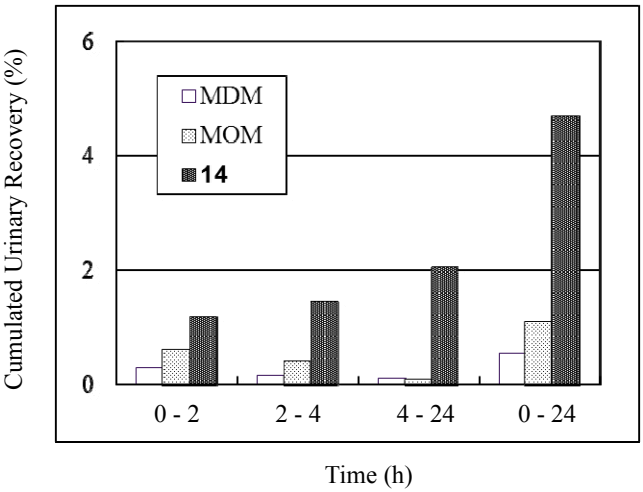


Figure 6. Time Course of Urinary Recovery of MDM, MOM, and **14** after Oral Dosing (200 mg/kg) in Mice (n = 6).



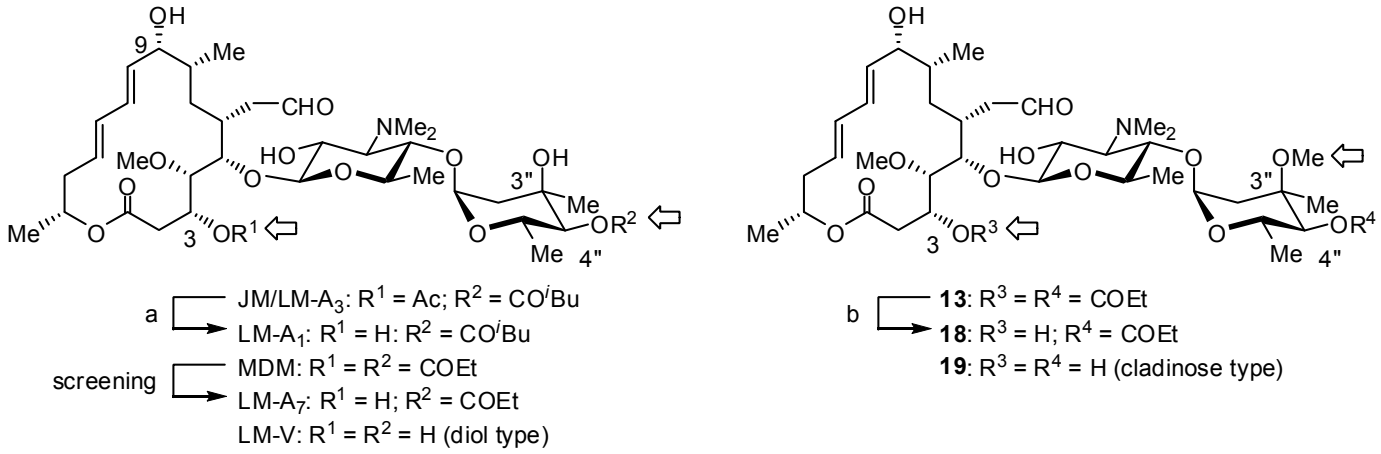
Serum concentrations of **14** and MOM after oral administration in mice are shown in Figure 5.[35] The serum level of compound **14** was higher than that of MOM. Analysis of urinary excretion of the compounds by a bioassay method using *Micrococcus luteus* ATCC9341 showed that the urinary excretion of **14** was larger than that of MOM or MDM (Figure 6).[35] These findings may be attributed to the strong antibacterial activities of cladinose-type metabolite(s). Then, we evaluated the *in vivo* potency of **14** using a mouse model of systemic infection. The median effective dose (ED_{50}) values showed that the *in vivo* activity of **14** against *Staphylococcus aureus* Smith I was 4 to 5 times greater than that of MOM. In addition, compound **14** was about 4 times more potent than MOM against *S. pneumoniae* DP-I Type I.[35] The MIC values of **14** were the same as those of MOM *i.e.* 0.20 μg/mL; therefore, the relatively strong *in vivo* efficacy of **14** deserved special mention, and we concluded that the antibacterial activities of some of major metabolites (for example, **2** *vs*. Mb-6) contributed to the strong *in vivo* efficacy. Strong *in vivo* efficacy at the clinical site is very important for suppressing the emergence of resistant bacteria. Then, we tried to develop novel cladinose analogues, which showed enhanced antibacterial activities.

1.4. Design and Synthesis of Metabolically Programmed 16-Membered Macrolides

Part 2: Optimization of Cladinose Analogues in 16-Membered Macrolides

The *in vitro* antibacterial activities of RKM are stronger than those of MOM. The C-3 position of RKM is a free hydroxyl group, and that of MOM is a propionyloxy group. The SAR studies of 16-membered macrolides showed that the *in vitro* potency of a molecule with a free hydroxyl group at the C-3 position is stronger than that with an acyloxy group at the C-3 position. Thus, we prepared 3-OH cladinose analogues of 16-membered macrolides to evaluate their biological activities. We planned to use the

biotransformation method to prepare the 3-OH cladinose analogues because many kinds of efficient biotransformation methods[36] have been used thus far to synthesize macrolide antibiotics.



[a]*Reagents and conditions:* (a) *Bacillus subtilis* ATCC 14593, 36 °C, 24 h, 7.0% as screening; (b) *Phialophora* sp. PF1083, 26 °C, 10 days, 28% of **18** plus 16% recovered **13**.

Figure 7. 3-De-*O*-acylation in Leucomycin Family by Biotransformation and Discovery of PF1083[a].

In the leucomycin family of macrolides, an acyl group at the C-3 position could not be efficiently cleaved by chemical reactions under acidic or basic conditions (The acetyl group at the C-3 position in the tylosin family can be chemically cleaved under acidic conditions). Under acidic conditions, a neutral sugar moiety and an allylic alcohol at the C-9 position are very labile, and two ester bonds in the lactone ring and at the C-4" position are quite unstable under basic conditions. Okamoto *et al.*[37] screened many types of microorganisms and found that *Bacillus subtilis* ATCC 14593 could convert JM to leucomycin A_1 (LM-A_1) (Figure 7). Although this practical 3-de-*O*-acylation in the leucomycin family of macrolides was reported for the first time, the reported conversion yield was 7.0%. Moreover, we had to remove a propionyl group (not an acetyl group); thus, we decided to screen novel microorganisms with powerful and regioselective 3-de-*O*-acylation potency.

We did not focus on bacteria or *Actinomycetes* species considering the antibacterial spectra of macrolide antibiotics. Shimizu *et al.*[38] screened more than 250 strains of fungi and discovered two microorganisms, *Phialophora* sp. PF1083 and *Preussia* sp. PF1086, which could convert MDM to leucomycin A_7 (LM-A_7) (Figure 7). Under optimized fermentation and purification conditions, the PF1083 strain could selectively remove the 3-*O*-propionyl group of MDM to consistently afford LM-A_7 in a yield of 30% or more. Finally, compound **13** was converted to compound **18** in a yield of 28% using the PF1083 strain. Substrate specificity studies[39] showed that an enzymatic reaction using the PF1083 strain selectively recognized the substrate with a 4"-*O*-acylated neutral sugar moiety (cf. section 1.6).

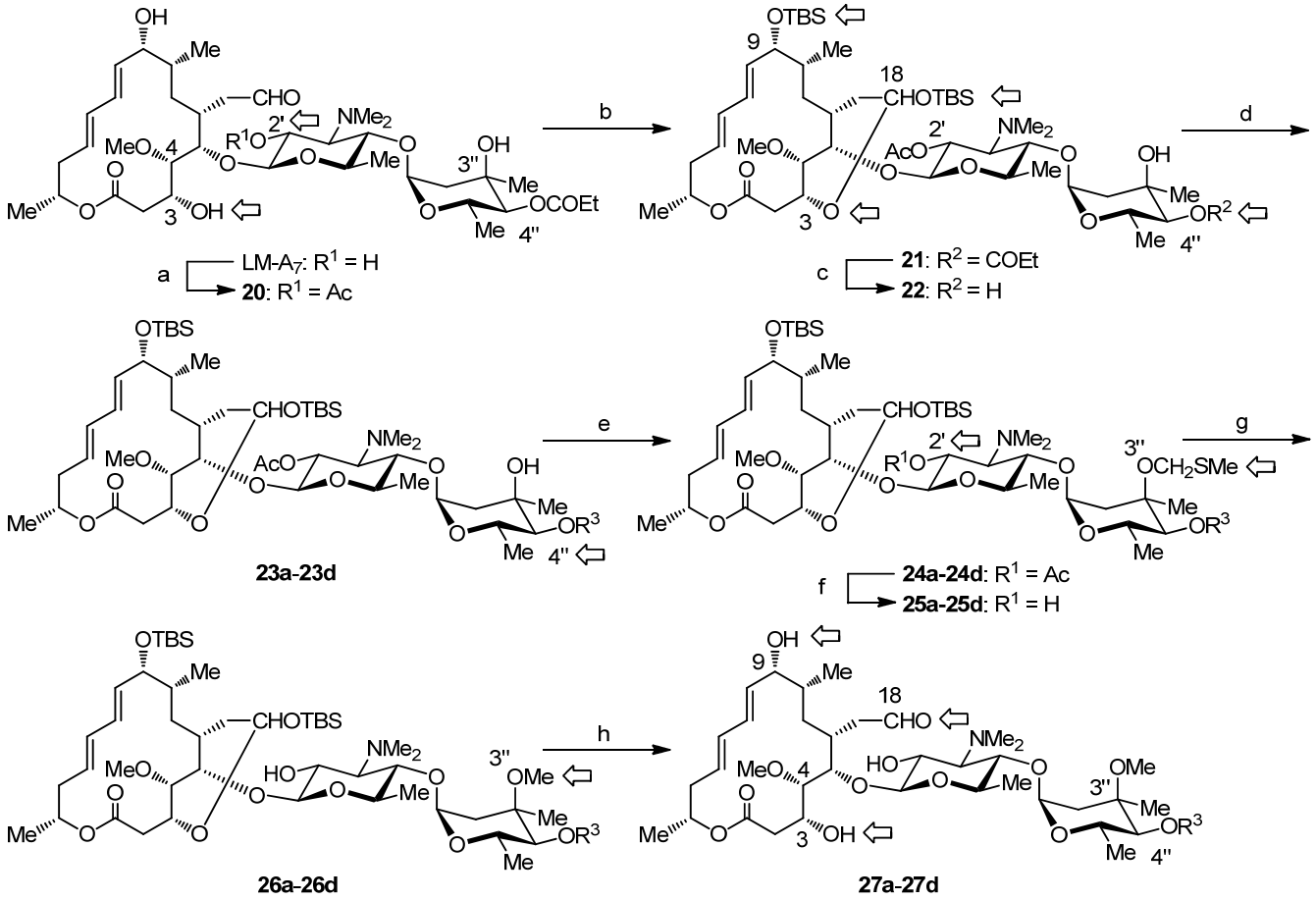
Table 3. Antibacterial Activities of 3-OH-L-Cladinose Analogues (**18** and **19**) and Corresponding Diol Analogues (MIC, μg/mL).

Test organisms	**18**	**19**	LM-A_7	LM-V
Enterococcus faecalis W-73	0.39	0.78	0.78	6.25
Streptococcus pneumoniae IP692	<0.025	0.10	0.10	0.39
S. pneumoniae Type I	0.05	0.05	0.20	0.39
Streptococcus pyogenes Cook	0.05	0.10	0.10	0.39
Moraxella catarrhalis W-0500	0.39	1.56	0.78	12.5
M. catarrhalis W-0506	0.78	1.56	0.78	12.5
Haemophilus influenzae 9334	0.78	1.56	0.78	12.5
H. influenzae Type b	6.25	12.5	6.25	50

18 *vs*. LM-A_7: 3-OH-4”-*O*-COEt derivatives; **19** *vs*. LM-V: 3-OH-4”-OH derivatives.

Compared to compound **13**, compound **18** showed markedly enhanced antibacterial activities (4 to 16 times stronger). Compound **18** was 4 times stronger than LM-A_7 (Table 3) against *S. pneumoniae*, and it was confirmed that a 4”-*O*-acylated cladinose-type neutral sugar was important for increasing the activities of these compounds against target pathogens. Moreover, the antibacterial activities of a tentative major metabolite of **18**, compound **19**, were remarkably stronger (4 to 8 times stronger) against clinically important pathogens in respiratory infections than those of a diol-type metabolite, leucomycin V[40] (LM-V) (Figure 7). Subsequently, we optimized 3-OH cladinose analogues in the 16-membered macrolides because **18** and its 4”-*O*-acyl analogues were supposed to exhibit stronger *in vivo* activities than the existing 16-membered macrolides, according to the above mentioned stronger antibacterial activities of both an intact molecule (**18**) and its tentative major metabolite.

To optimize an acyl group at the C-4” position, we had to prepare a key intermediate with a free hydroxyl group at the C-4” position. Moreover, two hydroxyl groups at the C-9 and the C-2’ positions and an aldehyde group had to be protected in the key intermediate. Although the hydroxyl groups at the C-3 and C-3” positions are less reactive, the C-3 hydroxyl group and the aldehyde group easily form a very reactive hemiacetal. Kurihara *et al.*[41] addressed this issue and synthesized a key intermediate, a diol (**22**), by using a combination of previously reported methods (Scheme 3).



[a]*Reagents and conditions:* (a) Ac_2O (2.0 eq), MeCN, 25 °C, 16 h, quant.; (b) TBSCl (3.0 eq), imidazole (6.0 eq), DMF, 45 °C, 24 h, 83%; (c) 25% aq. NaOH, *n*-Bu_4NHSO_4 (1.0 eq), PhH-H_2O (2:1), 25 °C, 1 h, 86%; (d) acyl chloride (1.2 eq), pyridine. 25 °C, 0.5 h, 90-92%; (e) DMSO-Bz_2O (3:1), 45 °C, 3 days, 58-64%; (f) MeOH, 25 °C, 16 h, 97-99%; (g) Raney nickel, EtOH, 25 °C, 20 min., 55-65%; (h) TBAF (2.0 M), THF, 45 °C, 1 h, 65-70%. R^3 = a: *n*-butyryl; b: *i*-butyryl; c: *n*-valeryl; d: *i*-valeryl.

Scheme 3. Optimization of an Acyl Group at the C-4" Position in Application of LM-A_7 as a Starting Material[a].

2'-*O*-Acetylleucomycin A_7 (**20**) was converted to bis-TBS intermediate by using the Kitasato method reported by Sano *et al*.,[42] which afforded the desired diol (**22**) by heterogeneous basic hydrolysis. Despite the highly basic conditions, the lactone ring was stabilized by a fused 7-membered silyl hemiacetal, and the 2'-*O*-acetyl group could be retained. The exceptional stability of the 2'-*O*-acetyl group under those phase transfer conditions[43] is worth mentioning. Yields of regioselective acylation at the C-4" were more than 90%, because the tertiary alcohol at the C-3" position was originally less reactive. Conversions from compounds **23a**-**23d** to **26a**-**26d** were performed on the basis of our previous experience (section 1.3. Scheme 2). The hydroxyl group at the C-3" position, however, was less active than the general tertiary hydroxyl group because of steric hindrance[44] by the TBS group at the C-18 position. Practical methylthiomethylation of **23** was finally accomplished by addition of benzoic anhydride[45] to afford **24** in a moderate yield. The final deprotection of the TBS groups of **26a**-**26d** by using tetrabutylammonium fluoride (TBAF) afforded desired 3-OH cladinose analogues (**27a**-**27d**).

Table 4. Antibacterial Activities of 4”-*O*-Acyl L-Cladinose Analogues (MIC, μg/mL)

Test organisms	**18**	**27a**	**27b**	**27c**	**27d**	LM-A_7	RKM
Enterococcus faecalis W-73	0.39	0.78	0.78	0.78	0.78	0.78	0.39
Streptococcus pneumoniae IP692	<0.025	<0.025	<0.025	<0.025	0.05	0.20	0.10
S. pneumoniae Type I	0.05	0.05	<0.025	<0.025	0.05	0.10	0.10
Streptococcus pyogenes Cook	0.05	0.05	<0.025	<0.025	0.05	0.05	0.05
Moraxella catarrhalis W-0500	0.39	0.20	0.20	0.10	0.20	0.20	0.20
M. catarrhalis W-0506	0.39	0.20	0.20	0.20	0.20	0.78	0.20
Haemophilus influenzae 9334	0.78	0.78	0.39	0.78	0.78	0.39	1.56

Antibacterial activities of compounds **27a**-**27d** against target pathogens are shown in Table 4. All five analogues showed very strong antibacterial activities, and the antibacterial activities of compounds **27b** and **27c** against *S. pneumoniae* were 2 to 4 times stronger than those of RKM. To our knowledge, these antibacterial activities are most potent among macrolides belonging to the leucomycin family. On the other hand, we could synthesize compound **27a** in only 6 steps using LM-A_5 (Figure 1) as the starting material, and we prepared the 9-*O*-acetyl derivative and 9-*O*-propionyl derivative of **27a**. The *in vivo* activities of **27a** and its 9-*O*-acyl derivatives against *S. pneumoniae* DP-I Type I,[41] however, were only two times potent than that of RKM.

Thus, we concluded that optimized 3-*O*-COEt cladinose-type 16-membered macrolide (**14**) and 3-OH cladinose-type 16-membered macrolides (**27**) and its 9-*O*-acyl derivatives showed characteristic pharmacokinetics and stronger *in vitro* and *in vivo* antibacterial activities against target pathogens than the corresponding existing 16-membered macrolides, MOM or RKM, respectively. These novel analogues, however, were still relatively unstable under physiological conditions, and the *in vivo* efficacy of these analogues was not as high as that of CAM in mice model. Then, we decided to synthesize a 16-membered macrolide with high metabolic stability, that is, a 16-membered macrolide in which the neutral sugar moiety is not metabolized at all.

1.5. Design and Synthesis of 16-Membered Macrolides Possessing a Metabolically Stable Neutral Sugar

Part 1: Discovery of 16-Membered Macrolides Possessing a Di-*O*-Alkyl-Neutral Sugar

Since we could generate only one candidate (**14**) using the cladinose-type 16-membered macrolide, we used another strategy to develop a new chemical class of 16-membered macrolides, which possess a di-*O*-alky-neutral sugar. Previous studies have reported the synthesis of 4”-*O*-alkyl 16-membered macrolides (Figure 8). Sano *et al*.[42] reported the synthesis and biological evaluation of

4”-*O*-alkylspiramycin I analogues (**28**). The original study described that “introduction of alkyl groups, which are poorly hydrolyzed to the corresponding 4”-hydroxyl group would be an interesting approach to further chemical modifications.” All the novel derivatives of **28**, except **28a**, showed *in vitro* antibacterial activities similar to or stronger than those of the parent spiramycin I (SPM I); however, the *in vivo* potency of **28b** as a selected compound was not improved. The 3, 3”-di-*O*-acetyl derivatives of **28a** and **28b** were then synthesized, and the derivative of **28a** showed enhanced antibacterial activity *in vivo*.[46]

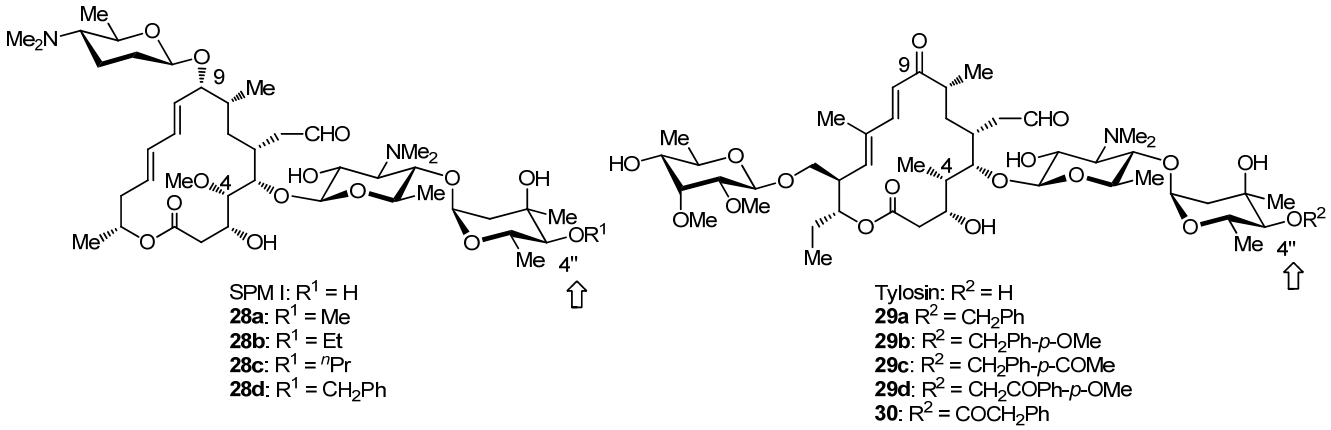
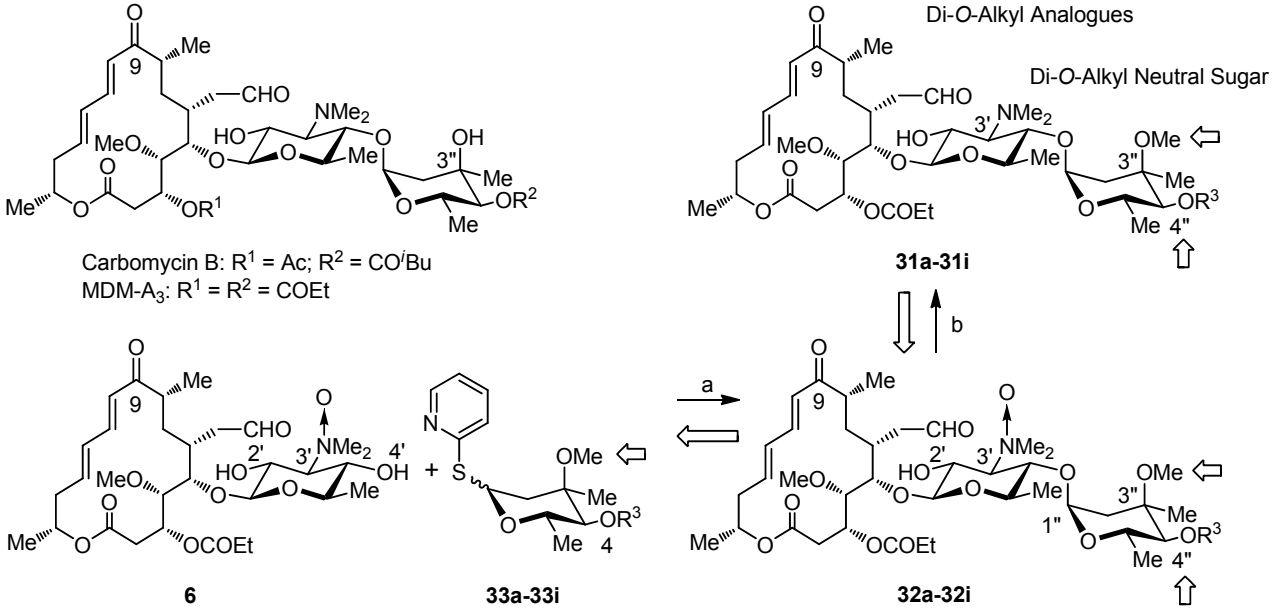


Figure 8. 4"-*O*-Alkylspiramycins Reported by Kitasato Institute and 4"-*O*-Alkyltylosins Reported by Sanraku Inc.

Kiyoshima *et al*.[47] reported synthesis of 4”-*O*-benzyltylosin analogues (**29**) using regioselective alkylation with the dibutyltin oxide method.[48] Compared to the control (**30**), these compounds (**29a**-**29d**) showed improved *in vitro* stability, and their *in vitro* antibacterial activities were similar to those of **30**. Synthesis of the above tylosin analogues did not seem to be quite difficult because of the (i) 9-dehydro structure (no allylic rearrangement) and (ii) reactive *O*-benzylation. On the other hand, synthesis of compounds **28b** and **28c** seemed to be rather difficult because of (i) sp^3 carbon at the C-9 position (possibility of allylic rearrangement) and (ii) less reactive aliphatic alkylation, but a fused 7-membered silyl hemiacetal enable aliphatic alkylation at the C-4” position. Initially, we wanted to synthesize 3-*O*-COEt analogues (no fused 7-membered silyl hemiacetal was available); therefore, we used the previously established glycosylation approach.

Glycosylation of compound **6** with 1-(2-pyridylthio) sugars (**33a-33i**) proceeded in the presence of anhydrous silver perchlorate followed by reduction of a tertiary amine oxide to afford the desired 9-dehydro-di-*O*-alkyl analogues (**31a-31i**)[49] (Scheme 4). Regioselectivity between the C-2’ and C-4’ positions was achieved (*i.e.* 2’-*O*-glycosylation did not occur), but stereochemistry at the C-1” position could not be controlled (the desired α-anomer, 38%; the undesired β-anomer, 38% based on consumption of compound **6**, respectively). Although a variety of 4-*O*-alkyl groups of **33** were accepted under these glycosylation conditions, only **33e**, which possesses a prenyl group resulted in a poor glycosylation yield (9.5%) because of its instability under acidic conditions.



[a]*Reagents and conditions:* (a) **33d** (6.0 eq), $AgClO_4$ (10 eq), MS4A, MeCN, -15 °C, 2 h and rt, 18 h in a dark place, 38% plus 38% of β-anomer based on consumed **6**, respectively; (b) Ph_3P (18 eq), CH_2Cl_2, 32 °C, 72 h, 72% of **31d** from **32d**.
R^3 = a: ethyl; b: allyl; c: *n*-butyl; d: $(CH_2)_2CHMe_2$; e: $CH_2CH{=}CMe_2$; f: $(CH_2)_3CHMe_2$; g: *n*-hexyl; h: $(CH_2)_2O(CH_2)_2OMe$; i: benzyl

Scheme 4. Preliminary Preparation of 9-Dehydro-16-Membered Macrolides with Di-*O*-Alkyl Neutral Sugar[a].

Table 5. Antibacterial Activities of **31c-31g**, **31i** and MDM-A_3 (MIC, μg/mL).

Test organisms	**31c**	**31d**	**31e**	**31f**	**31g**	**31i**	MDM-A_3
Enterococcus faecalis W-73	3.13	1.56	3.13	3.13	6.25	1.56	3.13
Streptococcus pneumoniae IP692	0.10	0.10	0.39	0.20	0.20	0.20	0.20
S. pneumoniae Type I	0.20	0.20	0.39	0.20	0.20	0.20	0.39
Streptococcus pyogenes Cook	0.10	0.05	0.20	0.05	0.20	0.20	0.20
Moraxella catarrhalis W-0500	0.78	0.78	1.56	1.56	1.56	1.56	1.56
M. catarrhalis W-0506	0.78	0.78	1.56	1.56	1.56	1.56	3.13
Haemophilus influenzae 9334	6.25	6.25	12.5	12.5	12.5	6.25	3.13

All 9-dehydro-di-*O*-alkyl analogues (**31a-31i**) showed clear antibacterial activities, but the compounds **31a** (ethyl), **31b** (allyl), and **31h** (methoxyethoxyethyl) showed rather weak potency (data not shown). Not only the length of the 4”-side chain but also its hydrophobicity affected the antibacterial activity. The antibacterial activities of other compounds were comparable to those of the parent compound midecamycin A_3 (MDM-A_3) (Scheme 4, Table 5). We selected compound **31d** for further evaluation as a prototype molecule, because a carbon framework of the 4”-sidechain of **31d** was the same as that of JM or carbomycin B.

Ishizuka[49] soon proved sustainable antibacterial activity of **31d** in rat plasma compared to the corresponding natural antibiotics, carbomycin B and MDM-A_3 as shown in Figure 9. Further, compared to the corresponding natural antibiotics, compound **31d** showed markedly improved pharmacokinetics,

i.e., serum concentration and urinary recovery (Figure 10) in *in vivo* mouse models.[49] The di-*O*-alkyl analogues, especially the 9-dehydro molecule (**31d**), showed sustainable antibacterial activities in the plasma, and their pharmacokinetics were superior to those of the existing macrolides; therefore, we decided to expand this research program and planned (i) stereoselective reduction at the C-9 ketone of **31d** and its analogues for improvement of pharmacokinetics and (ii) regioselective hydrolysis of the propionyl group at the C-3 position for enhancement of antibacterial activities in application of biotransformation.

Figure 9. Time Course of Relative Potency of **31d**, carbomycin B, and MDM-A_3 in Rat Plasma (t = 0; 100%, 37 deg).

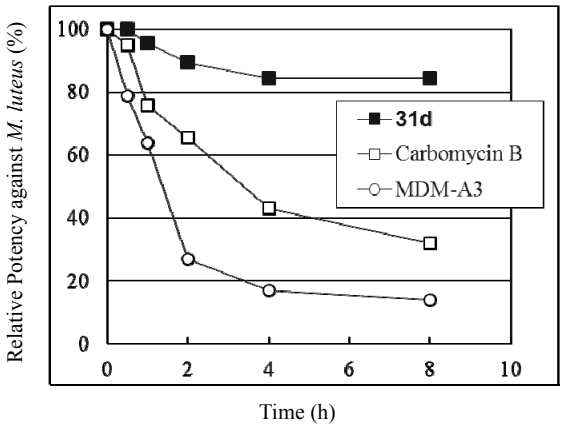
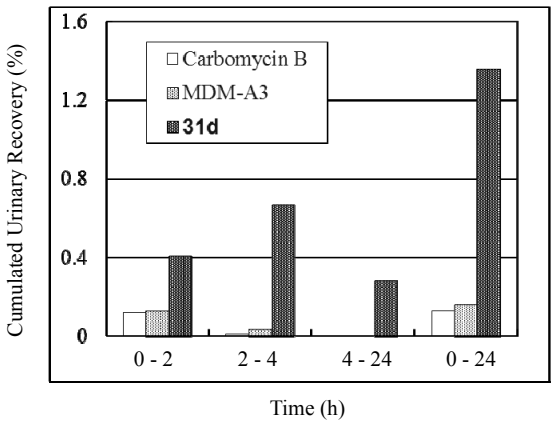


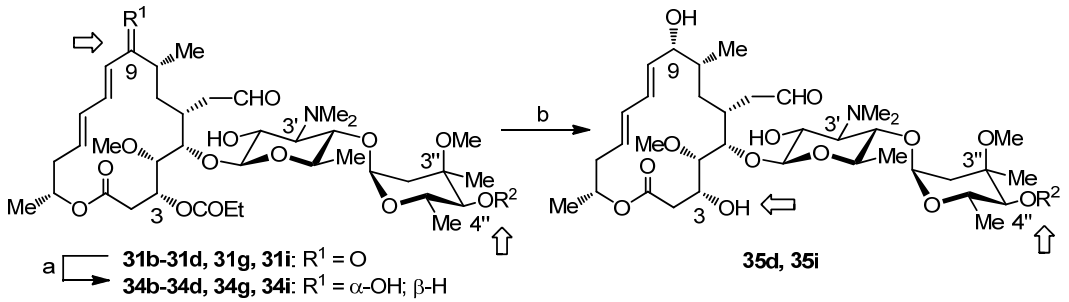
Figure 10. Time Course of Urinary Recovery of Carbomycin B, MDM-A_3, and **31d** after Oral Dosing (200 mg/kg) in Mice (n = 3).



1.6. Design and Synthesis of 16-Membered Macrolides Possessing a Metabolically Stable Neutral Sugar

Part 2: Optimization of 16-Membered Macrolides Possessing a Di-*O*-Alkyl-Neutral Sugar

A carbonyl group at the C-9 position of compounds **31** was converted to the corresponding α-hydroxyl group in a moderate yield by biotransformation using SF2772[25] (Scheme 5). The antibacterial activities of compound **34d** and some of its analogues were comparable and their stability in rat plasma was significantly improved compared to that of MDM[50] (data not shown).



[a]*Reagents and conditions:* (a) *Streptomyces mycarofaciens* SF2772, 28 °C, 24 h, 45-60%; (b) *Phialophora* sp. PF1083, 26 °C, 10 days, 8.2% of **35d** plus 7.3% recovered **34d** or 8.7% of **35i** plus 7.7% recovered **34i**.
R^2 = b: allyl; c: *n*-butyl; d: $(CH_2)_2CHMe_2$; g: *n*-hexyl; i: benzyl

Scheme 5. Biotransformation of Di-*O*-Alkyl Analogues of 16-Membered Macrolide[a].

Serum concentration of **34d** detected by microbial assay was extremely higher, and **34d** remained in circulation for a longer time than MDM, and its AUC was also larger than that of MDM. In particular, the markedly high maximum plasma concentration (Cmax) level of **34d** as the 16-membered macrolide antibiotic was comparable to that of CAM (Figure 11).[50] To date, only few analogues belonging to the leucomycin family have been reported to have such high serum concentrations. Moreover, the urinary recovery of **34d** within 24 hours in mice was 20% (Figure 12), while it was less than 1% for MDM, and 24% for CAM. Increased serum concentration and delayed excretion as well as high urinary recovery actually contributed to strong *in vivo* potency, especially against *S. pneumoniae* (cf. the last part of section 1.6).

Figure 11. Time Course of Serum Concentrations of **34d**, MDM, and CAM after Oral Dosing (200 mg/kg) in Mice (n = 2).

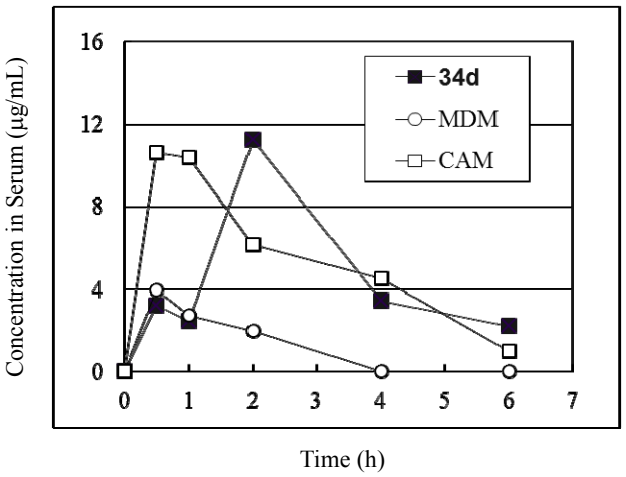
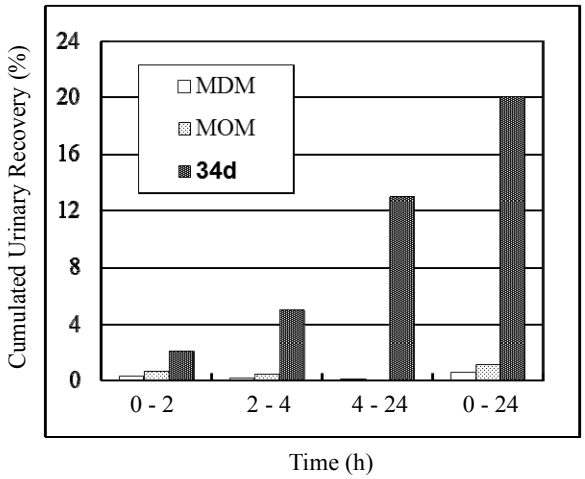
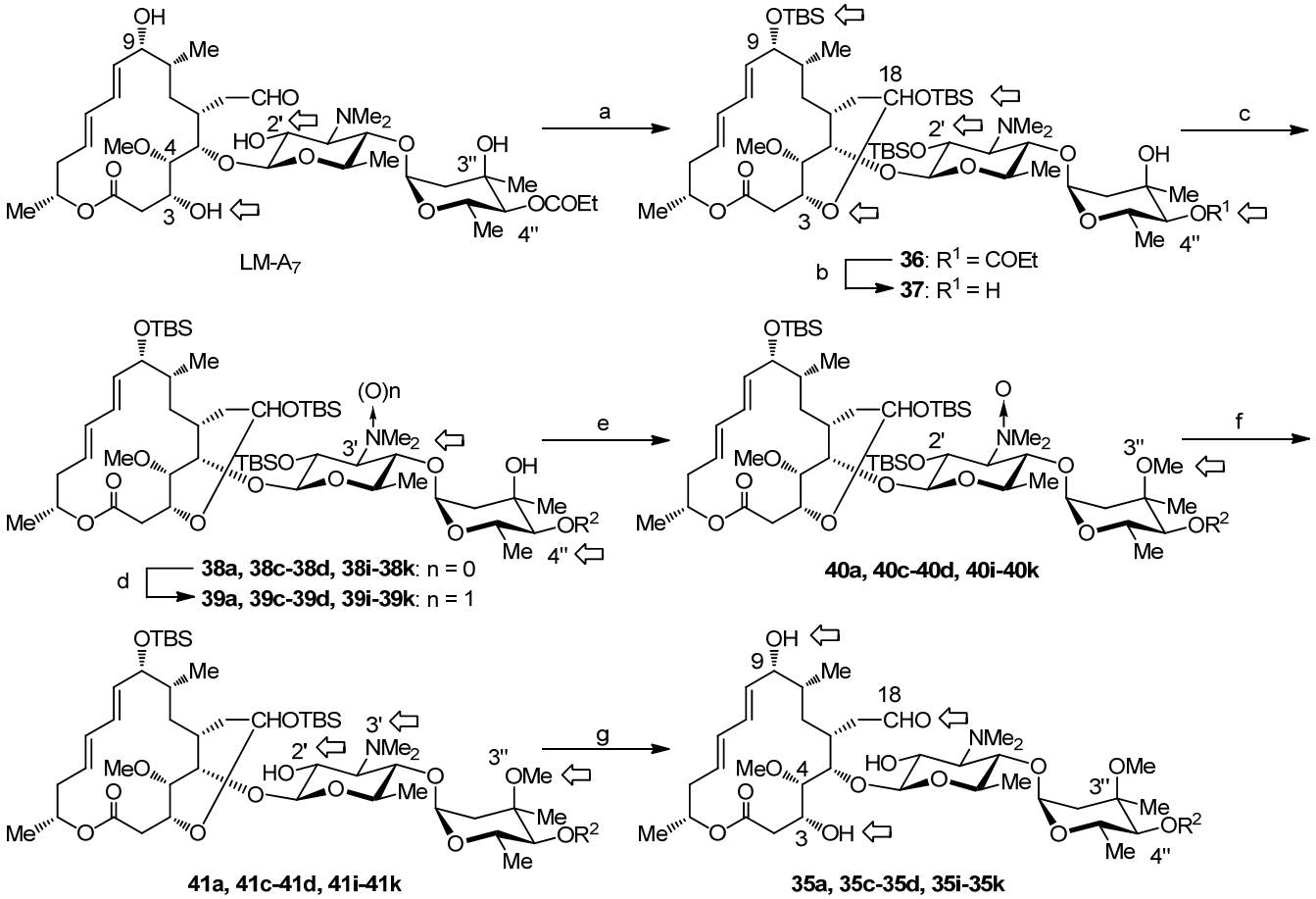


Figure 12. Time Course of Urinary Recovery of MDM, MOM, and **34d** after Oral Dosing (200 mg/kg) in Mice (n = 6).



Although we tried biotransformation of compound **34d** using *Phialophora* sp. PF1083 for the purpose of regioselective 3-de-*O*-propionylation, the conversion yield was less than 10% (Scheme 5). The yield of **35i** was also less than 10%, and we supposed that a low yield of this biotransformation is substrate specific, more precisely, 4”-*O*-substituent specific. Compounds **35d** and **35i** exhibited enhanced antibacterial activities against clinically important Gram-positive pathogens compared to those of LM-A_7,[51] and the half-life of **35d** in rat plasma was 3 to 4 times longer than that of LM-A_7 or RKM. Consideration 28% yield of compound **18** plus 16% recovery of starting material (**13**) using PF1083 (Figure 7), we were interested in the substrate specificity of PF1083. The studies performed by Shimizu[39] indicated that an efficient 3-de-*O*-propionylation of 16-membered macrolides using *Phialophora* sp. PF1083 required a 4”-acylated neutral sugar moiety. Even preliminarily optimized overall yield of compound **35d** from an intermediate (**6**) was almost 1%, and we had to develop a novel synthetic route of **35d** and its analogues in order to (i) prepare medium size of compound **35d** for *in vivo* studies using not only small rodent animals but large rodent animals also and (ii) optimize the 4”-*O*-alkyl group.



[a]*Reagents and conditions:* (a) TBSCl (6.0 eq), imidazole (12 eq), DMF, 50 °C, 16 h, 83%; (b) 25% aq NaOH, *n*-Bu_4NHSO_4 (1.0 eq), PhH-H_2O (2:1), 25 °C, 2 h, 87%; (c) alkyl halide (30 eq), NaH (5.0 eq), DMF, 45 °C, 1 h, 67-90%; (d) *m*CPBA (1.5 eq), $CHCl_3$, 25 °C, 5 min.; (e) MeI (30 eq), NaH (5.0 eq), 45 °C, 1 h; (f) silica gel, 25 °C, 16 h, 51-65% overall 3 steps; (g) 2.0 M of TBAF, THF, 45 °C, 1 h, 54-94%. R^2 = a: ethyl; j: *n*-propyl; c: *n*-butyl; k: *n*-pentyl; d: $(CH_2)_2CHMe_2$; i: benzyl.

Scheme 6. Optimization of an Alkyl Group at the C-4" Position in Application of LM-A_7 as a Starting Material[a].

Kurihara *et al.*[52] used LM-A_7 as a starting material and utilized Kitasato protection (silyl hemiacetal). Although the 2'-*O*-acetyl group was acceptable for 4"-*O*-acylation (Scheme 3), 4"-*O*-alkylation and direct 3"-*O*-methylation required a very stable 2'-*O*-protecting group, *i.e.* 2'-*O*-TBS group. In addition, direct 3"-*O*-methylation required protection of a dimethylamino group at the C-3' position. We synthesized completely protected desired 3",4"-di-*O*-alkyl analogues (**40**) (Scheme 6). According to the original information,[53] it was reported that a 2'-*O*-TMS protecting group could be hardly removed in 14-membered macrolide intermediates. Thus, a 2'-*O*-TBS group (a free dimethylamino group at the C-3' position) could not be removed even in different conditions, but the 2'-*O*-TBS group of compounds **40** was simultaneously removed when we reduced the dimethylamino *N*-oxide of **40**s to the free dimethylamino group. Although this mechanism could not be clarified yet, Professor Shuto[54] provided us with a tentative mechanism. Optimized overall yield of **35d** from LM-A_7 was around 20%, and we successfully completed scale up synthesis of **35d** and optimization of the 4"-*O*-alkyl group of **35d**.

Antibacterial activities of 16-membered macrolides, which possess a 3”, 4”-di-*O*-alkyl neutral sugar are shown in Table 6. Regarding to optimization of the 4”-*O*-alkyl group, an *n*-butyl group (**35c**), a 3-methylbutyl group (**35d**), and a benzyl group (**35i**) were selected according to the activities against clinically important Gram-positive pathogens, and compound **35d** was finally selected by the activities against clinically important Gram-negative pathogens. Antibacterial activities of **35d** against Gram-positive strains are comparable to those of RKM. Antibacterial activities of 3”-*O*-ethyl analogue and 3”-*O*-*n*-propyl analogue of **35d** were decreased compared to those of **35d**, and acidic stability of 3”-OH analogue of **35d** was extremely poor because of lack of 1,3-diaxial steric hindrance by the 3”-methoxy group. Then, we examined characteristic pharmacokinetics and *in vivo* efficacy of **35d**.

Table 6. Antibacterial Activities of 3-OH-3”-*O*-Me-4”-*O*-Alkyl Analogues (MIC, μg/mL).

Test organisms	**35j**	**35c**	**35k**	**35d**	**35i**	LM-A_7	RKM
Enterococcus faecalis W-73	0.78	0.78	0.39	0.39	0.39	0.78	0.39
Streptococcus pneumoniae IP692	0.20	0.10	0.05	0.10	0.10	0.20	0.10
S. pneumoniae Type I	0.20	0.20	0.20	0.10	0.10	0.20	0.10
Streptococcus pyogenes Cook	0.10	0.10	0.10	0.05	0.10	0.10	0.05
Moraxella catarrhalis W-0500	1.56	0.78	0.39	0.39	0.78	0.78	0.20
M. catarrhalis W-0506	3.13	1.56	0.78	0.39	0.78	1.56	0.20
Haemophilus influenzae 9334	6.25	3.13	3.13	1.56	3.13	1.56	1.56

Figure 13. Time Course of Serum Concentrations of **35d**, RKM, and CAM after Oral Dosing (200 mg/kg) in Mice (n = 2).

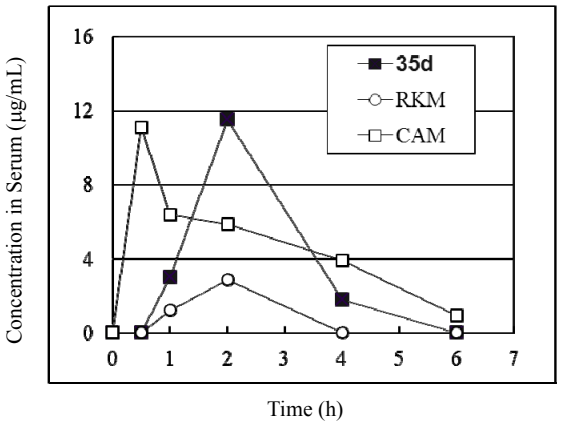
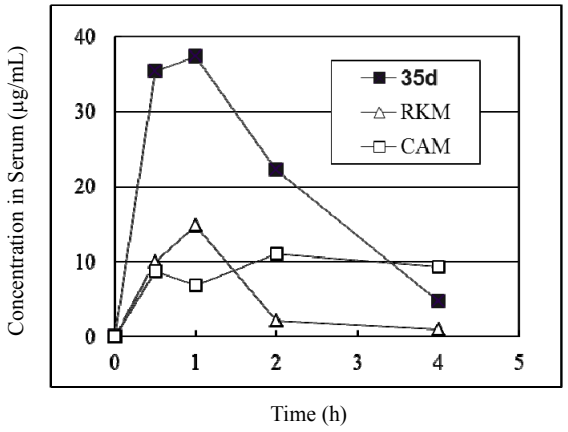


Figure 14. Time Course of Serum Concentrations of **35d**, RKM, and CAM after Oral Dosing (500 mg/kg) in Hamster.



We performed preliminary pharmacokinetic analysis of **35d**. We orally administered 200 mg/kg of **35d**, RKM, and CAM to mice. Time course of serum concentrations of these 3 molecules is shown in Figure 13. Absolute serum concentrations of **35d** detected by microbial assay were dramatically higher than those of RKM.[52] The serum level of LM-A_7 was lesser than that of RKM (data not shown). In particular, the markedly high Cmax level of **35d** as the 3-OH-type 16-membered macrolide antibiotic was comparable to that of CAM. These results led us to perform further pharmacokinetic study of **35d** using

hamsters as larger test animals. In the field of 16-membered macrolides, pharmacokinetics are often strongly affected by animal species.[9] We orally administered 500 mg/kg of these three molecules to hamsters. Compound **35d** exhibited a dramatically improved serum level detected by microbial assay than RKM (Figure 14). Moreover, the Cmax levels of **35d** at 30 minutes, 1 hour, and 2 hours after administration were quite higher than those of CAM. In conclusion, we discovered the novel 16-membered macrolide antibiotic (**35d**) with dramatically improved pharmacokinetics and strong *in vitro* potency.

Finally, we evaluated *in vivo* potency of 2 selected macrolides (**34d** and **35d**) by protective effect against systemic infections in mice, compared them with a 3-*O*-propionyl analogue, MDM for **34d**, and a 3-OH-type analogue, RKM for **35d**. The ED_{50} values indicated that the *in vivo* efficacy of **34d** and **35d** against both *S. pneumoniae* DP-I type I (n = 8) and *S. aureus* Smith I (n = 5) was superior to that of MDM and RKM, respectively. Further, the *in vivo* potency of **34d** against *S. pneumoniae* DP-I type I was 16 times stronger than that of MDM,[55,56] and **35d** was 4 to 8 times (6.5 times according to calculations) more active *in vivo* than RKM against the same pathogen. In conclusion, these novel 16-membered macrolide antibiotics (**34d** and **35d**) with a di-*O*-alkyl neutral sugar moiety exhibited enhanced *in vivo* efficacy and dramatically improved pharmacokinetics in mice and hamsters.

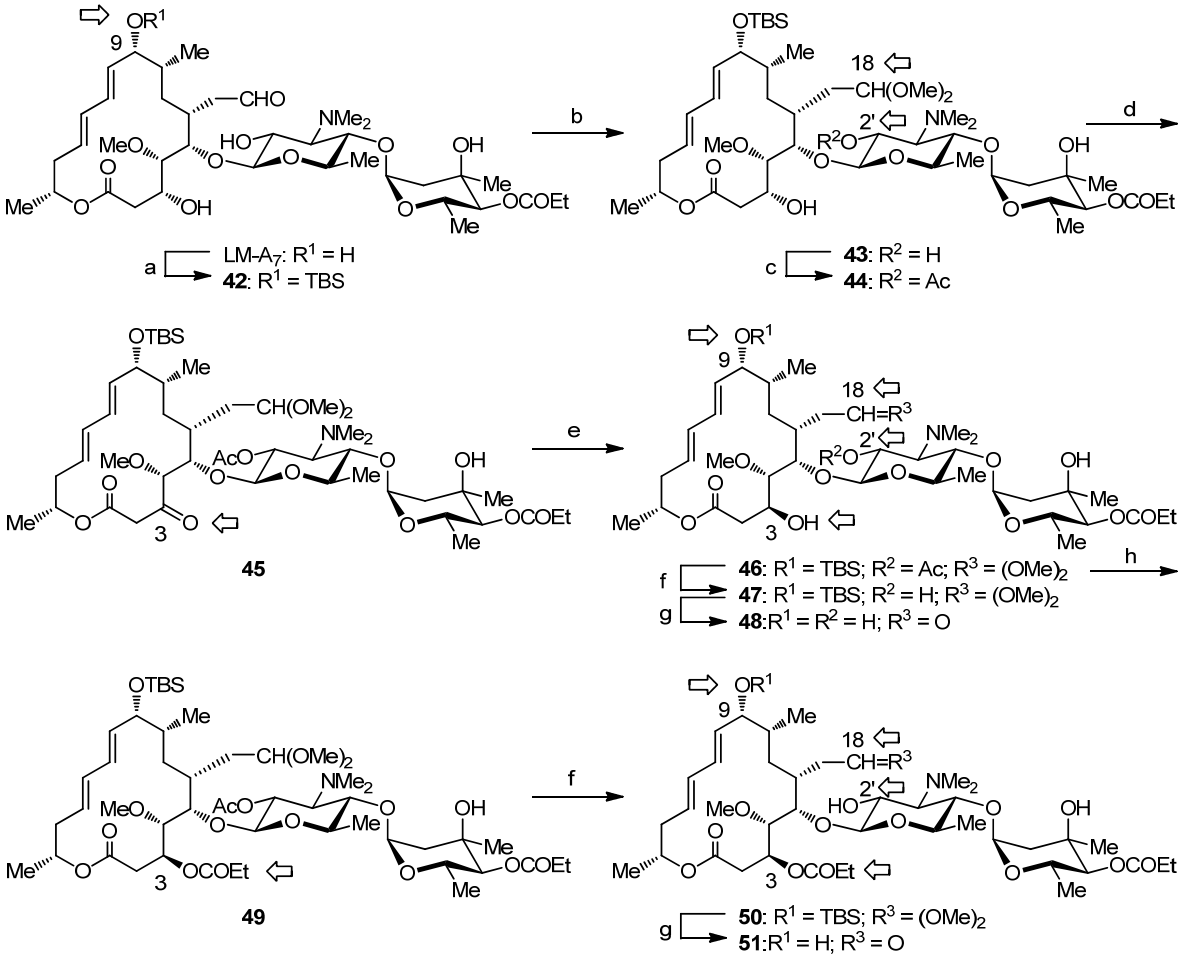
1.7. Chemical Transformation at the C-3 Position of 16-Membered Macrolides

Compared to 14-membered macrolides, 16-membered macrolides have a relatively unstable lactone linkage.[57] Steric hindrance around an ester bond in a 16-membered macrolactone (Figure 1, two broken circle lines) is relatively smaller than that in 14-membered macrolactone. Although a lactone linkage in 3-*O*-acyl-type 16-membered macrolides is relatively stable, a lactone linkage in the 3-OH-type 16-membered macrolides is relatively unstable. On the other hand, the *in vitro* potency of 3-OH-type 16-membered macrolides is stronger than that of 3-*O*-acyl-type analogues as described in the first part of section 1.4. We determined one approach for resolving this paradox at the C-3 position, where the *in vitro* potency and stabilization of the lactone linkage are inversely related.

We focused on the fact that in a three-dimensional structure, the hydroxyl group at the C-3 position was close to the aldehyde at the C-18 position and planned to synthesize 3-*epi* derivatives of 16-membered macrolides. In addition, the 3-*epi*-derivatives did not form a 3,18-hemiacetal because of the difference in the distance between the 3-*epi*-OH and the 18-aldehyde group in these derivatives and that between the 3-OH and 18-aldehyde group in the natural compounds. An important intermediate (**44**) was reacted with dimethyl sulfoxide, trifluoroacetic anhydride, and triethylamine to afford a ketone (**45**) in 30% yield (Scheme 7);[58] however, oxidation of **44** using alternative oxidation agents such as pyridinium dichromate (PDC) or other mild oxidants (*i.e.*, Dess-Martin periodinane or tetra-*n*-propylammonium

perruthenate-*N*-methylmorpholine *N*-oxide [TPAP-NMO]) was not successful. Treatment of **45** with sodium borohydride followed by deprotections yielded the desired β-alcohol, 3-*epi*-LM-A_7 (**48**). Remarkable β-selectivity can be explained by the following reasons: (i) the C-3 carbonyl group located at the β-site of the lactone and (ii) reagents generally attack a macrolactone from the outside of the lactone ring in macrolide chemistry. We prepared a 3-*O*-COEt congener, 3-*epi*-MDM (**51**), using **46**.

The antibacterial activities of 3-*epi*-analogues (**48** and **51**) against clinically important pathogens in the respiratory infections were similar to or lesser than those of the corresponding natural products, LM-A_7 and MDM (Table 7). The biological stability of **48** in rat plasma was significantly lower than that of LM-A_7 even in the preliminary study. Half-life of **48** was approximately 8 times shorter than that of LM-A_7. Thus, we developed an alternative approach for improvement of the biological stability of a lactone.



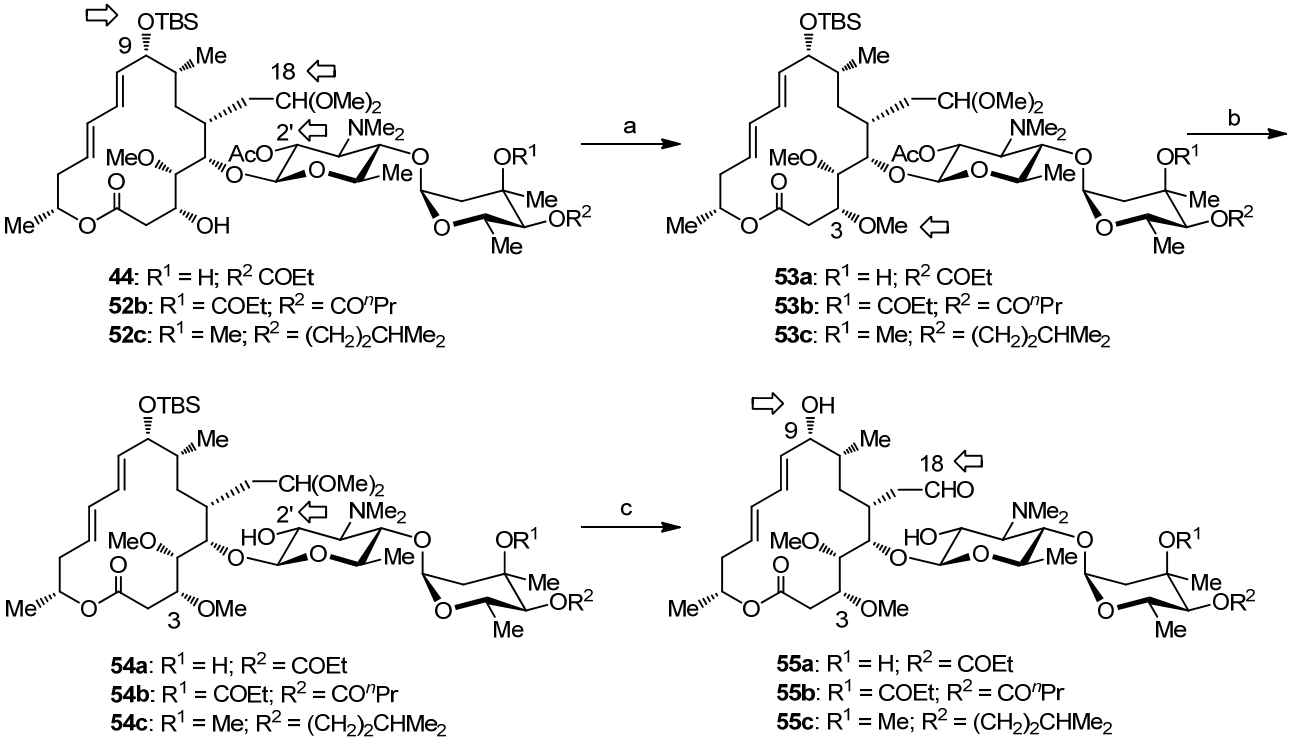
[a]*Reagents and conditions:* (a) (1) TBSCl (1.5 eq), imidazole (3.3 eq), DMF, 45 °C, 17 h; (2) TBAF (1.4 eq), THF, rt, 3 h; (b) $CH(OMe)_3$ (excess), PPTS (1.5 eq), 45 °C, 36 h; (c) Ac_2O (5.0 eq), MeCN, rt, 24 h, 62% overall 4 steps; (d) DMSO (5.7 eq), TFAA (3.5 eq), Et_3N (10 eq), CH_2Cl_2, -78 °C, 65 min., 30%; (e) $NaBH_4$ (4.8 eq), 1,4-dioxane, rt, 16 h, 75%; (f) 90% aq MeOH, 50 °C, 16 h, 98% for **47**; (g) CHF_2CO_2H (5.0 eq), MeCN-H_2O (1:1), rt, 16 h, 78% for **48**; (f) (g) 69% for **51** in 2 steps; (h) EtCOCl (5.5 eq), pyridine (15 eq), DMAP (1.0 eq), CH_2Cl_2, rt, 4 h, 53%.

Scheme 7. Synthesis of 3-*Epi*-LM-A_7 and 3-*Epi*-MDM[a].

Table 7. Antibacterial Activities of 3-*Epi*-Analogues of 16-Membered Macrolide (MIC, μg/mL).

Test organisms	**48**	LM-A_7	**51**	MDM
Streptococcus pneumoniae DP1 TypeI	0.39	0.20	0.20	0.20
S. pneumoniae IP692	0.78	0.20	0.39	0.39
Moraxella catarrhalis W-0500	0.78	0.78	0.78	1.56
Haemophilus influenzae 9334	0.78	1.56	3.13	6.25
H. influenzae PRC-44	6.25	6.25	12.5	25

We simultaneously focused on the two aspects for a side chain at the C-3 position as follows: (i) a small volume for *in vitro* potency and (ii) an electron donating character for increasing the basic stability of the lactone linkage. We used compound **44** as a key intermediate, and the corresponding RKM intermediate (**52b**) was prepared from RKM in 3 steps using procedures similar to those used for preparing compound **44**. An alternative important intermediate (**52c**) was synthesized from **41d** (Scheme 6) in 3 steps, including (i) regioselective deprotection of the TBS group at the C-18 position, (ii) 18-dimethylacetal formation, and (iii) regioselective 2'-*O*-acetylation. Regioselective 3-*O*-methylation of these intermediates (**44**, **52b**, **52c**) using methyl iodide with a strong base in dimethyl sulfoxide followed by deprotections afforded the desired 3-*O*-methyl analogues (**55a**, **55b**, **55c**) (Scheme 8).



44: R^1 = H; R^2 COEt
52b: R^1 = COEt; R^2 = CO^{n}Pr
52c: R^1 = Me; R^2 = $(CH_2)_2CHMe_2$

53a: R^1 = H; R^2 COEt
53b: R^1 = COEt; R^2 = CO^{n}Pr
53c: R^1 = Me; R^2 = $(CH_2)_2CHMe_2$

54a: R^1 = H; R^2 = COEt
54b: R^1 = COEt; R^2 = CO^{n}Pr
54c: R^1 = Me; R^2 = $(CH_2)_2CHMe_2$

55a: R^1 = H; R^2 = COEt
55b: R^1 = COEt; R^2 = CO^{n}Pr
55c: R^1 = Me; R^2 = $(CH_2)_2CHMe_2$

[a]*Reagents and conditions:* (a) MeI (4.8 eq), KOH (15 eq), DMSO, rt, 1 h, 40%, 80%, 55% for **53a**, **53b, 53c**, respectively; (b) 90% aq MeOH, rt, 16 h; (c) CHF_2CO_2H (5.6 eq), MeCN-H_2O (1:1), 40 °C, 16 h, 22%, 66%, 20% for **55a**, **55b**, **55c** in 2 steps, respectively.

Scheme 8. Synthesis of 3-*O*-Methyl Analogues of 16-Membered Macrolides[a].

The antibacterial activities of these 3-*O*-methyl analogues (**55a**, **55b**, **55c**)[58] against *S. pneumoniae* were similar to or slightly stronger than those of the corresponding 3-OH analogues, LM-A_7, RKM, and the promising derivative (**35d**), respectively (Table 8). Moreover, compounds **55b** and **55c** showed improved biological stability in rat plasma. After incubation for 2 hours at 37 °C, **55b** and **55c** were 2 to 3 times more stable than the corresponding 3-OH analogues, RKM, and **35d**. In conclusion, compound **55c** was proposed to be one of the most biologically stable 16-membered macrolide (6 to 8 times more stable than RKM) with antibacterial activities as strong as RKM. On the basis of these results, we concluded that methylation of the 3-OH group and 3", 4"-di-*O*-alkylation are one of the most promising modifications for improvement of the biological stability of the 16-membered macrolides.

Table 8. Antibacterial Activities of 3-*O*-Methyl Derivatives and Their 3-OH Analogues (MIC, μg/mL).

Test organisms	**55a**	LM-A_7	**55b**	RKM	**55c**	**35d**
Streptococcus pneumoniae DP1 TypeI	0.10	0.20	0.05	0.10	0.10	0.10
S. pneumoniae IP692	0.20	0.20	0.10	0.10	0.10	0.10
S. pneumoniae PRC-91	100	100	0.78	1.56	3.13	6.25
S. pneumoniae PRC-53	0.10	0.20	0.10	0.20	0.10	0.10
Moraxella catarrhalis W-0500	0.78	0.78	0.20	0.20	0.39	0.20
Haemophilus influenzae 9334	1.56	1.56	3.13	1.56	1.56	1.56
H. influenzae PRC-44	6.25	6.25	6.25	6.25	12.5	6.25

Among these characteristic molecules, compound **14** was assigned as a candidate for in-house development, and compound **35d** was discussed as a candidate in collaboration with a well-known pharmaceutical company in Japan.

2. Application of an Arylalkyl Group to 16-Membered Macrolides

2.1. Emergence of Resistant *S. pneumoniae* and Mechanisms of Resistance

Severe problems due to dangerous pathogens[59] at clinical site are important topics of consideration even today. In the 1990s, emergence of a resistant strain of clinically important pathogens in respiratory infections was an important cause of concern, especially in the pediatric field. Among them, a predominant type (with an *erm* gene) of ERSP could not be inhibited using CAM, AZM, or 16-membered macrolides. On the other hand, emergence of EM resistant *S. pyogenes*[60] was reported in 1991, and resistance to EM in *S. pyogenes* has become widespread.[61] Isolation frequency of ERSP in Japan has gradually increased[62] and reached 80% (Figure 15). High frequency of resistant *S. pneumoniae* is common in Japan, and this frequency is not so high in European countries or in the United States. The global clinical trial, Prospective Resistant Organism Tracking and Epidemiology for Ketolide Telithromycin (PROTEKT),[63] indicated that the frequency of ERSP was 77.9% in Japan, 30.9% in the US, and 24.4% in European countries. Another trial, Community-Acquired Pneumonia (CAP),[64] indicated that the frequency of ERSP was 81.4% in Japan.

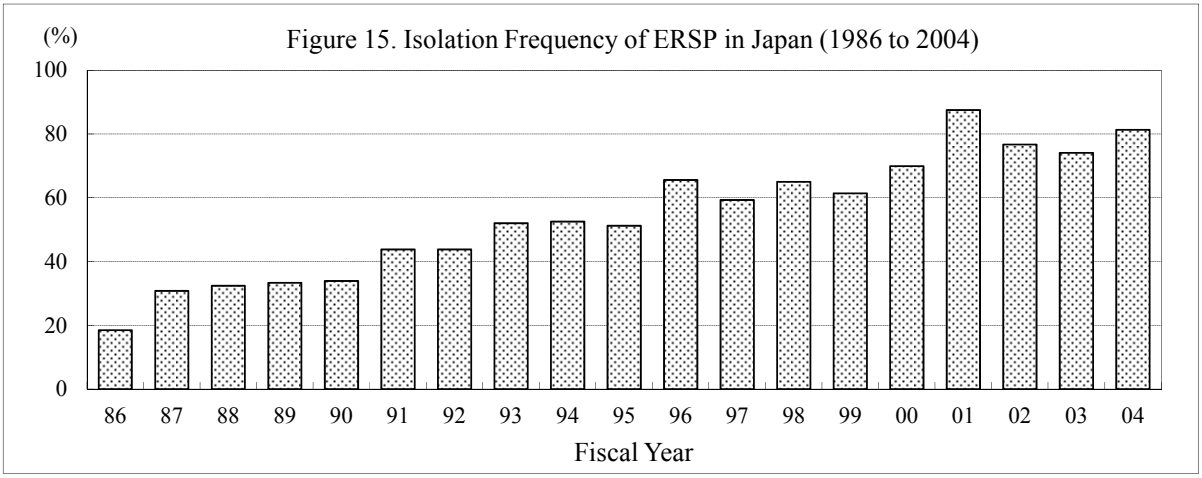


Figure 15. Isolation Frequency of ERSP in Japan (1986 to 2004)

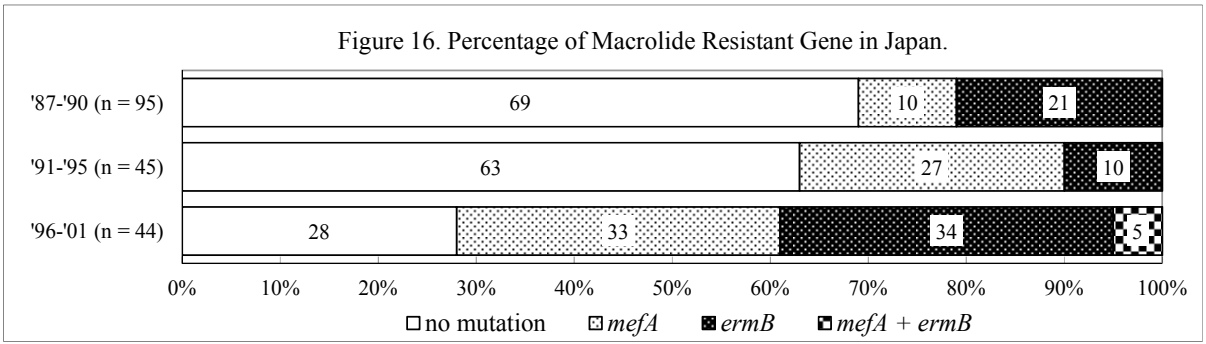


Figure 16. Percentage of Macrolide Resistant Gene in Japan.

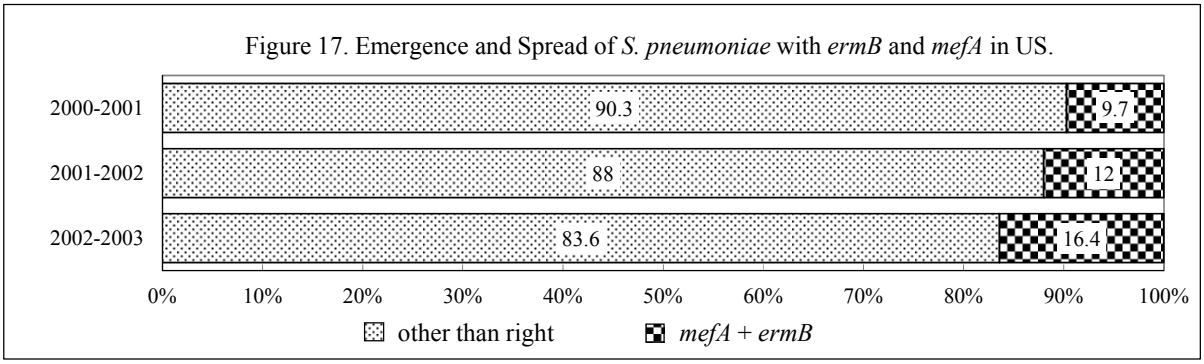
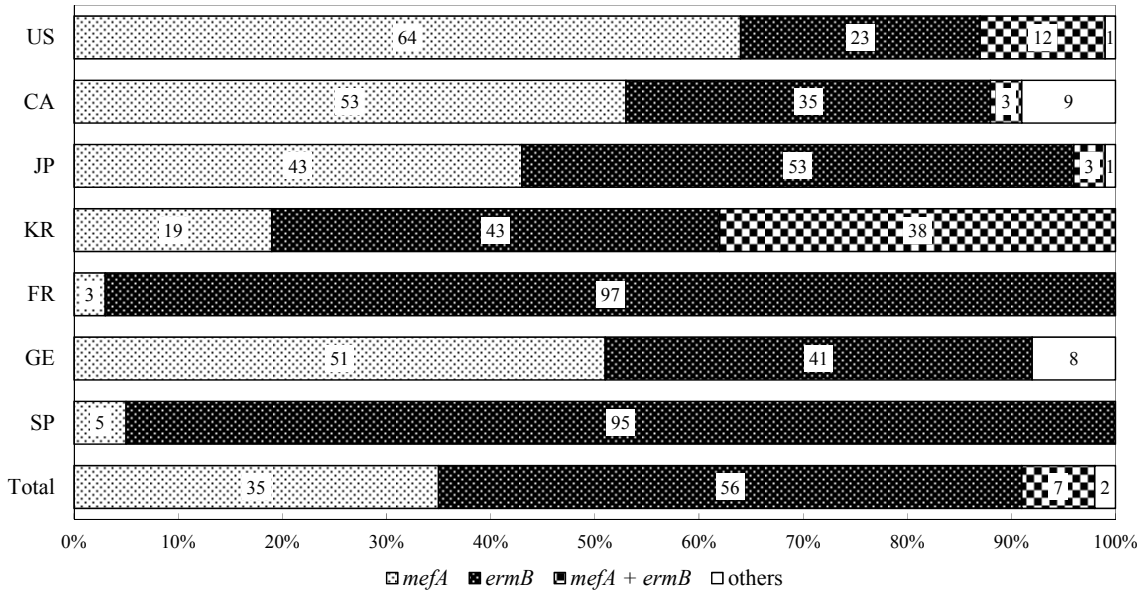


Figure 17. Emergence and Spread of *S. pneumoniae* with *ermB* and *mefA* in US.

Macrolide resistance in *S. pneumoniae* occurs mainly by two mechanisms, target-site modification (an *erm* gene) or efflux of the drug out of the cell (an *mef* gene). The most common form of target-site modification[65] includes dimethylation of a specific adenine residue on the 23S rRNA (A2058 in *Escherichia coli* numbering) by an rRNA methylase. Because macrolide antibiotics exert antibacterial activities by interaction of a ribosomal subunit,[66] dimethylation by an *erm* gene of the adenine residue on the rRNA is recognized as a mechanism of resistance. Kadota[67] reported that the frequency of both types of resistant *S. pneumoniae* (with an *erm* gene or an *mef* gene) was increasing in Japan (Figure 16), and Farrell *et al*.[68] concluded that resistant *S. pneumoniae* possessing both genes (an *erm* gene and an *mef* gene) markedly increased in only three years in the US (Figure 17). The distribution of macrolide resistance of *S. pneumoniae* isolates (*erm*-type vs. *mef*-type) differed depending on the country (Figure

18).[65] For example, development of resistance by target-site modification by methylase (an *erm* gene) was observed in France and Spain, and efflux of the drug (an *mef* gene) was the more common mechanism of development of resistance in the US. A worldwide review indicated that the frequency of target-site modification by methylase was more than 50%, thus, we focused on resistant *S. pneumoniae* possessing methylase (an *erm* gene) as a primary target. In addition, we decided to investigate the antibacterial activities of 16-membered macrolides against other clinically important Gram-positive pathogens in respiratory infections, such as *S. pyogenes*, which possesses an *erm* gene. Our study using 16-membered macrolides was advantageous because intact or chemically modified 16-membered macrolides[8] were effective against resistant *S. pneumoniae* possessing an *mef* gene. Therefore, they could not become a substrate for an efflux pump.

Figure 18. Distribution of Macrolide Resistance Mechanisms by Country among 1,043 Macrolide-Resistant Isolates *of S. pneumoniae* collected in the PROTEKT 1999-2000 Study.



2.2. Discovery of an Arylalkyl Group in 14-Membered Macrolides

In the early 1990s, major groups performing studies on macrolides began synthesis of novel macrolide derivatives with a focus on antibacterial activities against resistant bacteria. Kashimura *et al.*[69] reported the synthesis and antibacterial activities of TE-802 at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), which was held in San Francisco in 1995. The chemical structure of TE-802 included a fused tricyclic aglycone consisting of a diazaheptane ring and oxazolidinone accompanied with a 14-membered lactone (Figure 19). Agouridas *et al.*[70] reported the synthesis and antibacterial activities of Ru-004 (later HMR3004), which possesses a quinoline ring, and it was a prototype of telithromycin[71] (TEL). The C-3 position of these derivatives is a carbonyl group, and these

analogues are known as "ketolides." TE-802[72] showed antibacterial activities against EM resistant *Staphylococcus aureus* (MIC, 0.39 μg/mL), resistant *Klebsiella pneumoniae* (MIC, 6.26 μg/mL), and highly resistant *S. pneumoniae* (MIC, 6.25 μg/mL), although CAM was not effective against these strains. Ru-004[73] showed antibacterial activities not only against inducible resistant *S. pneumoniae* (MIC, 0.02 μg/mL) but also against resistant *S. pneumoniae* constitutively expressing an *erm* gene (constitutive resistant *S. pneumoniae*) (MIC, 0.15 μg/mL).

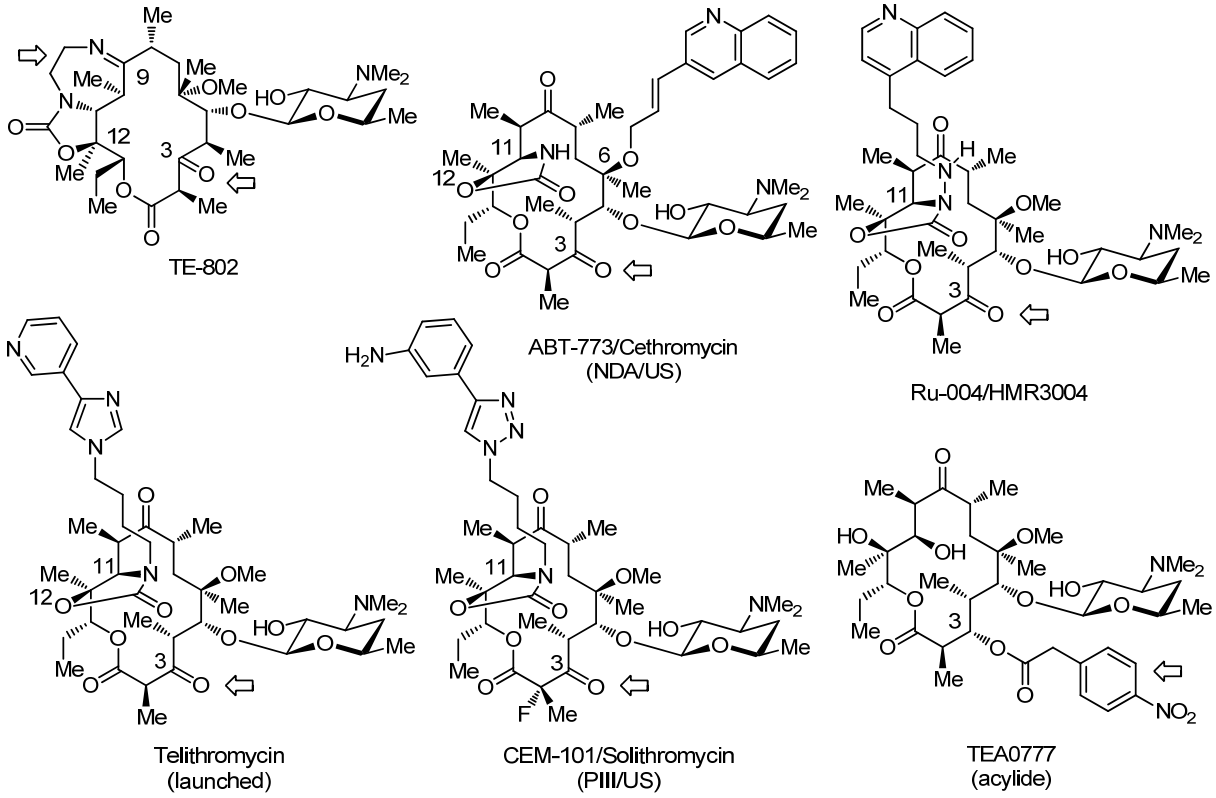


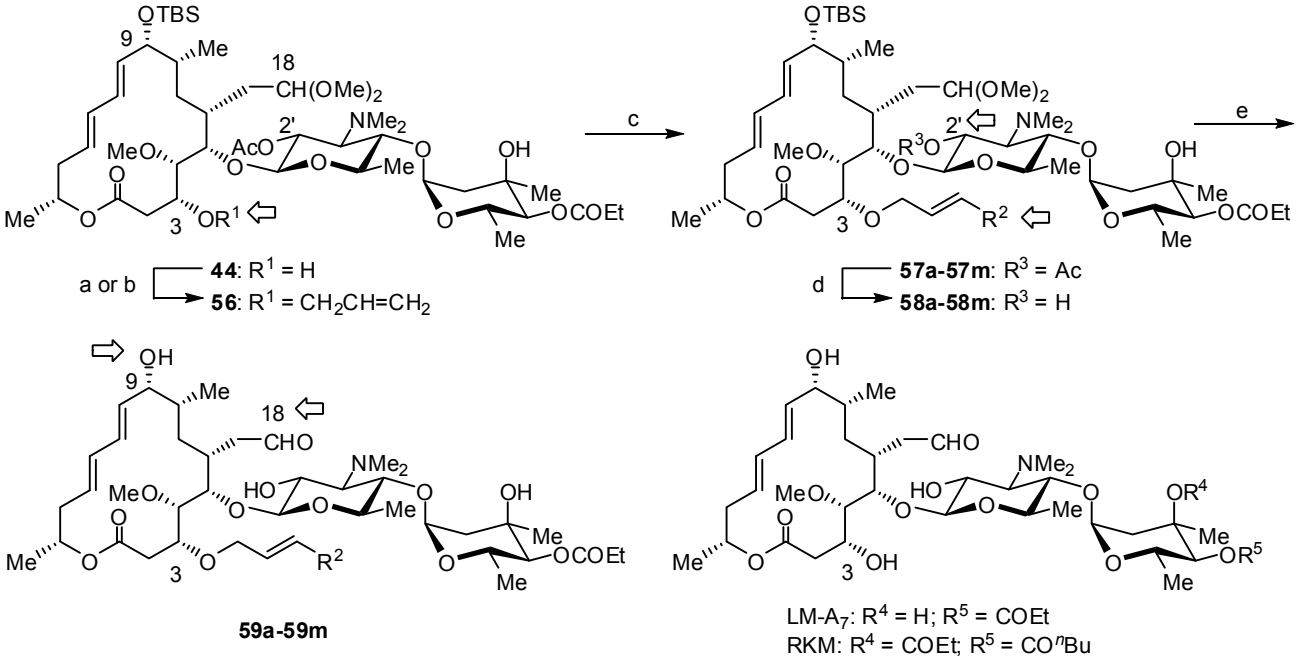
Figure 19. Ketolides and Acylide Focusing on Anti-Resistant Bacterial Activities.

Because TE-802 has a characteristic structure, generation of structurally related and patent-free lead compound(s) was difficult. On the other hand, a synthetic strategy to comprehensively introduce an arylalkyl group to the lactone ring was suggested to be appropriate if the arylalkyl group of Ru-004 was deeply concerned with the activity against constitutive resistant *S. pneumoniae*. According to this hypothesis, major macrolide research groups all over the world focused on the design, synthesis, and biological evaluation of novel arylalkylated 14-membered macrolides. Clinical trials of TEL[71] began in 1998 in European countries, and it was approved for the first time in France in 2001. The ketolides cethromycin[74] and solithromycin[75] (Figure 19), which possess an arylalkyl group are currently under a late stage of development. Cethromycin was granted the designation of an orphan drug for anthrax in the US in 2007. A current study has explained the scientific relationships[76] between these arylalkyl moieties

and their antibacterial activities against resistant bacteria. However, to date, no macrolide antibiotic without drawbacks, including serious side effects[77] or extremely bitter taste,[78] and which is effective against constitutive resistant *S. pneumoniae* has been launched. Thus, we decided to develop a 16-membered macrolide, which was safe, metabolically stable, and effective against resistant *S. pneumoniae* with an *erm* gene.

2.3. New Aspect and Limitations of Chemical Modifications at the C-3 Position of 16-Membered Macrolides.

We observed that replacement of a 3-*O*-propionyl group of MDM by a quinolin-2-yl-carbonyl group partially enhanced the antibacterial activities against both susceptible and resistant *S. pneumoniae*.[58] In addition, Tanikawa *et al*.[79] modified the 3-OH group using an acyl moiety and generated acylides (Figure 19). One of the acylides, TEA0777, showed increased antibacterial activities against resistant Gram-positive bacteria. A combination of NMR spectroscopy and molecular modeling of Ru-004 (Figure 19) indicated[80] that, in solution, the quinoline ring was anchored to the two carbonyl groups, 1-CO and 3-CO, by electrostatic interactions. On the basis of these findings, we decided to synthesize a variety of 3-*O*-(3-aryl-2-propenyl) derivatives of LM-A_7.



[a]*Reagents and conditions:* (a) KOH (14 eq), allyl iodide (10 eq), DMSO, rt, 2 h, 27%, 63% based on recovery of **44**; (b) $Pd_2(dba)_3$ (0.024 eq), 1,4-bis(diphenylphosphino)butane (0.10 eq), ally ethyl carbonate (10 eq), THF, 90 °C, sealed tube, 25 h, 44%, 71% based on recovery of **44**; (c) *trans*-di(μ-acetato)bis[*O*-(di-*O*-tolylphosphino)benzyl]dipalladium (II) (0.19 eq), TBAF (18 eq), aryl halide (3.0 eq), DMF, 100 °C, 17 h, 24-87%; (d) MeOH-H_2O (10:1), 50 °C, 36 h to 3 days; (e) CHF_2CO_2H (6.0 eq), MeCN-H_2O (1:1), rt, 37 h to 5 days, 53-78% in 2 steps.
R^2 = a: quinolin-3-yl; b: isoquinolin-4-yl; c: phenyl; d pyridin-3-yl; e: pyrimidin-5-yl; f: naphthalen-1-yl; g: naphthalen-2-yl; h: 4-nitrophenyl; i: 4-methoxyphenyl; j: 4-fluorophenyl; k: 4-(trifluoromethyl)phenyl; l: 4-biphenyl-1-yl; m: 4-(imidazol-1-yl)phenyl.

Scheme 9. Synthesis of 3-*O*-(3-Aryl-2-propenyl) Analogues[a] and Reference Compounds.

Initially, we introduced an allyl group to the 3-OH group of the appropriately protected intermediate (**44**) (Scheme 9). Compared to a conventional method, palladium-catalyzed allylation[81] provided **44** in an improved yield. Heck reactions of compound **56** with a variety of arylhalides afforded desired coupling products (**57a-57m**) accompanied with minor products with isomerized double bonds. Isomerization of the double bond in the allyl moiety at the C-3 position could be effectively prevented using palladacycle[82] or palladium dichloride. A couple of deprotection processes yielded structurally novel analogues, **59a-59m** in moderate yields.[83]

Table 9. Antibacterial Activities of 3-*O*-(3-Aryl-2-propenyl) Analogues (MIC, μg/mL).

Test organisms	Characteristics	**59a**	**59m**	LM-A_7	RKM	CAM
Streptococcus pneumoniae	standard	0.03	0.03	0.13	0.06	0.015
S. pneumoniae	susceptible	0.03	0.03	0.25	0.13	0.03
S. pneumoniae	*ermB* methylase (c)	64	16	>128	>128	>128
S. pneumoniae	*ermB* methylase (c)	64	32	>128	>128	>128
S. pneumoniae	*ermB* methylase (i)	32	0.5	>128	4	>128
S. pneumoniae	*ermB* methylase (i)	16	2	>128	4	>128
S. pneumoniae	*mefA* efflux	0.03	0.06	0.13	0.13	0.5
S. pneumoniae	*mefA* efflux	0.015	0.03	0.13	0.06	0.5
Streptococcus pyogenes	standard	0.03	0.06	0.13	0.06	0.015
S. pyogenes	*ermB* methylase (c)	64	>128	>128	>128	>128
S. pyogenes	*mefA* efflux	0.05	0.13	0.25	0.06	4

(c): constitutive; (i): inducible

Majority of the derivatives among **59a-59m** showed stronger antibacterial activities than LM-A_7, except **59k** (4-(trifluoromethyl)phenyl) and **59l** (biphenyl) (data not shown). Among compounds **59a-59j** and **59m**, **59a** (quinolin-3-yl) and **59m** (4-(imidazol-1-yl)phenyl) showed the strongest antibacterial activities against *S. pneunomiae* and *S. pyogenes*. Although these compounds had weak antibacterial activities against constitutive resistant *S. pneumoniae* (MIC, 16 to 64 μg/mL) (Table 9), they were the first examples in our research group, which could respond to constitutive resistant *S. pneumoniae* or *S. pyogenes*. Because compound **59a** showed a well-balanced antibacterial spectrum, we subsequently synthesized a 3-*O*-(3-quinolin-3-yl-2-propenyl) analogue of RKM, **60** (Figure 20) using a procedure similar to that used to synthesize **59**.

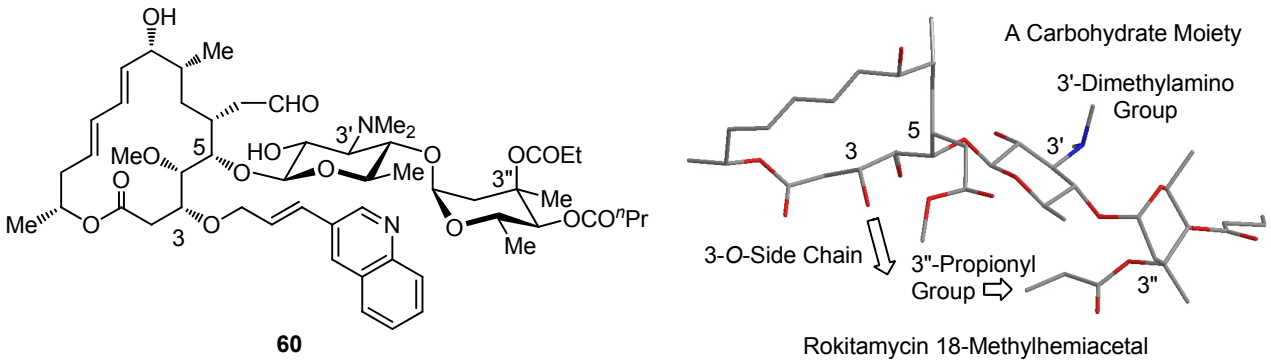


Figure 20. 3-*O*-(3-Quinolin-3-yl-2-propenyl) Analogue of RKM and X-Ray Data of RKM Methylhemiacetal.

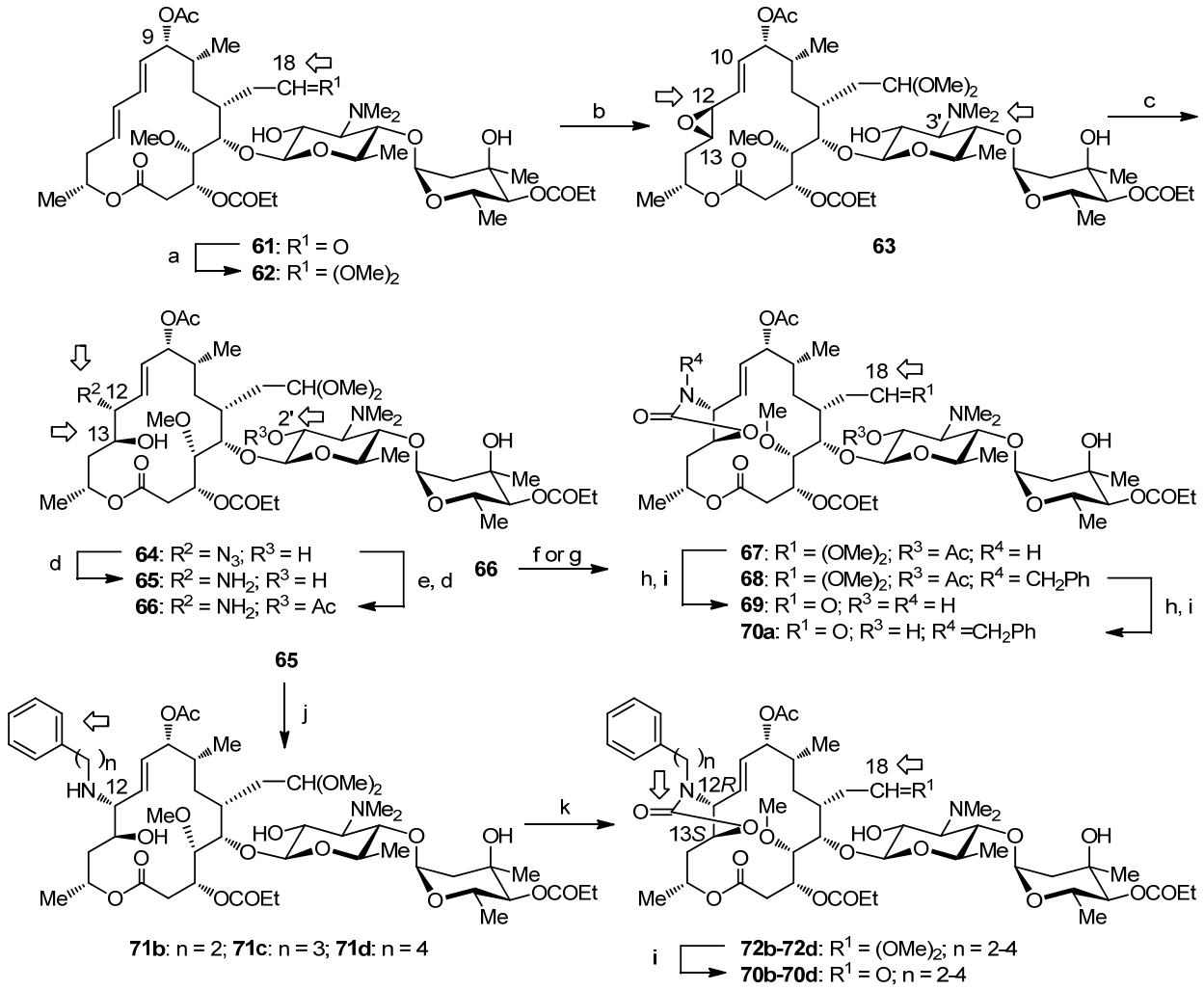
Contrary to our hypothesis, the antibacterial activities of **60** were lower than those of RKM. The antibacterial activities of **60** were about 2 to 4 times weaker against *S. pneumoniae* and 16 to 32 times weaker against clinically important Gram-negative pathogens in respiratory infections (data not shown). Thus, introduction of a 3-quinolin-3-yl-2-propenyl group to the 3-hydroxyl group of LM-A_7 increased the antibacterial activities, but introduction of the same group to the corresponding position of RKM showed a marked decrease in its antibacterial activities. These differences could be explained on the basis of findings reported Takenuki[84] in that the propionyl group at the C-3" position of RKM 18-methylhemiacetal was located in the space close to direction of the C-3 position (Figure 20). Conformational analysis of **60** showed that the 3-quinolin-3-yl-2-propenyl group of **60** overlapped with the original position of the propionyl group at the C-3" position, and thus, a carbohydrate moiety changed its three-dimensional position to avoid the quinoline ring. Changing of the three-dimensional structure of **60**, especially the relative positioning between the lactone ring and the disaccharide moiety, was supposed to reduce its biological activities. Because it was judged that chemical modification at the C-3 position would be difficult to discover a novel interaction with ribosomal RNA for enhancement of antibacterial activities against resistant *S. pneumoniae*, we had to reconsider and precisely analyze the important role of an arylalkyl moiety of Ru-004.

2.4. Introduction of an Arylalkyl Moiety onto the Western Hemisphere[85] in 16-Membered Macrolactones

Part 1: Regio- and Stereoselective Epoxidation and Regioselective Ring Opening Using Sodium Azide[86]

TEL and Ru-004 possess an arylalkyl moiety attached to 11, 12-cyclic carbamate in the western hemisphere of 14-membered macrolactone (Figure 19). The arylalkyl group protected[87] the A752 residue in domain II of ribosomal RNA against chemical modification. These observations suggested that the aryl side chain might interact with the second binding site in rRNA. Furthermore, X-ray crystallographic analysis of the ketolide-ribosome complex indicated[76] that the aryl group of TEL or

ABT-773/cethromycin bound to domain II through stacking or hydrogen bonding. Thus, the aryl moiety is significantly important for antibacterial activities against EM-resistant pathogens. Subsequently, we designed novel 16-membered macrolides, which possess an arylalkyl moiety attached to a cyclic carbamate in the western hemisphere.



[a]*Reagents and conditions:* (a) $(MeO)_3CH$ (excess), PPTS (1.0 eq), MeOH, 50 °C, 33 h, 80%; (b) (1) *m*CPBA (2.5 eq), $CHCl_3$, rt, 14 h; (2) aq $Na_2S_2O_4$ (2.9 eq), EtOH, 0 °C, 30 min., 62% in 2 steps; (c) NaN_3 (10 eq), NH_4Cl (5.0 eq), EtOH-H_2O (8:1), 80 °C, 21 h, 73%; (d) Ph_3P (1.5 eq), MeCN, rt, 23 h, then H_2O, r.t., 6 hrs, 68%; (e) Ac_2O (2.0 eq), MeCN, 50 °C, 8 h, 90%; (f) CDI (2.0 eq), THF, rt, 3 h, 88%; (g) (1) PhCHO (1.2 eq), $NaBH(OAc)_3$ (1.5 eq), AcOH (1.1 eq), $Cl(CH_2)_2Cl$, rt, 2 h, 80%; (2) CDI (2.0 eq), THF, reflux., 26 h, 81%; (h) MeOH-H_2O (9:1), 50 °C, 24-27 h, 97%; (i) CHF_2CO_2H (5.9 eq), MeCN-H_2O (1:1), rt-35 °C, 27-41 h. 61-69%; (j) $Ph(CH_2)_{n-1}CHO$ (1.3 eq), AcOH (4.0 eq), MeOH, rt, 1-1.5 h, 33-59%; (k) triphosgene (0.8 eq), Et_3N (5.0 eq), CH_2Cl_2, 0 °C, 45 min.-1.5 h, 42-90%.

Scheme 10. Synthesis of 12, 13-Cyclic Carbamate Analogues of 16-Membered Macrolides[a].

Muroi *et al.*[88] reported stereoselective and non-regioselective epoxidation at the C-10 to C-13 positions on JM/LM-A_3. To improve regioselectivity of epoxidation, we explored several substituents at the C-9 position. Our results showed that regioselectivity of epoxidation at the C-12 and C-13 positions was greatly improved when we used the 9-*O*-acetyl-type intermediate (**62**) with *m*CPBA. This oxidation step

also converted the 3'-dimethylamino group to its amine oxide and thus it was consequently reduced back to the original dimethyl amino group by treatment with sodium hydrosulfite. A structure of the epoxide (**63**) was confirmed by X-ray crystallographic analysis.[89]

Ring-opening reaction of the epoxide with sodium azide proceeded at the allylic position to afford an azide (**64**), which was converted to an amine (**65**) by triphenylphosphine. 2'-Acetate of **65** (**66**) was reacted with benzaldehyde in the presence of sodium triacetoxyborohydride followed by treatment of 1,1'-carbonyldiimidazole to afford *N*-benzyl-12,13-*N,O*-cyclic carbamate (**68**) (Scheme 10). On the other hand, alternative arylalkyl derivatives, **72b-72d**, were prepared from compound **65** *via* 12-*N*-arylalkyl-13-hydroxyl intermediates (**71b-71d**). The yields of **71** were not satisfactory because of formation of undesired 12-*N*,*N*-dialkyl derivatives. Deprotections of these intermediates gave the original molecules, **70a-70d**,[90] accompanied with a reference analogue (**69**). Absolute configurations at the C-12 and C-13 positions were determined by NOE experiments.[90]

Although the antibacterial activities of **70a-70d** against *S. pneumoniae* (susceptible and inducible resistant) were better than those of MDM (data not shown), their activities were generally comparable to those of MDM. We supposed that the construction of a cyclic carbamate in the western hemisphere in 16-membered macrolide was not always an appropriate approach for increasing the antibacterial activities against EM-resistant pathogens. Therefore, we decided to investigate the fundamental SAR of derivatives without a cyclic carbamate.

2.5. Introduction of an Arylalkyl Moiety onto the Western Hemisphere[85] in 16-Membered Macrolactones

Part 2: Optimization of an Arylalkylamino Moiety at the C-12 Position[86]

On the basis of the synthetic route described above (Scheme 10), we synthesized several analogues possessing an arylalkyl group at the western hemisphere without a cyclic carbamate. We synthesized a dimethylamino analogue (**73**) and seven phenyl alkylene analogues (**74a-74g**)[90] (Figure 21). Compound **74g** showed weaker antibacterial activities than **74a-74f** against *S. pneumoniae* (susceptible and inducible resistant). *In vitro* antibacterial activities against *S. pneumoniae* (Table 10) indicated that the length of an alkyl spacer was optimized as n = 3, *i.e.*, propylene. Because introduction of a substituent to the benzene ring of compound **74c** did not enhance their antibacterial activities, we therefore optimized an aryl moiety by synthesis and evaluation of aromatic analogues (**75** to **79**) (Figure 21). Chemical structures of aromatic rings in **77**, **78**, and **79** were referred to those of ABT-773/cethromycin, Ru-004, and TEL, respectively.

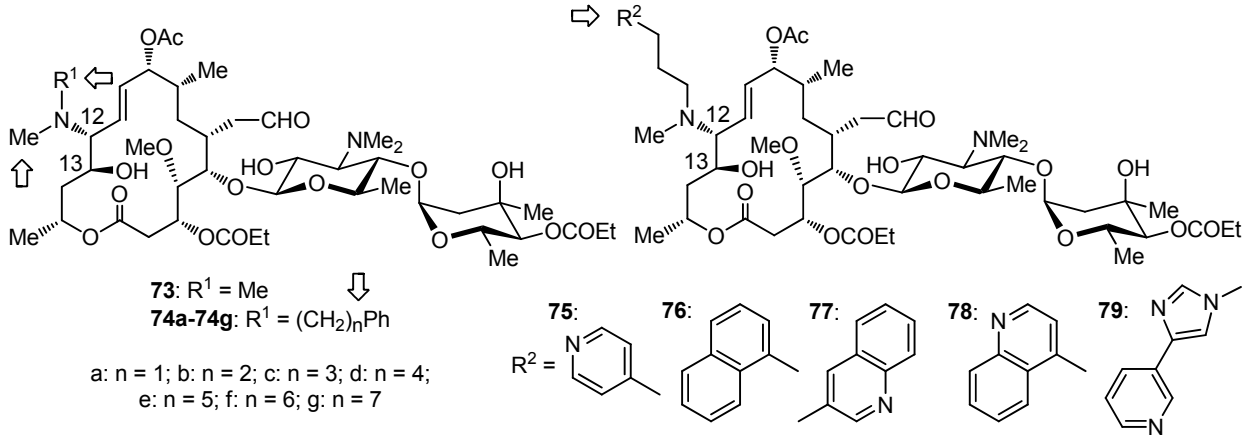


Figure 21. Optimization of a Spacer Length and an Aryl Moiety at the C-12 Position.

Table 10. Antibacterial Activities of 12-*N*-Methyl-12-*N*-Phenylalkyl Analogues (MIC, μg/mL).

Test organisms	Characteristics	**74a**	**74b**	**74c**	**74d**	**74e**	**74f**	MDM
Streptococcus pneumoniae	standard	0.1	0.1	0.05	0.05	0.1	0.1	0.39
S. pneumoniae	susceptible	0.2	0.2	0.1	0.1	0.2	0.2	0.78
S. pneumoniae	*ermB* methylase (c)	>100	>100	>100	>100	100	50	>100
S. pneumoniae	*ermB* methylase (i)	>100	50	12.5	25	12.5	6.25	>100
S. pneumoniae	*ermB* methylase (i)	>100	25	12.5	12.5	12.5	12.5	100
S. pneumoniae	*mefA* efflux	0.2	0.2	0.1	0.2	0.2	0.2	0.2
S. pneumoniae	*mefA* efflux	0.1	0.1	0.1	0.2	0.2	0.2	0.2
Streptococcus pyogenes	*ermB* methylase (c)	>100	>100	100	100	25	25	>100
S. pyogenes	*mefA* efflux	0.39	0.2	0.1	0.2	0.2	0.2	0.78

(c): constitutive; (i): inducible

Table 11. Antibacterial Activities of 12-*N*-Arylpropyl-12-*N*-methyl Analogues (MIC, μg/mL).

Test organisms	Characteristics	**75**	**76**	**77**	**78**	**79**
Streptococcus pneumoniae	standard	0.05	0.1	0.05	0.013	0.06
S. pneumoniae	susceptible	0.1	0.1	0.1	0.025	0.1
S. pneumoniae	*ermB* methylase (c)	>100	50	>100	50	>100
S. pneumoniae	*ermB* methylase (i)	>100	6.25	12.5	3.13	>100
S. pneumoniae	*ermB* methylase (i)	>100	25	50	0.78	100
S. pneumoniae	*mefA* efflux	0.1	0.1	0.1	0.025	0.1
S. pneumoniae	*mefA* efflux	0.1	0.1	0.1	0.05	0.1
Streptococcus pyogenes	standard	*	0.1	0.1	0.05	0.1
S. pyogenes	*ermB* methylase (c)	>100	50	>100	>100	>100
S. pyogenes	*mefA* efflux	0.1	0.2	0.2	0.1	0.2

(c): constitutive; (i): inducible; *not tested

Transformation of a benzene ring to a pyridine ring (**74c** to **75**) did not improve antibacterial activities, but introduction of a nitrogen atom to a naphthalene ring (**76** to **78**) enhanced antibacterial activities against important Gram-positive pathogens in respiratory infections (Table 11). In conclusion, a novel 16-membered macrolide possessing a [3-(quinolin-4-yl)propyl]amino moiety at the C-12 and a hydroxyl group at the C-13 position showed moderate antibacterial activities against inducible resistant *S. pneumoniae* and responded to constitutive resistant *S. pneumoniae*. To consider whether we should continue to investigate the western hemisphere of 16-membered lactone, we discussed about possibilities that an arylalkyl moiety of the western hemisphere interacted with the domain II in the rRNA.

Then, we synthesized two tailor-made analogues of **74c**, compounds **80**[91] and **81** (Figure 22). Compound **80** had a phenylpropenyl group at the C-3 position and compound **81** had an acetyl group at the C-3" position. Compounds **80** and **81** showed 4 to 8 times stronger antibacterial activities than **74c** against inducible resistant *S. pneumoniae*. Moreover, while compound **80** showed weak activities against constitutive resistant *S. pneumoniae* and constitutive resistant *S. pyogenes*, RKM, CAM, or compound **81** had no effect on these pathogens.

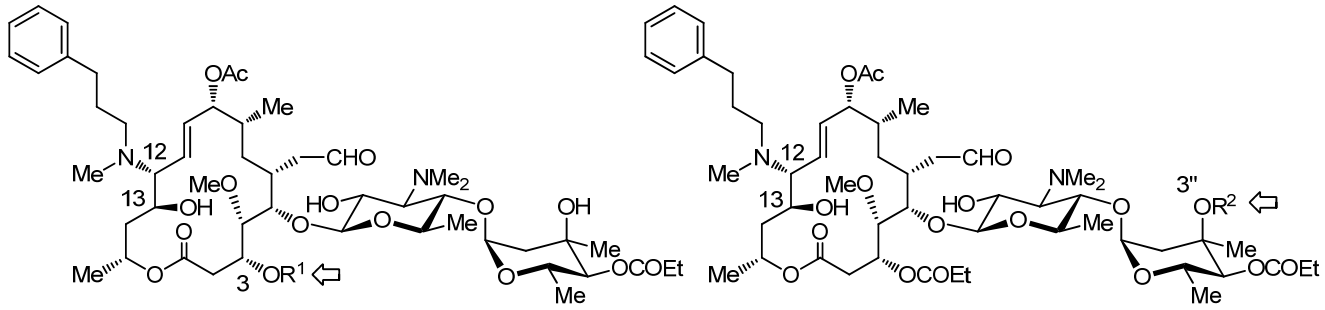


Figure 22. Modifications at the C-3 or C-3" Position of Compound **74c**.

Our results indicated that the antibacterial activities of our novel 16-membered macrolides modified at the western hemisphere against resistant Gram-positive bacteria could be enhanced with further modifications at the C-3 or at the C-3" positions. Therefore, we performed novel modifications at the western hemisphere of 16-membered macrolides.

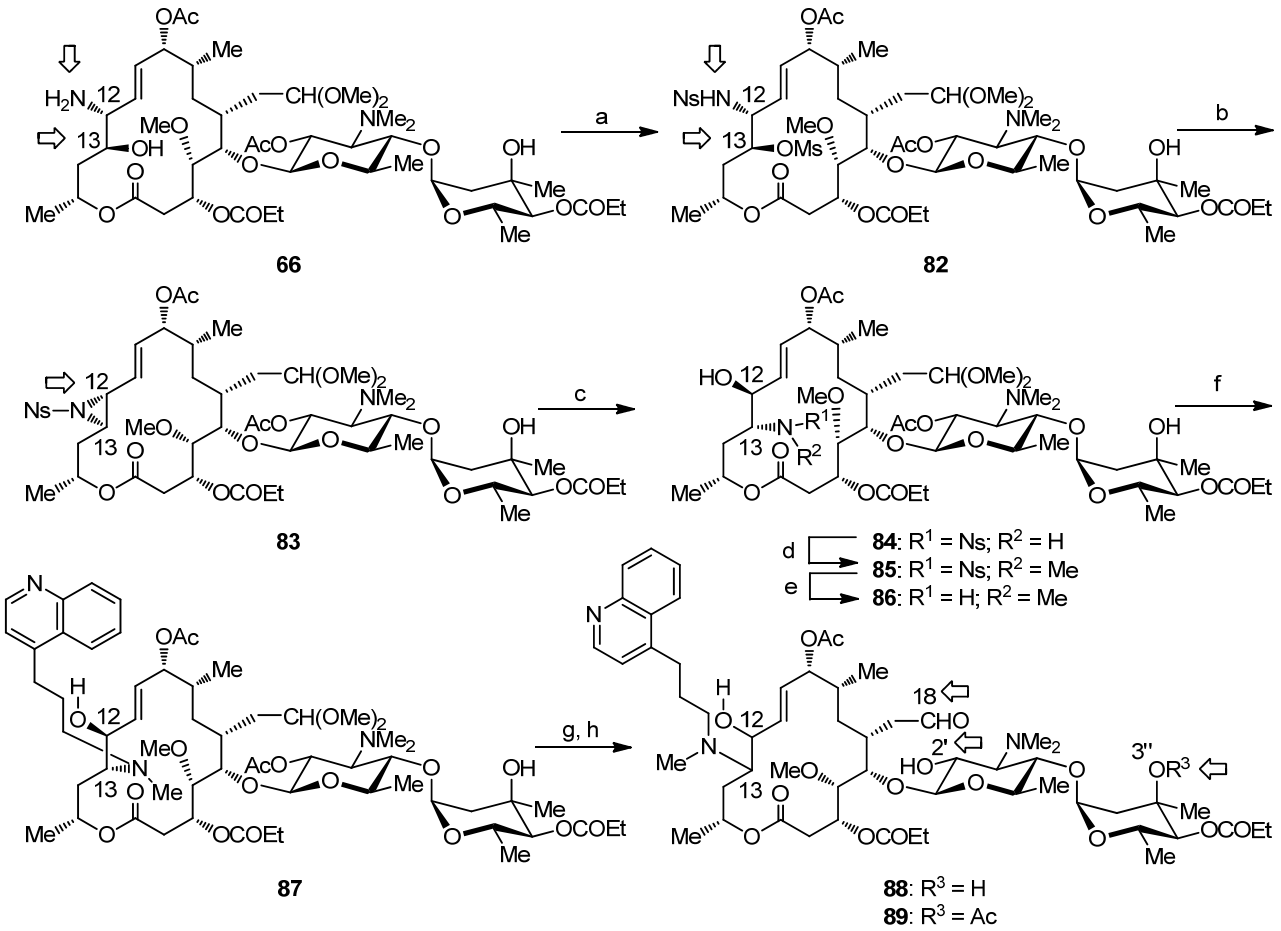
2.6. Introduction of an Arylalkyl Moiety onto the Western Hemisphere in 16-Membered Macrolactones

Part 3: An Alternative Approach, Synthesis of 13-Amino-12-Hydroxyl Analogues[86]

The results of our SAR studies indicated that (i) the structure of an aryl moiety and (ii) the length of an alkyl spacer were very important for strong antibacterial activities. Promising derivatives synthesized to

date (compounds **59a**, **59m**, **74f**, **76**, **78**, and **80**) could only respond to constitutive resistant *S. pneumoniae*, and their antibacterial activities were weaker than those of ketolides. Therefore, we decided to increase the antibacterial activities against constitutive resistant *S. pneumoniae* using 16-membered macrolides. We introduced an arylalkyl group only to the hydroxyl group at the C-3 position and/or the nitrogen atom at the C-12 positions in our derivatives. On the other hand, (i) introduction of an arylalkyl group to the 9-hydroxyl group and (ii) application of a 10,11-epoxide were not found to be useful as per the results of our in-house SAR studies. Therefore, we explored the synthesis and biological evaluation of 13-arylalkylamino-12-hydroxyl analogues[92] as a new class of macrolides.

12-Amino-13-hydroxyl intermediate (**66**) was consequently treated with 2-nitrobenzenesulfonyl chloride and then methanesufonyl chloride to afford a 12-nosylamino-13-mesylate (**82**), which was converted to an *N*-nosylaziridine (**83**) under basic conditions (Scheme 11). A trifluoroacetate anion regioselectively



[a]*Reagents and conditions:* (a) 2-NsCl (1.6 eq), TEA (10 eq), CH_2Cl_2, rt, 4 h, then, MsCl (1.7 eq), rt, 1 h, 99%; (b) K_2CO_3 (20 eq), acetone, 50 °C, 7 h, 87%; (c) CF_3CO_2K (15 eq), DMF, 50 °C, 32 h, 83%; (d) MeI (5.0 eq), K_2CO_3 (10 eq), MeCN, 50 °C, 5 h, 83%; (e) PhSH (5.0 eq), DMSO (6.2 eq), K_2CO_3 (4.0 eq), MeCN, rt, 2 h, 45%; (f) 3-(quinolin-4-yl)propanal (1.2 eq), $NaBH_3CN$ (3.0 eq), AcOH (10 eq), EtOH, 0 °C, 2 h, 48%; (g) (1) Dess-Martin Periodinane (2.0 eq), pyridine (15 eq), CH_2Cl_2, rt, 1 h; (2) $NaBH_4$ (2.0 eq), $CeCl_3$•$7H_2O$ (2.0 eq), MeOH, rt, 30 min., 8% in 2 steps; (h) (1) MeOH-H_2O (9:1), 50 °C, 14 h; (2) CHF_2CO_2H (22 eq), MeCN-H_2O (1:1), rt, 28 h, 41% in 2 steps.

Scheme 11. Synthesis of 13-Arylalkylamino-12-hydroxyl Analogue of 16-Membered Macrolide[a].

attacked at the C-12 position from the β-face and work up treatment gave a 12-hydroxyl-13-nosylamino analogue (**84**). Methylation of the 13-amino group and deprotection of the nosyl group followed by reductive alkylation in application of 3-(quinolin-4-yl)propanal and sodium cyanoborohydride constructed a total framework of a desired 13-arylalkylamino-12-hydroxyl intermediate (**87**). After isolation of **87**, the 12-hydroxyl group was oxidized by Dess-Martin periodinane to a ketone, and then was reduced to a hydroxyl group by sodium borohydride. In this 2-step process (reaction condition [g] in Scheme 11), there were some possibilities that the C-13 position was isomerized. The yield of oxidation was very low, and the stereochemistry at the C-12 and C-13 positions could not be determined even after deprotections. The final product (**88**), however, could be isolated with high purity. On the other hand, 3”-*O*-acetyl analogue of **88**, compound **89**, was independently synthesized from MOM using a procedure similar to that used for **88**.

Compounds **88** and **89** showed strong antibacterial activities against *S. pneumoniae* and *S. pyogenes* and their activities against constitutive resistant *S. pneumoniae* were markedly stronger than those of relatively potent 16-membered macrolides synthesized thus far (compounds **59a**, **59m**, **74f**, **76**, **78**, and **80**). Although we analyzed the possibility of performing further studies on 13-arylalkylamino analogues in the future, we did not decide to focus on further optimization of these novel analogues because of the following reasons: (i) many synthetic steps, (ii) unclear stereochemistry at the C-12 and C-13 positions, and (iii) very low yield of the oxidation step (reaction condition (g) (1) in Scheme 11). Therefore, we had to explore a novel research strategy.

Table 12. Antibacterial Activities of 13-Arylalkylamino-12-Hydroxyl Analogues (MIC, μg/mL).

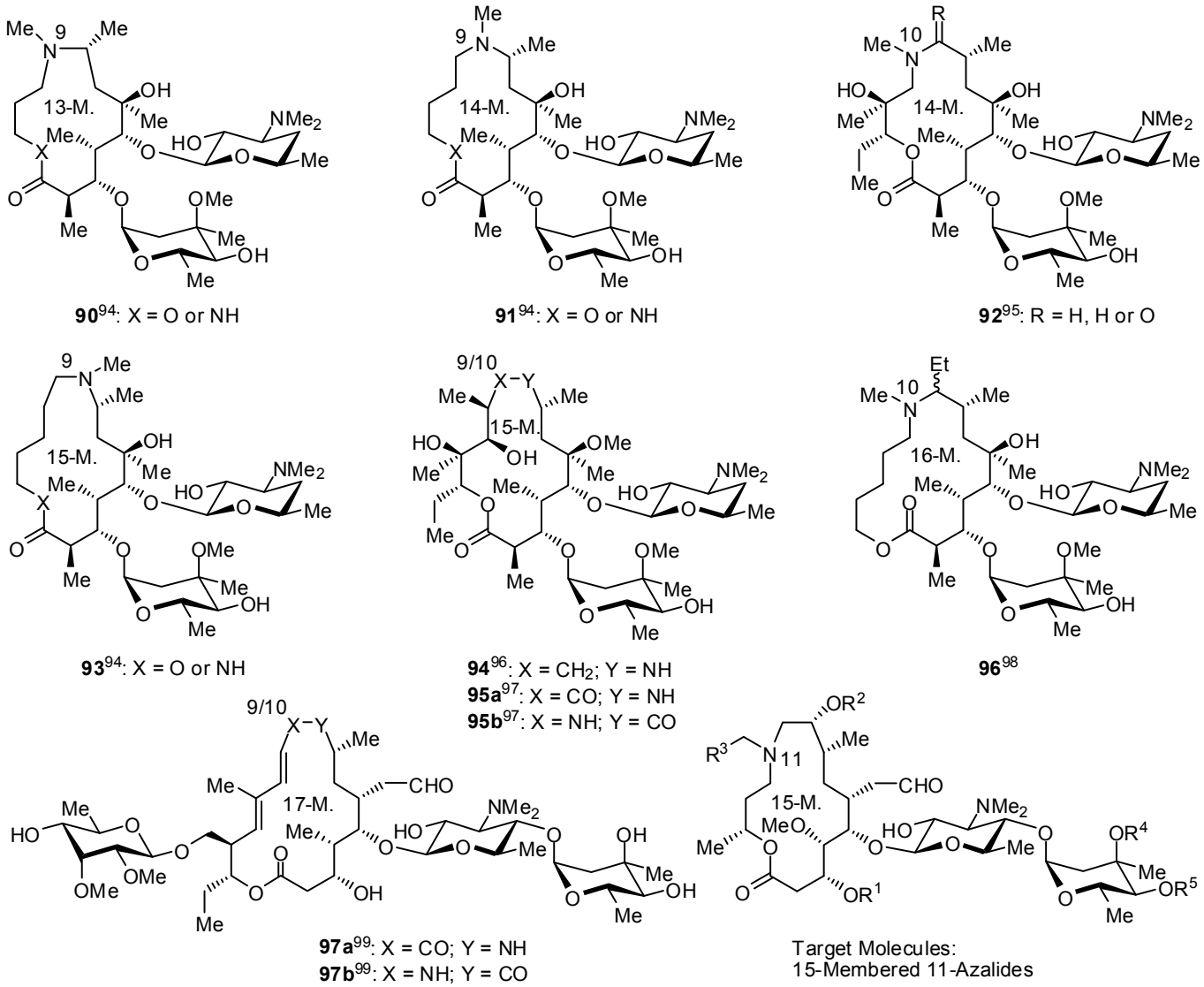
Test organisms	Characteristics	**88**	**89**	RKM	CAM
Streptococcus pneumoniae	standard	0.015	0.06	0.06	0.015
S. pneumoniae	susceptible	0.03	0.06	0.06	0.15
S. pneumoniae	*ermB* methylase (c)	16	8	>128	>128
S. pneumoniae	*ermB* methylase (c)	16	8	>128	>128
S. pneumoniae	*ermB* methylase (i)	2	0.5	1	>128
S. pneumoniae	*ermB* methylase (i)	2	1	1	>128
S. pneumoniae	*mefA* efflux	0.03	0.13	0.13	0.5
S. pneumoniae	*mefA* efflux	0.03	0.13	0.13	0.5
Streptococcus pyogenes	standard	0.06	0.13	0.03	0.015
S. pyogenes	*ermB* methylase (c)	64	8	>128	>128
S. pyogenes	*mefA* efflux	0.13	0.25	0.06	4

(c): constitutive; (i): inducible

3. From Natural Products to Innovative Transformation of Macrolide Framework: Synthesis of Novel Azalides[86]

3.1. Reported Azalides[93] and Selection of an Alternative Macrocyclization Reaction

In the previous chapter (chapter 2), we confirmed that introduction of an arylalkyl group to the western hemisphere of the 16-membered lactone was very important for its antibacterial activities against EM-resistant *Streptococcus* species. Because we could establish a synthetic method to introduce a substituent to an α-amino group at the C-12 position, we subsequently planned to synthesize 15-membered 11-azalides, which possess an arylalkyl group at the N-11 position (Figure 23, right bottom). Since the method of synthesis of AZM reported by Pliva in 1981 (foreign application priority data of the original substrate patent of AZM is March 6, 1981 in Yugoslavia), many novel azalides have been synthesized[94-99] (Figure 23). These examples included 13-membered to 17-membered azalides. Because the position of the nitrogen atom in all derivatives was "9" (next to the C-8 position) or "10" (next to the C-9 position), our target molecules, 11-azalide, were not known with a lactone ring of any size. On the other hand, several examples of synthesis including chemical reconstruction of a lactone ring,



90[94]: X = O or NH

91[94]: X = O or NH

92[95]: R = H, H or O

93[94]: X = O or NH

94[96]: X = CH_2; Y = NH

95a[97]: X = CO; Y = NH

95b[97]: X = NH; Y = CO

96[98]

97a[99]: X = CO; Y = NH

97b[99]: X = NH; Y = CO

Figure 23. Structures of Azalides Reported as Novel Macrolide Framework and Target Molecules.

were reported in compounds **90**, **91**, **93**, and **96**, but the introduction of an arylalkyl group to the lactone ring has not been reported thus far. Moreover, any azalides, which were effective against constitutive resistant *S. pneumoniae* were previously unknown.

After we started research on azalides, Taisho Pharmaceutical Co., Ltd. reported synthesis of 15-membered 12-azalides.[100] Sugimoto *et al.*[101] introduced a variety of substituents into an azalactone (C-9, C-13, or C-14 positions) and a neutral sugar moiety, and generated compounds **98-100** (Figure 24), which were effective against EM-resistant *S. pneumoniae*. The derivatives are also known as 11a-azalides. Although novel azalides have been recently reported,[102] the novel structure of an azalide, except that of the above 15-membered 12-azalide, has not been reported thus far.

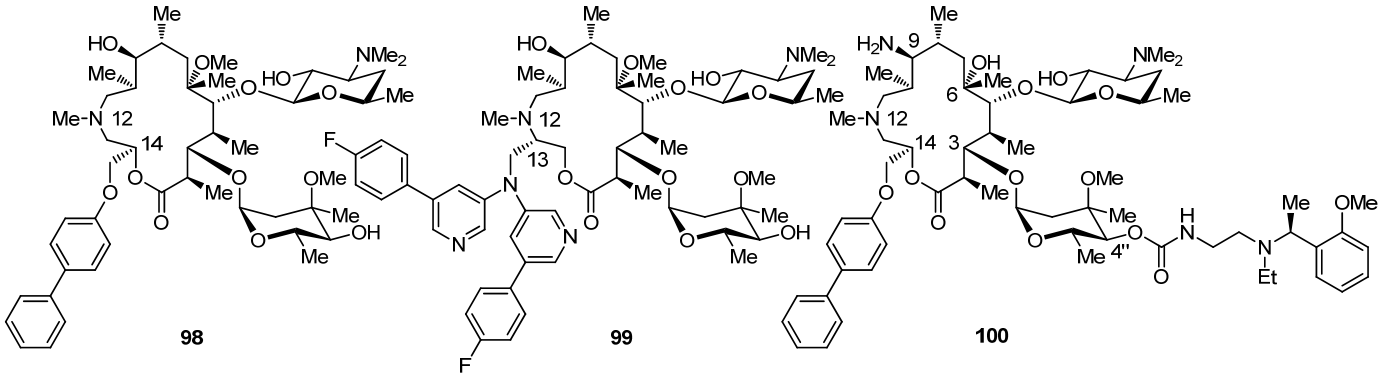
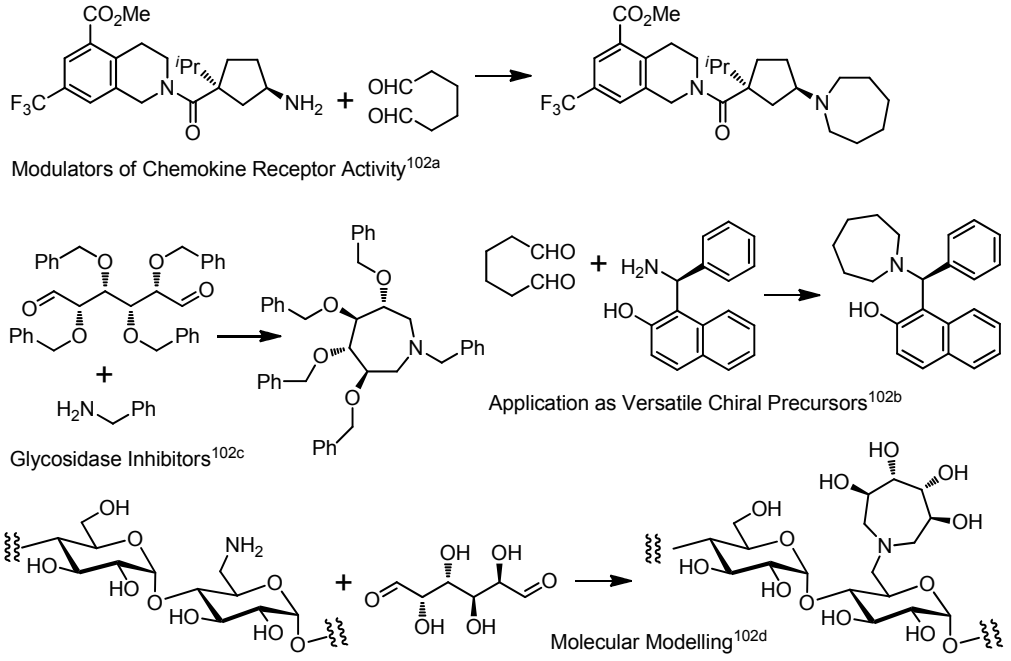


Figure 24. Structures of 15-Membered 12-Azalides Reported by Taisho Pharmaceutical.

To synthesize our target molecules, 15-membered 11-azalides (Figure 23), macrolactonization was supposed to be a general approach as the key cyclization reaction. However, in 1990s, total synthesis[103] of important natural products, including rapamycin (double Stille coupling) and epothilone A (ring closing metathesis) was achieved without macrolactonization, because this process did not always provide a satisfactory yield. Moreover, macrolactonization was believed to be too complicated or result in insufficient yield of antiinfective agents in pharmaceutical industries. Therefore, we tried to apply reductive alkylation as a macrocyclization reaction. This means one-step macrocyclization using "a linear dialdehyde and an amine." Synthesis of at least 4 compounds[104] with a 7-membered ring was performed using the one-step cyclization using a linear dialdehyde and an amine in 2009 (Scheme 12); however, to our knowledge, no compounds with an 8-membered ring or more were synthesized using this method. In 1982, Ōmura *et al.*[105] reported that the basic framework of the 16-membered macrolide, JM/LM-A_3, except an aldehyde group at the C-18 position, was quite stable under hydride reduction conditions for reductive alkylation/reductive amination.



Scheme 12. One-Step Cyclization in Application of a Linear Dialdehyde and an Amine.

Typically, 8- and 9-membered lactones[106] are very difficult to be synthesized using lactonization (Figure 25). In the case of cyclic ether formation, a specific ring size is not observed (Figure 26). On the other hand, 8- to 11-membered cyclanes[107] are highly strained (Figure 27). However, no information is available about relationships between the ring size and the difficulties associated with one-step macrocyclization using a linear dialdehyde and an amine. In our collaborative conformational analysis[108] about one-step macrocyclization, 14- and 16-membered azacyclanes would be easily formed compared to 8-, 10-, and 12-membered azacyclanes in a model study. The results of these analyses are still preliminary, and more results are required to publish these data elsewhere.

Figure 25. Lactone Formation.

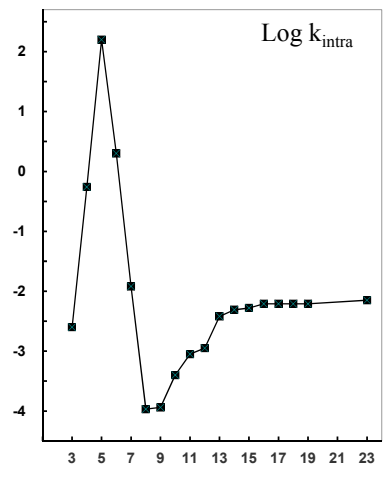


Figure 26. Cyclic Ether Formation.

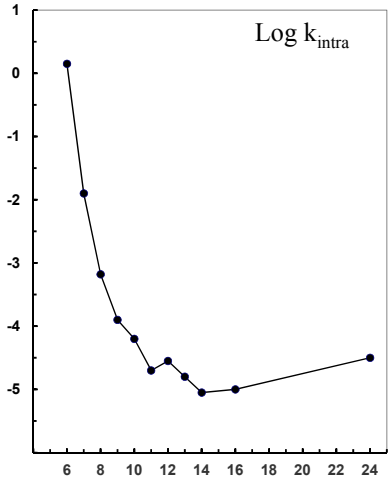
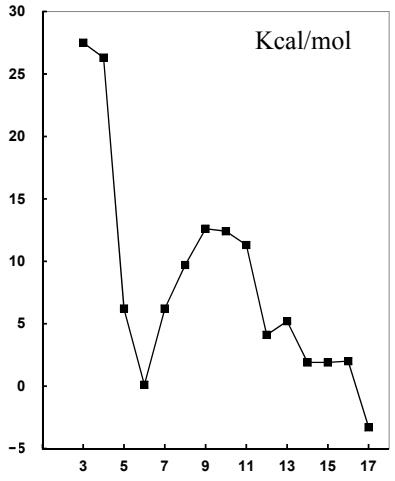
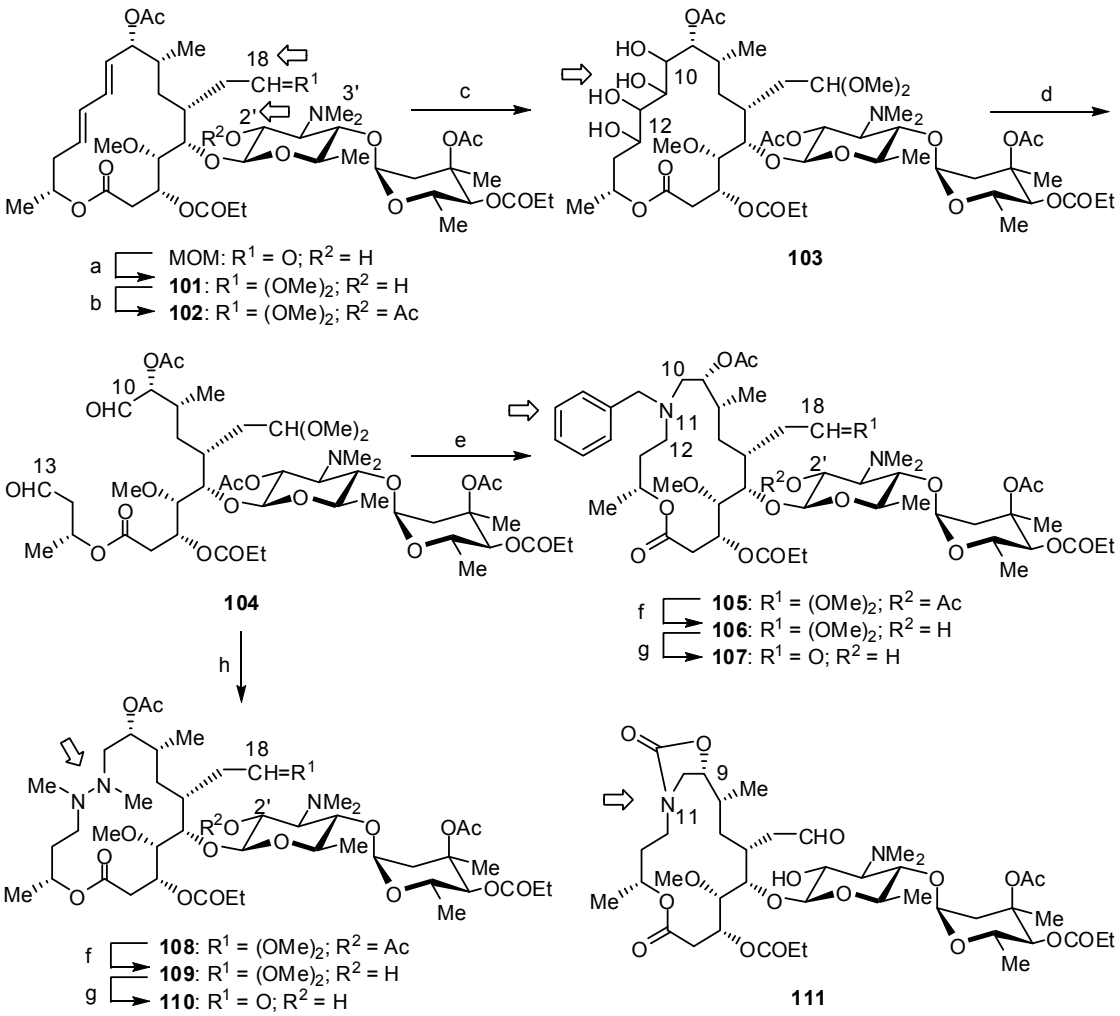


Figure 27. Ring Strain for Cyclanes.



3.2. Isolation of a Linear Dialdehyde and its One-Step Macrocyclization[109]

We selected a 15-membered 11-azalide (**107**) and a 16-membered diazalide (**110**) as prototype compounds and investigated their synthetic route (Scheme 13). Because we planned to use oxidative ring opening reaction using lead tetraacetate for formation of the dialdehyde (**104**), the 2'-hydroxyl group or 3'-dimethylamino group in addition to the aldehyde group at the C-18 position required to be protected. Although we have already established an *N*-oxide protection of the 3'-dimethylamino group, poor reproducibility was often observed in the deprotection process with triphenylphosphine, particularly on a large scale. Thus, we decided to protect the 2'-position and prepared a completely protected intermediate (**102**). Oxidation of **102** by using osmium tetroxide with *N*-methylmorpholine-*N*-oxide as a cooxidant yielded a tetraol (**103**), which was converted to the desired key intermediate (**104**) by ring cleavage using lead tetraacetate. This dialdehyde was very reactive and unstable, but it could be purified by silica gel

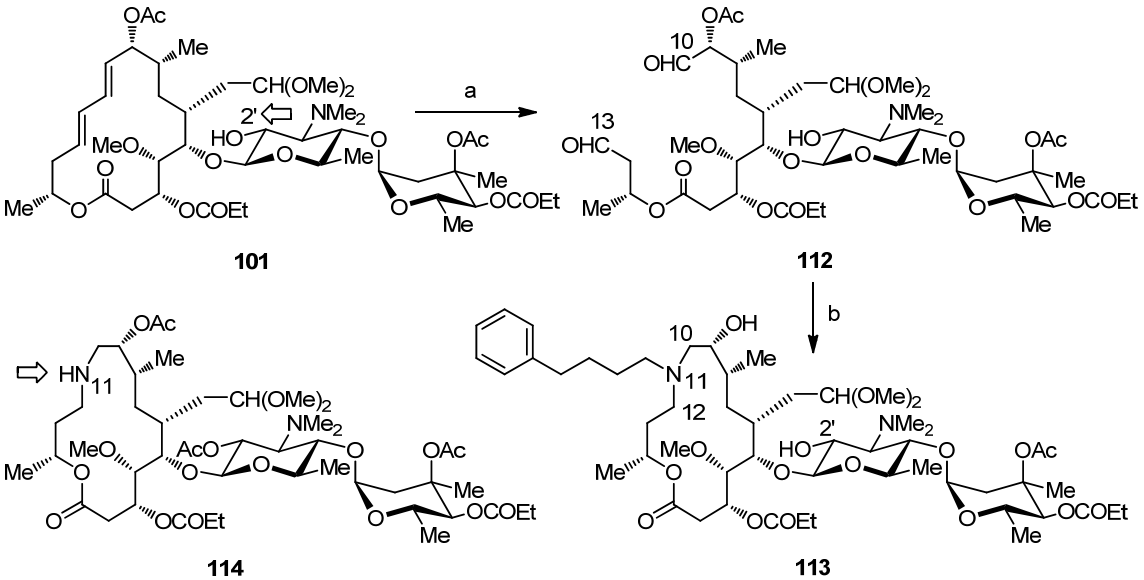


[a]*Reagents and conditions:* (a) $(MeO)_3CH$ (80 eq), PPTS (1.2 eq), MeOH, 40-50 °C, 4 days; (b) Ac_2O (5 eq), MeCN, 40 °C, 16 h; (c) OsO_4 (0.15 eq), NMO (2.0 eq), aq. acetone, rt, 24 h, 30% in 3 steps; (d) $Pb(OAc)_4$ (2.1 eq), PhH, Na_2CO_3 (8.0 eq), rt, 10 min.; (e) $BnNH_2$ (1.1 eq), $NaBH_3CN$ (3.9 eq), AcOH (15 eq), EtOH, 0 °C to rt, 33 h, 10% in 2 steps; (f) MeOH:H_2O (9:1), 55 °C, 24 h, 79-90%; (g) CHF_2CO_2H (20 eq), MeCN-H_2O, rt, 24 h, 84-94%; (h) 1,2-dimethylhydrazine hydrochloride (1.1 eq), others are same as (e), 13% in 2 steps.

Scheme 13. Isolation of a Dialdehyde (**104**) and Its Macrocyclization with an Amine or a Diamine[a].

column chromatography to afford pure **104**, which showed one doublet (δ 9.59, H-10) and one triplet (δ 9.75, H-13 as the numbering before ring opening) in ^{1}H NMR spectroscopy ($CDCl_3$). Owing to its instability, we used **104** without purification after determination of its structure. One-step macrocyclization of **104** with 1.1 eq. of benzylamine and 3.9 eq. of sodium cyanoborohydride in ethanol in the presence of excess acetic acid afforded an 11-azalactone (**105**) possessing a novel framework in a 10% yield. Sequential deprotections yielded the desired 15-membered 11-azalide (**107**) as the first proto-type molecule. In NMR analysis of compound **107**, NOEs were observed between methylene protons in the benzyl group and the protons at the C-10 and C-12 positions, respectively, and interactions between the methylene protons in the benzyl group and the carbons (C-10 and C-12 positions) were observed in the HMBC experiment. The structure of compound **107** was confirmed by these magnetic resonance data. Similarly, one-step cyclization of **104** with 1.1 eq. of 1,2-dimethylhydrazine hydrochloride afforded a 16-membered diazalactone (**108**) and following deprotections yielded the 16-membered diazalide (**110**).

Compared to MOM, compounds **107**[109] and **110** showed slightly reduced antibacterial activities (data not shown), and further modifications were suggested to use these molecules as lead compounds. On the other hand, antibacterial activity of a fused cyclic carbamate analogue (**111**) against inducible resistant *S. pneumoniae* was 16 to 32 times weaker than that of MOM. Thus, we determined whether **107** or **110** should be selected on a priority basis as a lead compound. When we used "unsymmetrical" dialkylhydrazine instead of (symmetrical) dimethylhydrazine, we could not isolate each regioisomer even before or after deprotection(s). Therefore, we decided to select compound **107** as the lead molecule.



[a]*Reagents and conditions:* (a) (1) O_3, anhydrous MeOH, -78 °C, 15 min.; (2) O_2 bubbling, 5-10 min.; (3) Me_2S, -78 °C, 30 min.; (b) $Ph(CH_2)_4NH_2$ (1.1 eq), $NaBH(OAc)_3$ (3.0 eq), AcOH (15 eq), MeOH, -78 °C to 0 °C 1 h, 8.0 % in 2 steps.

Scheme 14. An Alternative Synthetic Route of 15-Membered Azalide by Ozone Oxidation[a].

The first synthetic route of 15-membered azalides had two major problems as follows: (i) very low yields of key oxidation steps and (ii) negative impact on human health (osmium tetroxide) and the environment (lead tetraacetate) because of the oxidative reagents (Scheme 13). Sasai *et al.*[110] reported an alternative synthetic route of 15-membered azalide by using ozone oxidation (Scheme 14). This route of synthesis did not require protection of the 2'-hydroxyl group and markedly improved the total yield. Although ozone oxidation was difficult to control in a large-scale reaction, LONZA[111] has already industrialized the ozone oxidation process to a ton-scale. On the other hand, we prepared the 11-NH derivative (**114**) as the common intermediate in the downstream for efficient medicinal chemistry, *via* hydrogenolysis of an 11-*N*-*p*-methoxybenzyl analogue of **105**.

3.3. Optimization of a 15-Membered 11-Azalide and Expansion of the Scope of Azalide Research

Initially, we optimized the length of an alkyl spacer between a benzene ring and a 15-membered azalactone. When the substituent at the N-11 position was a benzyl group, deacetylation of the 2'-hydroxyl group proceeded in a regioselective manner (Scheme 13). In the case of substituents longer than the benzyl group (a phenethyl group or longer), deacetylation of the 9-hydroxyl group was partially observed along with complete deacetylation at the 2'-position. Therefore, we prepared compounds **115a**-**115c** and **116a**-**116d** (Figure 28).

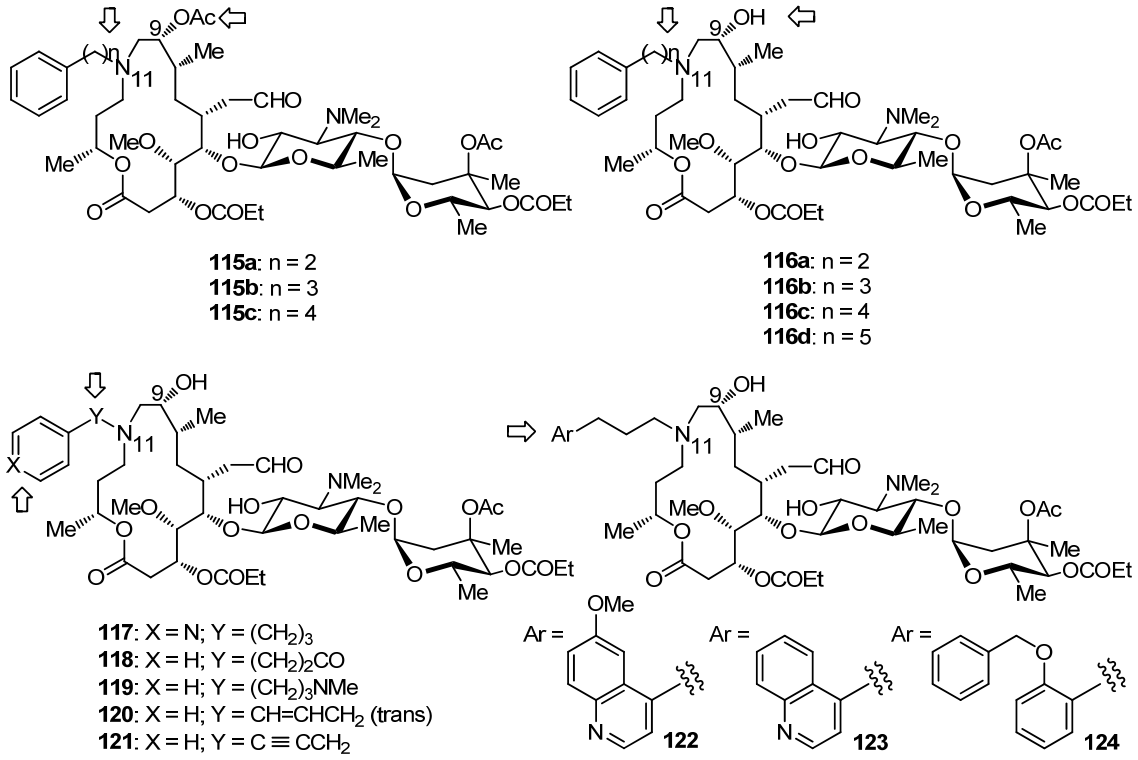


Figure 28. SAR Studies and Optimization of 15-Membered 11-Azalides.

Almost all derivatives (**115** and **116**) except **115c** showed strong antibacterial activities against susceptible and resistant *Streptococcus* species with an *mef* gene (Table 13). Because compounds **116b** and **116c** (a C_3 or C_4 spacer) showed optimum antibacterial activities, including those against inducible resistant *S. pneumoniae*, we optimized an aromatic part with a spacer of a C_3 length (Figure 28). Additionally, we focused on (i) the nature of the bond between a substituent and an azalactone and (ii) the degree of unsaturation of a spacer moiety. Among compounds **117** to **124**, compounds **119** (a hydrazine type) and **121** (a triple bond type) did not possess antibacterial activities against inducible resistant *S. pneumoniae*, and the antibacterial activities against resistant *S. pneumoniae* were greatly affected not only by a structure of an aromatic ring but also by that of a spacer. All 15-membered 11-azalides similar to 16-membered macrolides showed strong activities against resistant *S. pneumoniae* with an *mef* gene. Compounds **117** (pyridin-4-yl), **122** (6-methoxyquinolin-4-yl), and **123** (quinolin-4-yl) showed relatively stronger antibacterial activities against inducible resistant *S. pneumoniae* (Table 14). Our working hypothesis that introduction of an arylalkyl group to the western hemisphere of azalide can generate a novel macrolide effective against resistant *S. pneumoniae* was supposed to be scientifically valid. Therefore, we continued to perform chemical modifications at the western hemisphere, but even the strongest analogue (**122**) did not respond to constitutive resistant *S. pneumoniae* and *S. pyogenes*.

The comprehensive SAR data about 16-membered macrolide derivatives obtained in our laboratory showed that 3-*O*-modified **59a** and **59m** responded to constitutive resistant *S. pneumoniae* (MIC, 16 to 64 μg/mL), and 13-*N*-modified **88** and **89** showed moderate antibacterial activities against constitutive resistant *S. pneumoniae* (MIC, 8 to 16 μg/mL). Because the optimized 15-membered 11-azalide did not respond to constitutive resistant *S. pneumoniae*, we had to develop an advanced or alternative chemical modification at the western hemisphere of the 16-membered azalactone.

Table 13. Antibacterial Activities of 15-Membered Azalides with a Different Spacer (MIC, μg/mL).

Test organisms	Characteristics	**115a**	**115b**	**116a**	**116b**	**116c**	**116d**
Streptococcus pneumoniae	standard	0.25	0.25	0.13	0.06	0.06	0.13
S. pneumoniae	susceptible	0.25	0.25	0.13	0.13	0.13	0.13
S. pneumoniae	*ermB* methylase (c)	>128	>128	>128	128	128	64
S. pneumoniae	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128
S. pneumoniae	*ermB* methylase (i)	>128	64	32	32	8	4
S. pneumoniae	*ermB* methylase (i)	32	8	16	8	8	8
S. pneumoniae	*mefA* efflux	0.25	0.25	0.13	0.13	0.06	0.13
S. pneumoniae	*mefA* efflux	0.25	0.25	0.13	0.13	0.06	0.13
Streptococcus pyogenes	standard	0.13	0.13	0.06	0.06	0.06	0.13
S. pyogenes	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128
S. pyogenes	*mefA* efflux	0.25	0.25	0.13	0.13	0.13	0.25

(c): constitutive; (i): inducible

Table 14. Antibacterial Activities of 15-Membered Azalides with a Different Aromatic Ring (MIC, μg/mL).

Test organisms	Characteristics	**116b**	**117**	**118**	**120**	**122**	**123**	**124**	MOM
Streptococcus pneumoniae	standard	0.06	0.06	0.13	0.13	0.03	0.06	0.25	0.25
S. pneumoniae	susceptible	0.13	0.06	0.25	0.13	0.06	0.06	0.25	0.5
S. pneumoniae	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pneumonia	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pneumonia	*ermB* methylase (i)	8	4	32	16	2	4	8	64
S. pneumonia	*ermB* methylase (i)	8	4	64	16	2	8	8	128
S. pneumoniae	*mefA* efflux	0.13	0.13	0.25	0.25	0.13	0.13	0.5	0.5
S. pneumoniae	*mefA* efflux	0.13	0.13	0.25	0.13	0.06	0.13	0.5	0.5
Streptococcus pyogenes	standard	0.03	0.06	0.06	0.06	0.06	0.06	0.25	0.25
S. pyogenes	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pyogenes	*mefA* efflux	0.25	0.13	0.5	0.25	0.25	0.13	1.0	0.5

(c): constitutive; (i): inducible

Thus, our results indicate that three-dimensional design of an aryl moiety is supposed to be very important to generate a novel macrolide, which exhibits strong antibacterial activities against resistant *Streptococcus* species. Our first approach (sections 3.2. and 3.3.) using a dialdehyde (**104**) (Figure 29), however, enabled us to structurally diversify only a small area in an original lactone moiety. Then, we used 9-formylcarboxylic acid (**125**) as a key intermediate for sequential macrocyclization to provide a variety of novel templates.

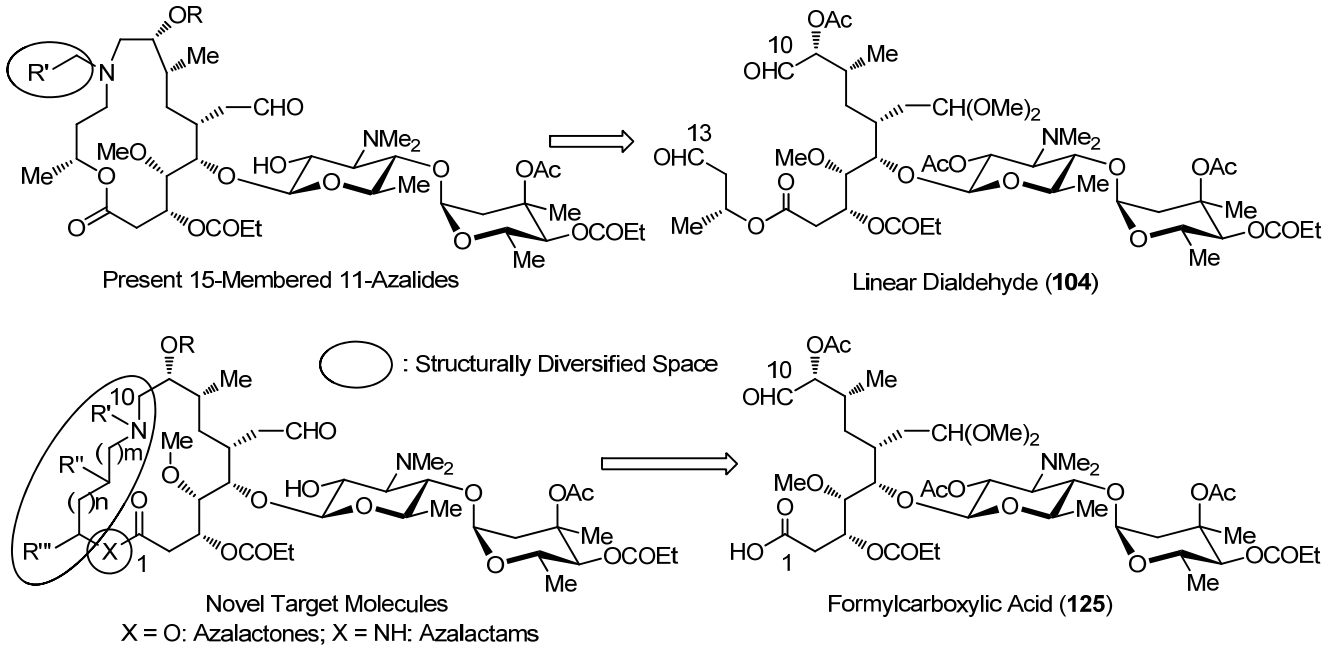
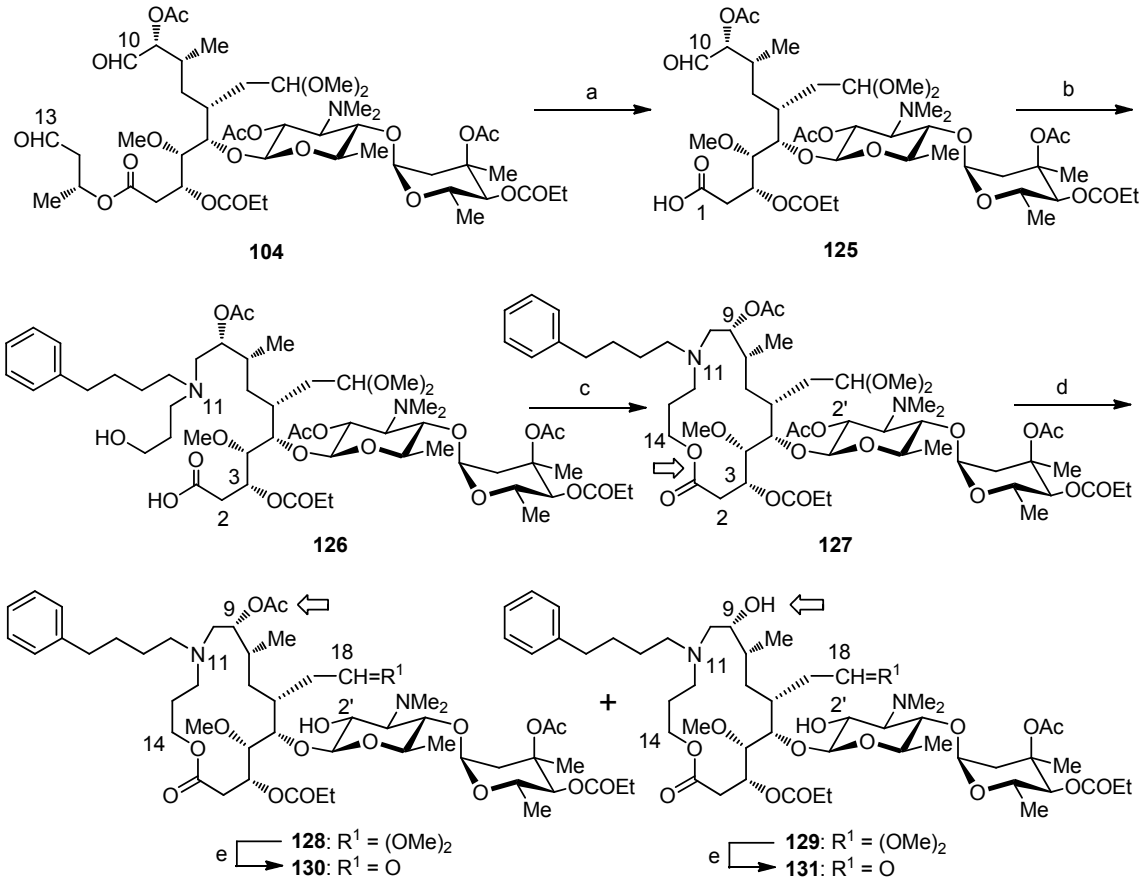


Figure 29. Extended Research Concept in Application of Formylcarboxylic Acid (**125**).

3.4. Preparation of 14- to 16-Membered Novel Azalides and Azalactams[112] and Template Selection



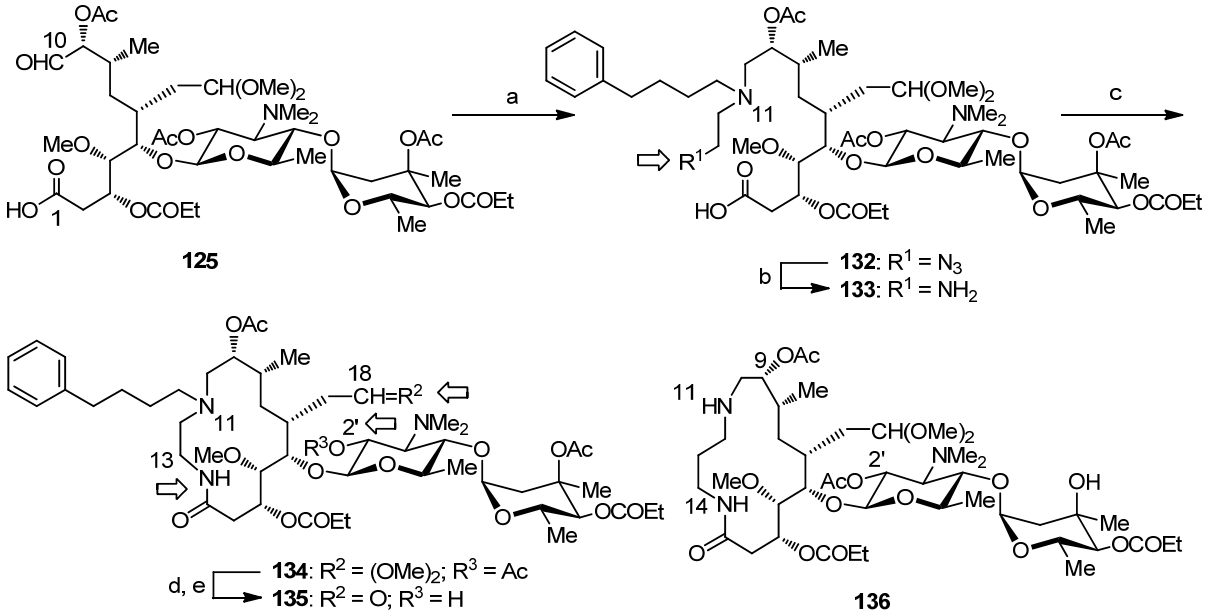
[a]*Reagents and conditions:* (a) DBU (1.5 eq), MeCN, rt, 6 h, 35% (52% in 2 steps based on **103**); (b) (1) 3-(4-phenylbutylamino)propanol (1.5 eq), AcOH (10 eq), molecular sieves 3A, DMF, rt, 4 h; (2) $NaBH_4$ (1.0 eq), rt, 1.5 h, 65%; (c) (1) 2,4,6-Cl_3-C_6H_2COCl (1.2 eq), Et_3N (2.0 eq), THF, rt, 2 h; (2) DMAP (6.0 eq) in PhH, slowly added, rt, 2 h, 43%; (d) MeOH, rt, 76 h, 32% for **128**, 24% for **129**, trace of ring-opened methyl ester; (e) CHF_2CO_2H (30 eq), MeCN-H_2O, rt, 25 h, 78% for **130**, 73% for **131**.

Scheme 15. Synthesis of 15-Membered 11-Azalides (**130** and **131**)[a].

A reactive linear dialdehyde (**104**) was reacted with 1.5 eq. of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) at room temperature and yielded a key intermediate formylcarboxylic acid (**125**) (Scheme 15). Although the yield of this β-elimination was 35%, the overall two-step yield starting from the tetraol (**103**) without isolation of **104** was 52%. Compound **125** could be prepared from **104** by only standing at room temperature as a chloroform solution in the presence of anhydrous sodium sulfate. An aldehyde of **125** was reacted with an amino alcohol possessing an arylalkyl moiety under reductive amination conditions to afford a seco acid (**126**) in 65% yield. We used the Yamaguchi protocol to convert the seco acid to a desired 15-membered azalactone (**127**) in 43% yield followed by deprotections to yield novel 15-membered azalides (**130** and **131**). The yield was not optimized in the macrolactonization reaction because a 2,3-double bond-type analogue was formed as a byproduct. Slow addition of a DMAP solution could suppress the formation of a dimer during macrolactonization (Scheme 15). Addition of the solution at a normal speed resulted in 29% of **127** and 22% of the dimer.[112] The acetyl group at the C-9 position

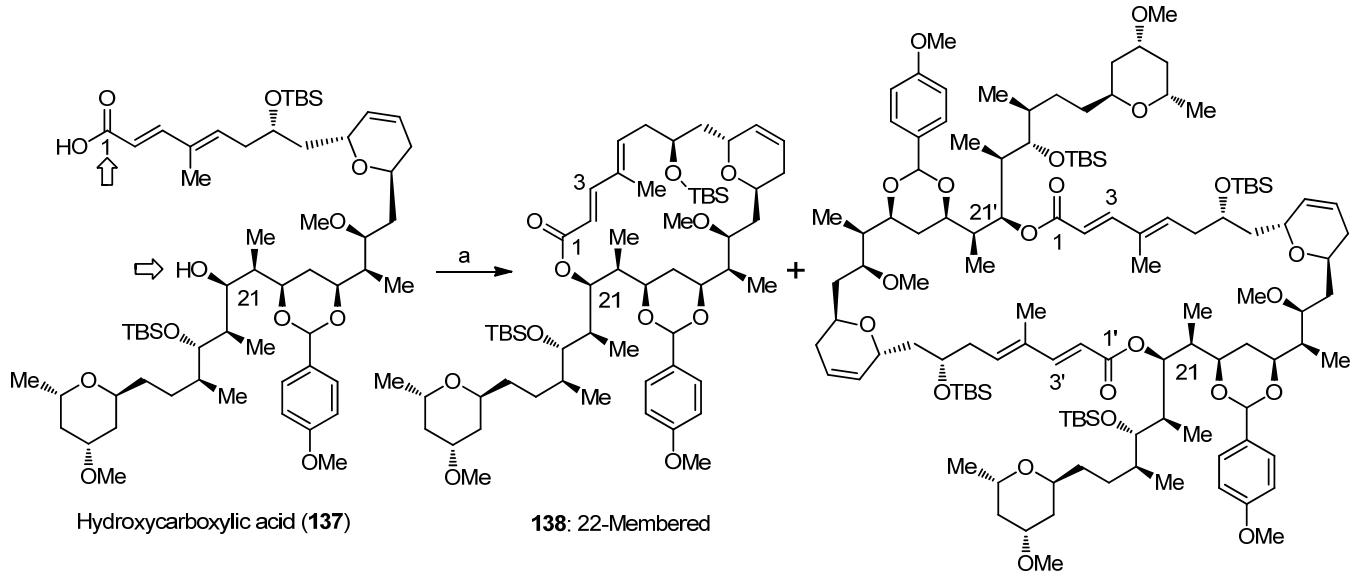
was partially removed according to a neighboring effect by a tertiary amine at the N-11 position. Meanwhile, a deprotection step of the acetyl group at the C-2' position gave two series of analogues, a 9-OAc type and a 9-OH type, and these phenomena were ideal for our comprehensive SAR studies. Longer reaction time for deacetylation using methanol resulted in methanolysis of the ester bond in the lactone. Synthesis of a 15-membered azalide by using this method was superior to the one-step macrocyclization using the linear dialdehyde (**104**) because of (i) the possibility of preparation of azalides with different structures and (ii) an improved total yield.

The 14-membered azalactam (**135**) could be prepared using a procedure similar to that used to prepare **130** and **131** starting from **125** (Scheme 16). When we introduced a diamine moiety, an aminoazide ($Ar(CH_2)_4NH(CH_2)_2\underline{N_3}$) provided a better yield under reductive amination conditions than a mono-*N*-protected diamine ($Ar(CH_2)_4NH(CH_2)_2\underline{NHR}$). In addition, we prepared a common 11-NH-type intermediate (**136**) for efficient medicinal chemistry. Because compounds **130** and **131** showed moderate antibacterial activities against inducible resistant *S. pneumoniae* (Table 15), we planned to perform comprehensive medicinal chemistry in application of the macrolactonization methodology (Scheme 15). We disclosed our plan for the first time at an in-house research conference, and some time was required before implementation of our research plan. As mentioned in section 3.1., macrolactonization did not always provide a satisfactory yield, and it was believed to be too complicated or result in insufficient yield for medicinal chemistry of antiinfective agents in pharmaceutical industries at that time.



[a]*Reagents and conditions:* (a) (1) *N*-(2-azidoethyl)-4-phenylbutylamine (1.5 eq), $NaBH_3CN$ (0.45 eq), AcOH (8.4 eq), EtOH, rt, 21 h, 52%; (b) PPh_3 (5.0 eq), 10% aq THF, 60 °C, 36 h, 64%; (c) $(PhO)_2P(=O)N_3$ (3.4 eq), $NaHCO_3$ (18 eq), DMF, rt, 19 h, 46%; (d) MeOH, rt, 5 h, 70%; (e) CHF_2CO_2H (30 eq), MeCN-H_2O, rt, 25 h, 85%.

Scheme 16. Synthesis of 14-Membered 11-Azalactam (**135**)[a].

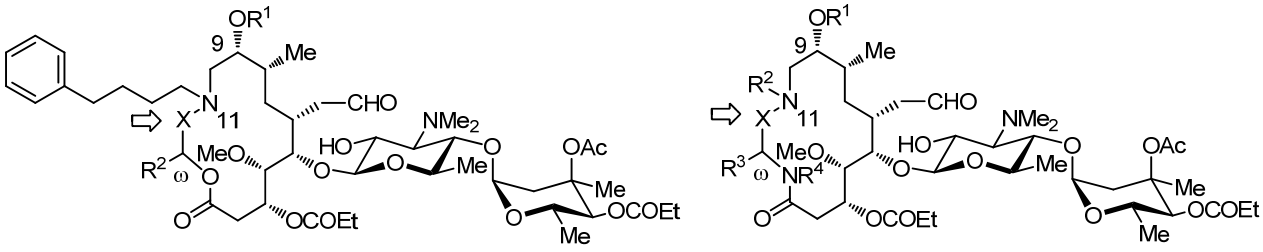


[a]*Reagents and conditions:* (a) 2,4,6-Cl_3-C_6H_2COCl (15 eq), Et_3N (18 eq), PhMe (0.005 M), 25 °C, 2 h, then add DMAP (1.7 eq), PhMe (0.001 M), 110 °C, 24 h, 69% for **138**, 8% for **139**.

Scheme 17. Acceptable Isolated Yield in Macrolactonization of **137** by Yamaguchi Protocol[a].

A member of our research group has joined a research project called "total synthesis of swinholide A" in the K. C. Nicolaou[113] group at The Scripps Research Institute. To optimize macrolactonization of a seco acid (**137**) for preparation of a dimer (**139**), they evaluated different approaches. One of these approaches was an "original" Yamaguchi protocol, which could consistently afford a 22-membered lactone (**138**) in 69% yield (Scheme 17). These results provided us with a good opportunity to continue our research using a formylcarboxylic acid (**125**), because (i) 14- to 16-membered lactones were supposed to be as easily cyclized as a 22-membered lactone (Figure 25) and (ii) many improved Yamaguchi protocols had already been reported.

On the basis of the synthetic routes shown in Schemes 15 and 16, we synthesized a variety of 14- to 16-membered azalides and azalactams (Figure 30). The 14-membered azalactone analogue (**140**) did not show strong antibacterial activities. A prototype azalactam (**135**) and an *N*-methylamide analogue (**148**) did not respond to inducible resistant *S. pneumoniae*.



Compd	R^1	R^2	X	Azalactone
140	Ac	α-Me	CH_2	14-membered
141	Ac	α-Me	$(CH_2)_2$	15-membered
142	H	α-Me	$(CH_2)_2$	15-
130	Ac	H	$(CH_2)_2$	15-
131	H	H	$(CH_2)_2$	15-
143	Ac	α-Me	$(CH_2)_3$	16-membered
144	H	α-Me	$(CH_2)_3$	16-
145*	H	Me	Y	16-
146*	H	Me	Y	16-

Compd	R^1	R^2	R^3	R^4	X	Azalactam
147	Ac	$(CH_2)_4Ph$	α-Me	H	CH_2	14-membered
135	Ac	$(CH_2)_4Ph$	H	H	CH_2	14-
148	Ac	$(CH_2)_4Ph$	H	Me	CH_2	14-
149	Ac	Me	H	$(CH_2)_4Ph$	CH_2	14-
150*	H	$(CH_2)_4Ph$	Me	H	$(CH_2)_2$	15-membered
151*	H	$(CH_2)_4Ph$	Me	H	$(CH_2)_2$	15-
152	Ac	$(CH_2)_4Ph$	H	H	$(CH_2)_2$	15-
153	H	$(CH_2)_4Ph$	H	H	$(CH_2)_2$	15-
154	H	$(CH_2)_4Ph$	H	H	$(CH_2)_3$	16-membered

Y: 11-CH_2CH=CH- (trans)

*Stereochemistry at the ω position is not defined.

Figure 30. 11-Substituted 14- to 16-Membered 11-Azalides and 11-Azalactams.

Table 15. Antibacterial Activities of 15- and 16-Membered Azalides with a Phenylbutyl Group (MIC, μg/mL).

Test organisms	Characteristics	**141**	**142**	**130**	**131**	**143**	**144**	**145**	**146**
Streptococcus pneumoniae	standard	0.25	0.06	0.25	0.13	0.25	0.06	0.03	0.03
S. pneumoniae	susceptible	0.25	0.13	0.25	0.13	0.25	0.06	0.06	0.06
S. pneumoniae	*ermB* methylase + *mefA* (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pneumonia	*ermB* methylase (c)	>128	>128	N.T.	N.T.	>128	64	>128	>128
S. pneumoniae	*ermB* methylase (i)	>128	8	8	4	4	1	2	8
S. pneumoniae	*ermB* methylase (i)	>128	8	32	16	4	1	2	4
S. pneumoniae	*mefA* efflux	0.5	0.06	0.5	0.25	0.25	0.13	0.06	0.06
S. pneumoniae	*mefA* efflux	0.25	0.06	0.25	0.13	0.13	0.06	0.06	0.06
Streptococcus pyogenes	standard	0.25	0.06	0.25	0.06	0.25	0.13	0.06	0.06
S. pyogenes	*ermB* methylase (c)	>128	>128	>128	>128	>128	64	32	>128
S. pyogenes	*mefA* efflux	0.5	0.13	0.25	0.13	1	0.25	0.25	0.25

N.T.: not tested; (c): constitutive; (i): inducible

Antibacterial activities of the selected azalides and azalactams are showed in Tables 15 and 16. Generally, the azalides had stronger antibacterial activities against *Streptococcus* species than the azalactams. On the other hand, azalactams showed relatively stronger activities against clinically important Gram-negative pathogens in respiratory infections[112] (data not shown). Among azalides, the 16-membered analogues (**143** to **146**) showed remarkable antibacterial activities against inducible resistant *S. pneumoniae*. Therefore, we decided to select the 16-membered azalide (azalactone derivative) as a template for further medicinal chemistry.

Table 16. Antibacterial Activities of 14- to 16-Membered Azalactams with a Phenylbutyl Group (MIC, μg/mL).

Test organisms	Characteristics	**147**	**135**	**149**	**150**	**151**	**152**	**153**	**154**
Streptococcus pneumoniae	standard	0.5	0.5	0.13	0.06	0.13	0.25	0.13	0.13
S. pneumoniae	susceptible	1	0.5	0.5	0.13	0.25	0.5	0.25	0.25
S. pneumoniae	*ermB* methylase + *mefA* (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pneumoniae	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pneumoniae	*ermB* methylase (i)	32	>128	16	128	32	32	16	8
S. pneumoniae	*ermB* methylase (i)	32	>128	16	128	32	32	16	8
S. pneumoniae	*mefA* efflux	1	1	0.5	0.25	0.25	0.25	0.25	0.13
S. pneumoniae	*mefA* efflux	1	0.5	0.5	0.25	0.25	0.25	0.25	0.13
Streptococcus pyogenes	standard	0.5	0.5	0.5	0.06	0.06	0.13	0.13	0.06
S. pyogenes	*ermB* methylase (c)	>128	>128	>128	>128	>128	>128	>128	>128
S. pyogenes	*mefA* efflux	1	1	2	0.25	0.25	0.5	0.5	0.25

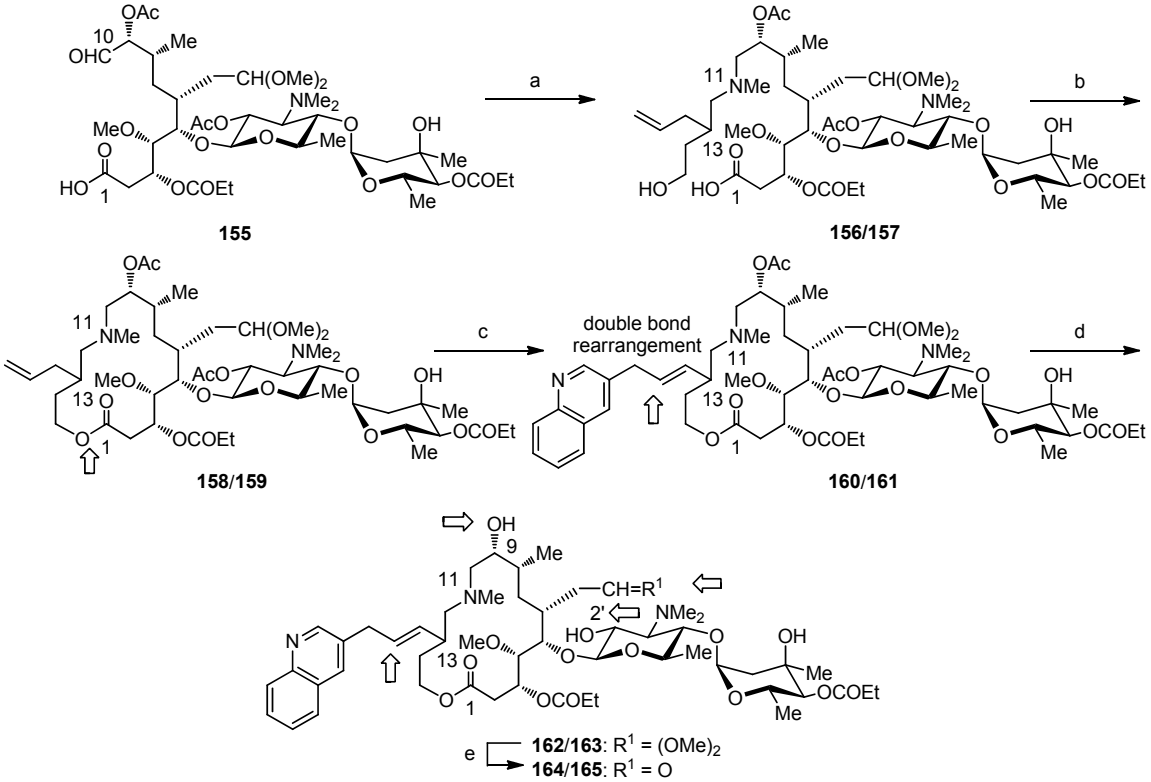
(c): constitutive; (i): inducible

3.5. Discussion about the Position for Introduction of an Arylalkyl Moiety at the Western Hemisphere[112]

We were able to select a 16-membered 11-azalide as a template for further medicinal chemistry; thus, we subsequently determined the position at the western hemisphere at which an arylalkyl moiety could be introduced. The SAR studies performed in-house indicated that the biological activities of the derivatives could be increased only to a limited extent by the introduction of a substituent at the 9-hydroxyl group or the 11-nitrogen atom. Therefore, we planned to introduce the arylalkyl moiety at the C-13 position and the C-15 position by carbon-carbon bonding.

Initially, we synthesized 13-substituted analogues (Scheme 18). There are various strategies to construct a meaningful and powerful "focused library by small molecules" derived from structurally complicated natural products such as anticancer agents or antibiotics. As characteristic examples, one-by-one synthesis and its exact structure determination by conventional approach or split-and-pool methodology for peptide synthesis are well known. On the other hand, attractive strategies such as "chemical biology studies"[114] and "click chemistry"[115] were subsequently introduced as novel approaches to construct a high-quality library. Then, we used (±)-3-methylaminomethyl-5-hexen-1-ol[116] for reductive amination of **155** and isolated the diastereoisomers **156** and **157**; compound **156** was a less polar isomer with an R_f value 0.34, and compound **157** was a polar isomer with an R_f value 0.14 determined using chloroform:methanol (5:1). Finally, each diastereoisomer was separately converted to the desired 13-substituted 16-membered azalide, but their absolute configurations at the C-13 were not determined. Macrolactonization was successfully performed using the Shiina protocol[117] using 2-methyl-6-nitrobenzoic anhydride (MNBA) instead of the acid chloride. When we used a novel approach[118] by using tri-*tert*-butylphosphine in the Heck reaction, we mainly detected an isomer generated by rearrangement of the double bond. Moreover, in some cases, small amounts of *cis* isomers were detected along with major *trans* isomers. In this series (Scheme 18),

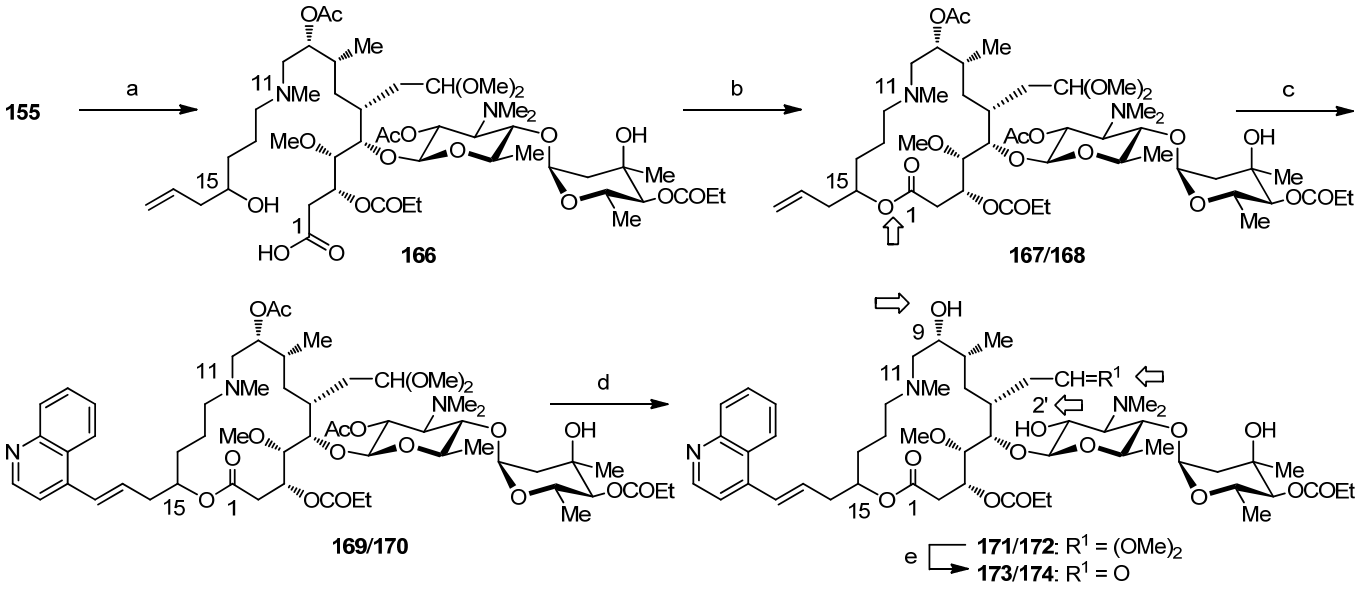
Heck reactions with other bromoquinolines, for example, 6-bromoquinoline, 8-bromoquinoline, or 3-bromoisoquinoline, resulted in a very low yield (15% to 25%).



[a]*Reagents and conditions:* (a) (1) 3-methylaminomethyl-5-hexenol (1.5 eq), AcOH (10 eq), molecular sieves 3A, DMF, rt, 4 h; (2) $NaBH_4$ (1.0 eq), rt, 1.5 h, 40% for **156** and 30% for **157** (total yield: 70%); (b) MNBA (1.5 eq), DMAP (3.0 eq), CH_2Cl_2, 0 °C, 4.5 h, 63% for **158** and 54% for **159**; (c) 3-bromoquinoline (2.0 eq), $Pd_2(dba)_3$ (0.3 eq), $^tBu_3P$ (0.6 eq), Cy_2NMe (2.0 eq), 1,4-dioxane, 50 °C, 48 h, 5.8% for **160** accompanied with 2.8% for its *cis* isomer, total yield of this Heck reaction was 8.6%, 28% for **161**; (d) MeOH, 40 °C, 48 h, 33% for **162**, 21% for **163**; (e) CHF_2CO_2H (30 eq), MeCN-H_2O (1:1), rt, 25 h, 51% for **164**, 45% for **165**.

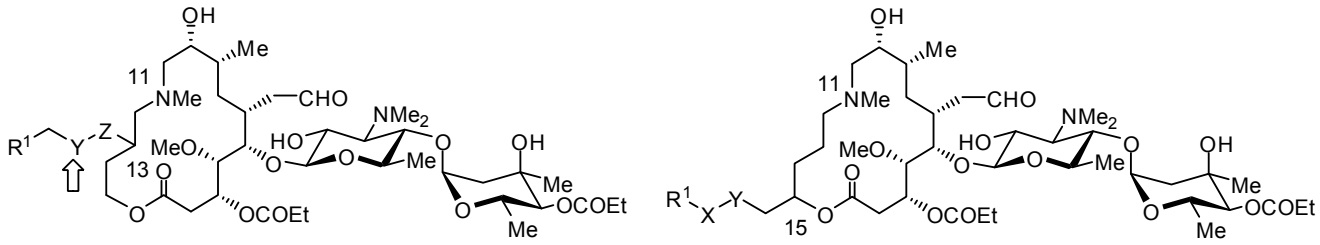
Scheme 18. Synthesis of 13-Substituted 16-Membered Azalides[a].

We simultaneously performed synthetic studies of 15-substituted 16-membered azalides (Scheme 19). Heck reaction of **167** or **168** with several kinds of bromoheterocyclic compounds afforded the desired coupling products with higher yields than those obtained after the reactions with **158** or **159**; further, we did not detect product generated through double bond rearrangement. We prepared both diastereoisomers at the C-15 position and did not determine their stereochemistry. However, these compounds had sufficient antibacterial activities (*vide post*, Table 18) for further modifications, and thus we determined their stereochemistry at the C-15 and established the optimal method for synthesis of the single diastereoisomer of 15-substituted 16-membered azalides. To synthesize the β-isomer only at the C-15, we prepared compound **168** in a stereoselective manner. The coupling reaction of **155** with (*R*)-7-methylamino-1-hepten-4-ol[119] yielded (15*R*)-**166** (a seco acid of **168**) as a single diastereoisomer, which was converted into the key intermediate (**168**). Kanemoto[119] prepared (*R*)-7-methylamino-1-hepten-4-ol from D-ornithine hydrochloride in 8 steps.



[a]*Reagents and conditions:* (a) (1) 7-methylamino-1-hepten-4-ol (1.5 eq), AcOH (10 eq), molecular sieves 3A, DMF, rt, 4 h; (2) $NaBH_4$ (1.0 eq), rt, 1.5 h, 66%; (b) MNBA (1.5 eq), DMAP (3.0 eq), CH_2Cl_2, 0 °C, 2.5 h and rt, 2 h, 21% for **167** and 47% for **168** (total yield: 68%); (c) 4-bromoquinoline (2.0 eq), $Pd_2(dba)_3$ (0.3 eq), $^tBu_3P$ (0.6 eq), Cy_2NMe (2.0 eq), 1,4-dioxane, 50 °C, 48 h, 25% for **169** and 42% for **170**; (d) MeOH, rt, 5 h, 61% for **171**, 41% for **172**; (e) CHF_2CO_2H (30 eq), MeCN-H_2O (1:1), rt, 25 h, 51% for **173**, 61% for **174**.

Scheme 19. Synthesis of 15-Substituted 16-Membered Azalides[a].



Compd	R^1	Y-Z
164*	quinolin-3-yl	CH=CH (*trans*)
165*	quinolin-3-yl	CH=CH (*trans*)
175*	quinolin-3-yl	CH=CH (*cis*)
176*	quinolin-4-yl	CH=CH (*trans*)
177*	quinolin-4-yl	CH=CH (*trans*)
178*	quinolin-4-yl	CH=CH (*cis*)
179*	quinolin-4-yl	CH=CH (*cis*)
180*	quinolin-4-yl	CH_2CH_2
181*	quinolin-4-yl	CH_2CH_2
182*,**	quinolin-6-yl	CH=CH (*trans*)
183*,**	quinolin-8-yl	CH=CH (*trans*)
184*,**	isoquinolin-4-yl	CH=CH (*trans*)

Compd	R^1	X-Y	Configuration at the C-15
173	quinolin-4-yl	CH=CH (*trans*)	α
174	quinolin-4-yl	CH=CH (*trans*)	β
185	quinolin-4-yl	CH_2CH_2	α
186	quinolin-4-yl	CH_2CH_2	β
187	phenyl	CH=CH (*trans*)	β
188	pyridin-3-yl	CH=CH (*trans*)	β
189	naphthalen-1-yl	CH=CH (*trans*)	β
190	naphthalen-2-yl	CH=CH (*trans*)	β
191	quinolin-3-yl	CH=CH (*trans*)	β
192	quinolin-3-yl	CH_2CH_2	β
193	quinolin-6-yl	CH=CH (*trans*)	β

*Stereochemistry at the C-13 position is not defined.
**Only one diastereoisomer was synthesized.

Figure 31. A Variety of 13-Substituted and 15-Substituted 16-Membered Azalides.

We comprehensively synthesized a variety of 13-substituted and 15-substituted 16-membered azalides (Figure 31) according to the synthetic routes indicated in Scheme 18 and Scheme 19. 13-Substituted

analogues and 15-substituted analogues typically showed comparable antibacterial activities. Among the 13-substituted analogues, compounds **165**, **177**, and **179** did not show antibacterial activities against inducible resistant *S. pneumoniae*. Among the 15-substituted analogues, compounds **173**, **185** (both α-configuration at the C-15), and **188** (pyridinyl) did not show antibacterial activities against inducible resistant *S. pneumoniae*. Compound **174** (15-β-configuration) was 2 to 4 times more potent against a variety of pathogens than compound **173** (15-α-configuration).

Table 17. Antibacterial Activities of 13-Substituted 16-Membered Azalides (MIC, μg/mL).

Test organisms	Characteristics	**164**	**175**	**176**	**178**	**180**	**181**	**182**	**183**	**184**
Streptococcus pneumoniae	standard	0.008	0.06	0.015	0.03	0.015	0.015	0.015	0.015	0.015
S. pneumoniae	susceptible	0.03	0.25	0.03	0.06	0.03	0.06	0.03	0.015	0.03
S. pneumoniae	*ermB* methylase (c)	128	>64	>128	>128	>128	>128	>128	128	128
S. pneumoniae	*ermB* methylase (c)	128	>64	>128	>128	>128	>128	128	128	128
S. pneumoniae	*ermB* methylase (i)	1	8	8	8	16	16	2	8	8
S. pneumoniae	*ermB* methylase (i)	1	8	8	8	16	16	2	8	8
S. pneumoniae	*mefA* efflux	0.015	0.13	0.03	0.06	0.03	0.06	0.015	0.03	0.015
S. pneumoniae	*mefA* efflux	0.015	0.13	0.03	0.06	0.03	0.06	0.015	0.03	0.03
Streptococcus pyogenes	standard	0.03	0.13	0.03	0.13	0.06	0.06	0.03	0.06	0.03
S. pyogenes	*ermB* methylase (c)	>128	>64	>128	>128	>128	>128	>128	>128	>128
S. pyogenes	*mefA* efflux	0.13	0.5	0.13	0.5	0.25	0.25	0.25	0.25	0.25

(c): constitutive; (i): inducible

Although the antibacterial activities of the 13-substituted analogues were comparable to the 15-substituted analogues (Tables 17 and 18), the 15-substituted analogues were slightly more potent. Compound **190** was the first example among 16-membered azalides, which showed weak antibacterial activity against constitutive resistant *S. pneumoniae*. Moreover, the problems associated with the 13-substituted analogues were very low yields and partial rearrangements of the double bond in Heck reactions. We performed conformational analyses of the β-isomer of **180** or **181**, **186** (β-isomer), and ABT-773/cethromycin.[89] The three-dimensional position of a quinoline ring of the minimized structure of **186** (a 15-substituted analogue) significantly overlapped with that of ABT-773/cethromycin. Therefore, we finally selected the 15-β-substituted 16-membered 11-azalide as a preferable template for the final optimization. Generally, the absolute configuration of the ω-position (the position next to an oxygen atom of the lactone linkage) in natural macromolecules is very important for their biological activities. In our 15-substituted 16-membered azalides, "unnatural" β-configuration showed stronger antibacterial activity than the natural α-configuration. These phenomena seem to be somehow rare in the chemistry of natural products. Kosan[120] reported the synthesis and strong antibacterial activity of a 13-substituted 14-membered ketolide against resistant *S. pneumoniae*.

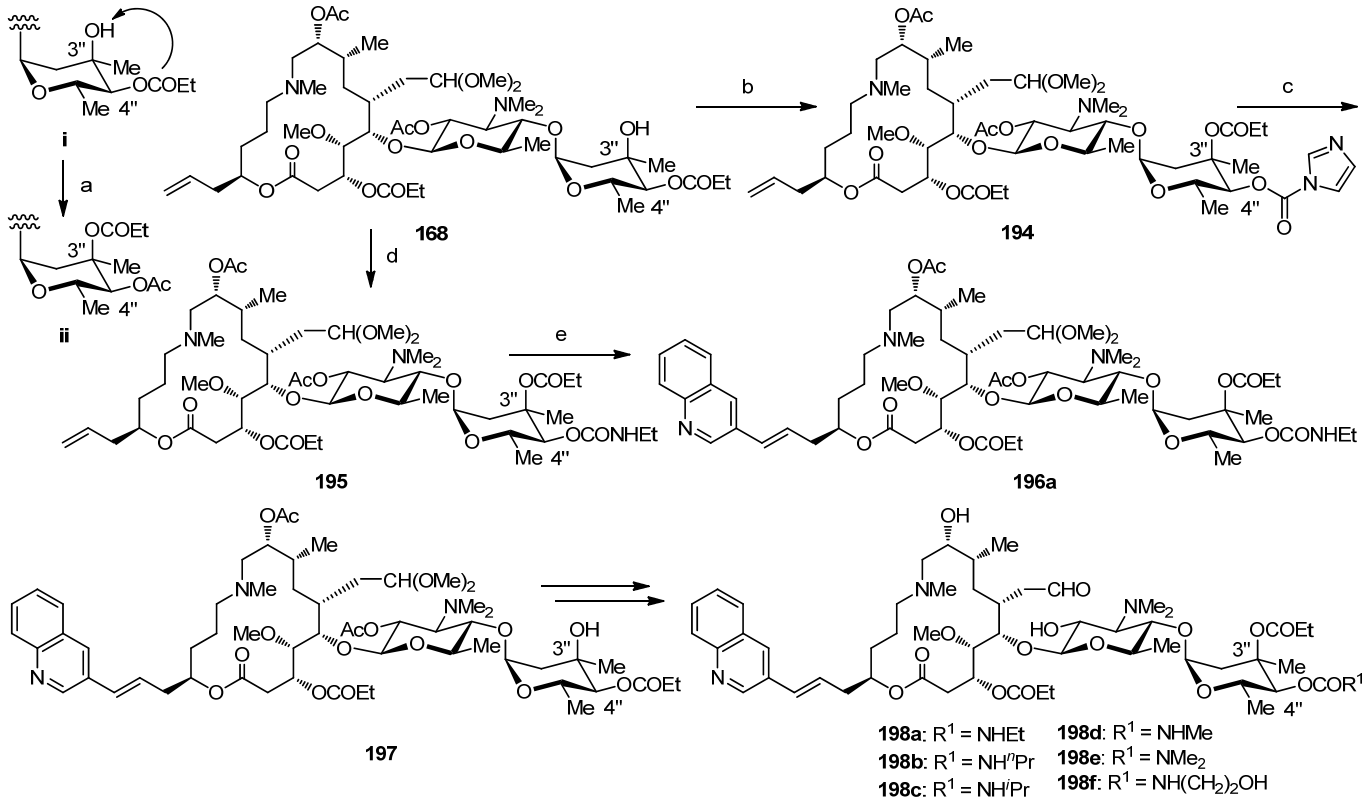
Table 18. Antibacterial Activities of 15-Substituted 16-Membered Azalides (MIC, μg/mL).

Test organisms	Characteristics	**174**	**186**	**187**	**189**	**190**	**191**	**192**	**193**
Streptococcus pneumonia	standard	0.03	0.03	0.03	0.06	0.06	0.015	0.08	0.015
S. pneumoniae	susceptible	0.06	0.06	0.06	0.13	0.06	0.03	0.015	0.03
S. pneumoniae	*ermB* methylase (c)	>128	>128	128	64	32	128	128	>128
S. pneumoniae	*ermB* methylase (c)	>128	>128	>128	64	32	128	128	>128
S. pneumoniae	*ermB* methylase (i)	8	8	2	4	8	8	4	8
S. pneumoniae	*ermB* methylase (i)	4	8	4	2	8	8	4	8
S. pneumoniae	*mefA* efflux	0.06	0.06	0.06	0.06	0.13	0.06	0.015	0.03
S. pneumoniae	*mefA* efflux	0.06	0.06	0.06	0.06	0.13	0.03	0.015	0.03
Streptococcus pyogenes	standard	0.13	0.06	0.13	0.25	0.25	0.06	0.015	0.06
S. pyogenes	*ermB* methylase (c)	>128	>128	>128	64	32	>128	>128	>128
S. pyogenes	*mefA* efflux	0.5	0.5	0.5	1	1	0.5	0.13	0.25

(c): constitutive; (i): inducible

3.6. Molecular Design of a Neutral Sugar Moiety, which is Stable under Physiological Conditions[121]

Although a carbon framework of prototype compounds (**174** and **186** to **193**) of 15-β-substituted 16-membered 11-azalides (Figure 31) had been optimized, their C-3 position and a neutral sugar moiety in addition to their aryl moiety remained to be optimized. As described in sections 1.4. and 1.6., the *in vitro* potency of a 16-membered macrolide with a free hydroxyl group at the C-3 position was stronger than that with an acyloxy group at the C-3 position. Thus, we had to synthesize 3-OH-type analogues of the 15-substituted 16-membered azalides. The antibacterial activities of these azalides against inducible resistant *S. pneumoniae* were moderate or slightly weak (MIC, 2 to 8 μg/mL), and their neutral sugar moieties were unstable under physiological conditions, because the structure of a neutral sugar moiety of the above-mentioned prototype compounds was 3”-OH and 4”-*O*-COEt type. We therefore optimized the neutral sugar moiety before synthesizing 3-OH analogues or optimizing an aryl moiety in a substituent at the C-15 position. To build up a metabolically stable neutral sugar moiety, we designed and synthesized a macrolide belonging to a new chemical class, for example, compound **35d** (Scheme 6), which possessed 4”-*O*-alkyl cladinose. Synthesis of a 4”-*O*-alkyl cladinose-type analogue of the 16-membered macrolide, however, was relatively difficult, and its *in vitro* antibacterial activities were not superior to those of the corresponding 4”-*O*-acyl cladinose-type molecule. Therefore, we explored a novel neutral sugar moiety that (i) could be easily synthesized, (ii) had enhanced activities, and (iii) was relatively stable against metabolism.



[a]*Reagents and conditions:* (a) Ac_2O, pyridine, 100 °C; (b) CDI (5.1 eq), DABCO (30 eq), pyridine (0.25 eq), sealed tube, 60 °C, 69 h; (c) $EtNH_2$·HCl (7.0 eq), Et_3N (7.3 eq), DMF, 0 °C, 56 h, 61% in 2 steps; (d) ethyl isocyanate (30 eq), DABCO (10 eq), pyridine (0.03 eq), sealed tube, 60°C, 47 hrs, 93%; (e) 3-bromoquinoline (2.0 eq), $Pd_2(dba)_3$ (0.15 + 0.25 eq), $^tBu_3P$ (0.30 + 0.50 eq), Cy_2NMe (2.0 eq), 1,4-dioxane, 50 °C, 52 h, 68%.

Scheme 20. Introduction of Carbamoyl Group to the C-4" Position with Intramolecular Migration of Propionyl Group[a].

Omoto *et al.*[122] reported that acetylation of a 4”-*O*-COEt neutral sugar (i) by using acetic anhydride and pyridine at a high temperature afforded a 4”-*O*-Ac-3”-*O*-COEt neutral sugar (ii) accompanied by intramolecular migration of a propionyl group (Scheme 20, left top). Considering this migration of the propionyl group, compound **168** was reacted with ethyl isocyanate in pyridine at 100 °C in a sealed tube to obtain a mixture of 3”-*O*-CONHEt derivative of **168** and a desired compound **195**. After optimization of this reaction condition, we finally treated **168** with ethyl isocyanate in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) in pyridine at 60 °C in a sealed tube to obtain a 93% yield of compound **195**. In addition, compound **195** was prepared using an imidazolide (**194**). Heck reaction at the allyl group of compound **195**, which possesses an unnatural neutral sugar, smoothly proceeded to afford a 68% yield of a quinolin-3-yl derivative (**196a**). Metabolic stability of the 15-substituted 16-membered azalide, which possesses a 3”-*O*-COEt-4”-*O*-CONHEt-type neutral sugar moiety, was significantly higher than that of an azalide, which possesses a 3”-OH-4”-*O*-COEt-type neutral sugar moiety. The 3”-OH-4”-*O*-COEt-type analogue was decomposed more than 90% in only 5 minutes in mouse liver S9, but about 50% of the 3”-*O*-COEt-4”-*O*-CONHEt-type analogue remained intact even after 1 hour in the same condition.[123] Then, we optimized a carbamoyl group at the C-4” position.

A neutral sugar moiety of a key intermediate (**197**) was converted to 3”-*O*-COEt-4”-*O*-CONHR (or 3”-*O*-COEt-4”-*O*-CONMe$_2$) type derivatives, and after deprotections, afforded compounds **198a** to **198f** (Scheme 20). All these analogues showed strong antibacterial activities against inducible resistant *S. pneumoniae* (MIC, 0.13 to 0.5 μg/mL) and resistant *S. pneumoniae* with an *mef* gene (MIC, 0.03 to 0.06 μg/mL).[121] Further, almost all these analogues responded to constitutive resistant *S. pneumoniae*. Among them, compound **198a** showed the strongest activities against the target pathogens, and the structure of the neutral sugar moiety was decided as 3”-*O*-COEt-4”-*O*-CONHEt. This indicated that the size of the substituents in the neutral sugar moiety of **198a** was quite similar to that of RKM. Moreover, the metabolic stability of **198a** in mouse liver S9 was markedly greater than that of MOM (Figure 32) and its stability in human liver S9 was completely comparable to that of TEL (Figure 33).[89]

Figure 32. Time Course of Relative Concentration of **198a**, MOM, and TEL in Mouse Liver S9 (t = 0; 100%).

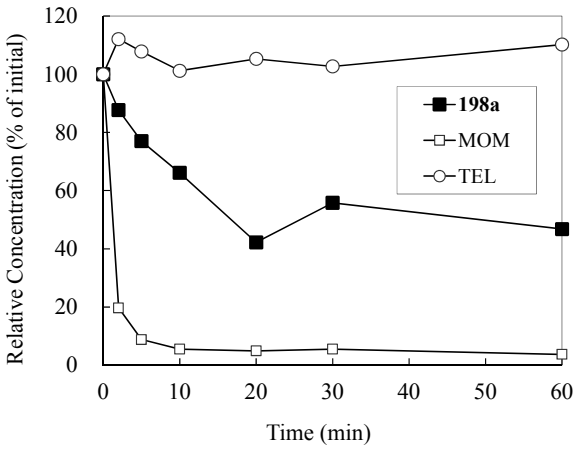
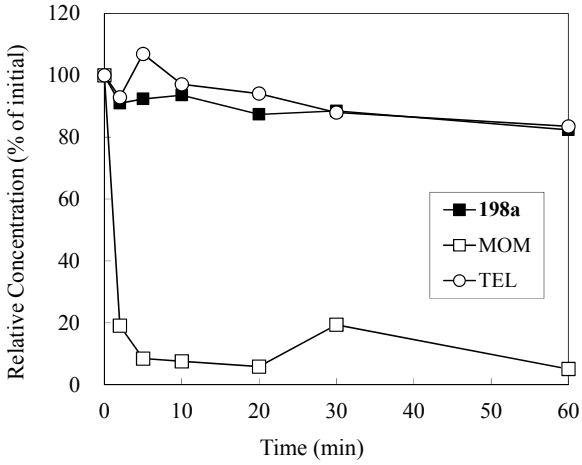
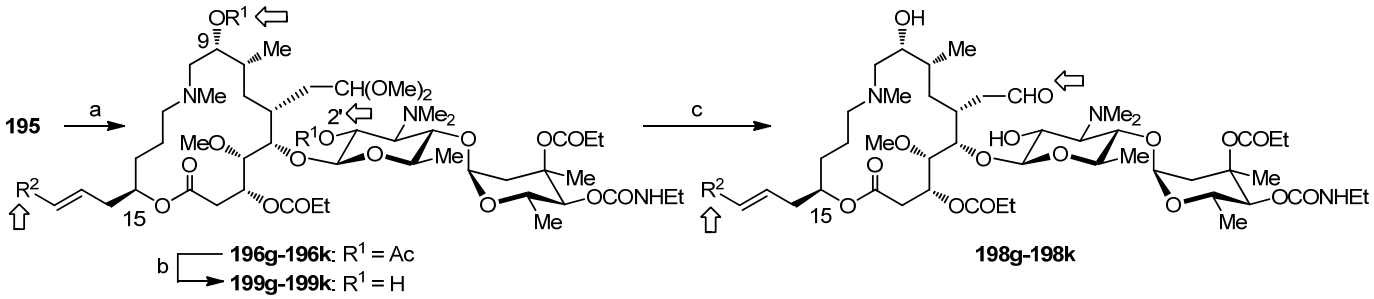


Figure 33. Time Course of Relative Concentration of **198a**, MOM, and TEL in Human Liver S9 (t = 0; 100%).



3.7. Final Optimization of 15-β-Substituted 16-Membered 11-Azalides[121]



[a]*Reagents and conditions:* (a) arylbromide (2.0 eq), $Pd_2(dba)_3$ (0.15 + 0.25 eq), $^tBu_3P$ (0.30 + 0.50 eq), Cy_2NMe (2.0 eq), 1,4-dioxane, 50-80 °C, 16-51 h,17-67% for **196g**, **196h**, **196j** and **196k**, or microwave, 130-160 °C, 25 min., 42% for **196i**; (b) MeOH, rt, to 40 °C, 36-89 h, 36-69%; (c) CHF_2CO_2H (16 eq), MeCN-H_2O (1:1), rt, 8.5-28 h, 66-91%. R^2 = g: naphthalen-2-yl; h: quinolin-4-yl; i: isoquinolin-4-yl; j: pyridin-3-yl; k: 6-aminopyridin-3-yl.

Scheme 21. Partial Optimization of an Aryl Moiety in the 15-Substituent[a].

We used a key intermediate (**195**) with a metabolically stable neutral sugar moiety and screened an aryl moiety at the C-15 substituent (Scheme 21). Heck reaction of **195** and deprotections afforded five analogues. For the preparation of **196i** only, Heck reaction required high temperature and activation by microwave.

Table 19. Antibacterial Activities of 3-*O*-COEt Type 15-β-Substituted 16-Membered Azalides (MIC, μg/mL).

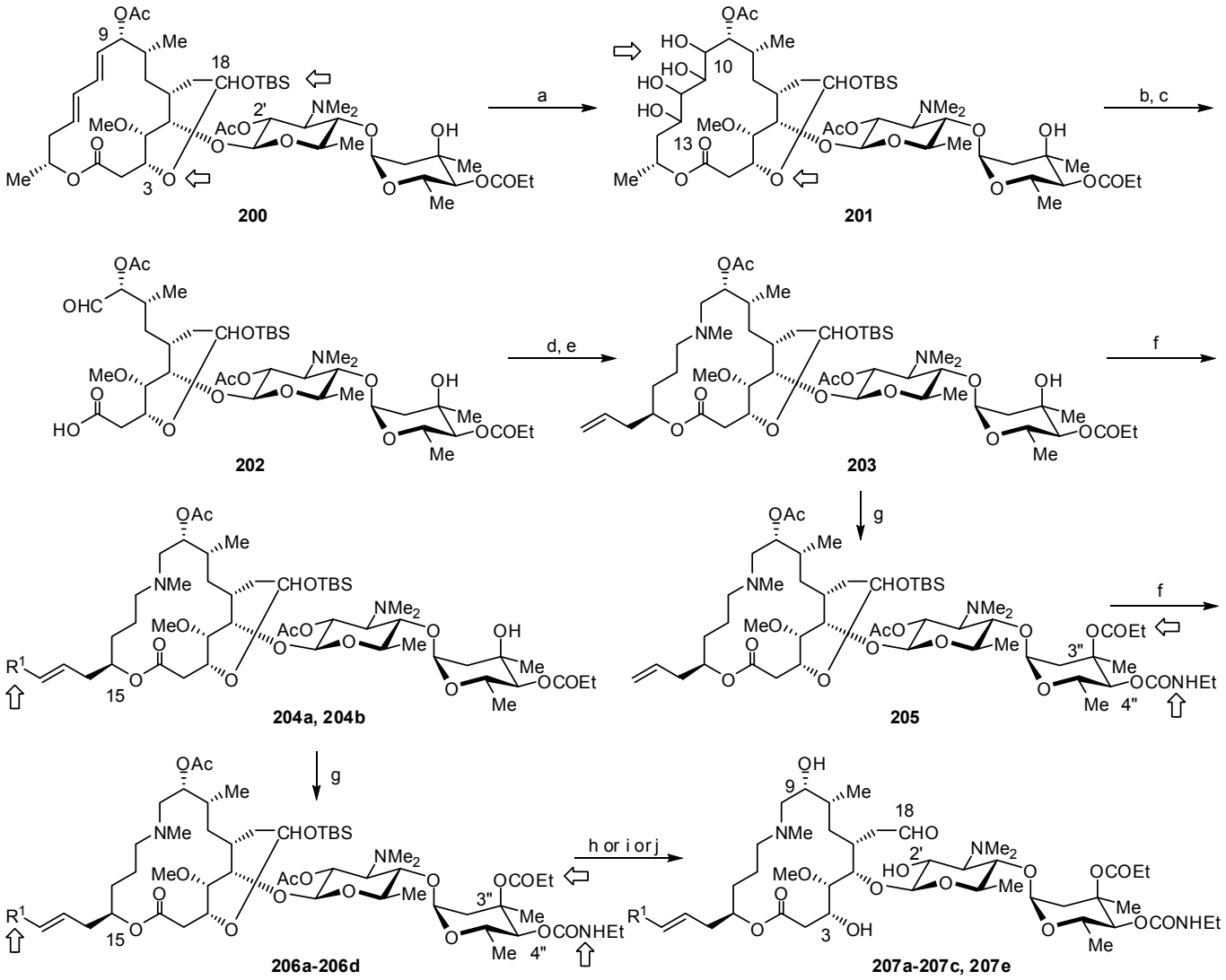
Test organisms	Characteristics	**198a**	**198g**	**198h**	**198i**	**198j**	**198k**	**191**
Streptococcus pneumoniae	standard	0.015	0.06	0.015	0.015	0.015	0.03	0.015
S. pneumoniae	*ermB* methylase (c)	16	16	64	8	32	8	128
S. pneumoniae	*ermB* methylase + *mefA* (c)	16	32	64	8	32	8	128
S. pneumoniae	*ermB* methylase (c)	16	32	64	8	32	8	128
S. pneumoniae	*ermB* methylase (i)	0.13	0.5	0.5	0.13	0.5	0.25	8
S. pneumoniae	*ermB* methylase (i)	0.25	0.5	0.5	0.25	0.5	0.5	8
S. pneumoniae	*mefA* efflux	0.015	0.13	0.03	0.03	0.06	0.06	0.06
S. pneumoniae	*mefA* efflux	0.015	0.13	0.03	0.03	0.06	0.06	0.03
Streptococcus pyogenes	standard	0.06	0.25	0.06	0.06	0.13	0.13	0.06
S. pyogenes	*ermB* methylase (c)	32	16	>128	128	>64	>32	>128
S. pyogenes	*mefA* efflux	0.13	0.5	0.25	0.25	0.5	0.5	0.5

(c): constitutive; (i): inducible

Compared to **191** (3”-OH-4”-*O*-COEt), **198a** (3”-*O*-COEt-4”-*O*-CONHEt) showed markedly increased antibacterial activities against resistant *Streptococcus* species after chemical transformation of the neutral sugar moiety (Table 19). Among these analogues, compounds **198i** and **198k** showed the strongest activities against constitutive resistant *S. pneumoniae*, and we continued the final optimization focusing on an isoquinoline ring (**198i**) and a 6-aminopyridine group (**198k**).

To achieve improved antibacterial activities, we synthesized 3-OH-type of 15-β-substituted 16-membered 11-azalides, which possess a metabolically stable neutral sugar moiety, focusing on an isoquinoline ring and a 6-aminopyridine group. This method of synthesis involved several approaches discussed thus far (Scheme 22). Starting from LM-A_7 prepared by biotransformation[38] of MDM with PF1083, a tetraol (**201**) was synthesized in application of Kitasato protection method[42] (3,18-silyl hemiacetal). Subsequently, we converted the tetraol (**201**) to a key intermediate, 15-β-allyllactone **(203)** using our original method. The efficiency of macrolactonization of a seco acid was reported[124] to be highly affected by the three-dimensional conformation and protecting groups of the seco acid. In our case, fortunately, macrolactonization proceeded to afford a 68% yield of a lactone (**203**) despite the presence of a fused 7-membered silyl hemiacetal.

There were two plausible routes for synthesis of **206**, which were (i) condition (f) then (g) (Heck reaction, and then, neutral sugar transformation), and (ii) condition (g) then (f) (neutral sugar transformation, and then, Heck reaction).



[a]*Reagents and conditions:* (a) OsO_4 (0.15 eq), NMO (3.0 eq), aq acetone, rt, 31 h; (b) $Pb(OAc)_4$ (2.5 eq), Na_2CO_3 (8.3 eq), PhH, rt, 15 min; (c) DBU (1.6 eq), EtOAc, rt, 30 min, 44% in 3 steps from **200**; (d) (*R*)-7-methylamino-1-hepten-4-ol (1.3 eq), molecular sieves 3A, DMF, rt, 5 h, then $NaBH_4$ (1.0 eq), rt, 2 h, 36%; (e) MNBA (1.5 eq), DMAP (3.0 eq), THF, rt, 16 h, 68%; (f) arylbromide (2.0 eq), $Pd_2(dba)_3$ (0.15 + 0.25 eq), $^tBu_3P$ (0.30 + 0.50 eq), Cy_2NMe (2.0 eq), 1,4-dioxane, 50-90 °C, 17-69 h, 53-80%; (g) ethyl isocyanate (30 eq), DABCO (10 eq), pyridine (0.03 eq), sealed tube, 60 °C, 27-46 h, 78-93%; (h) (1) MeOH, 50 °C, 24 h, 23% from **206a** and 63% from **206b**; (2) CHF_2CO_2H (45 eq), MeCN-H_2O (1:1), rt, 15-40 h, 87% for **207a** and 72% for **207b**; (i) (1) MeOH, rt, 15 h; (2) HCl (20 eq), MeCN-H_2O (1:1), rt, 5.5 h, 7.4% for **207c** in 3 steps based on **205**; (j) (1) MeOH, rt to 40 °C, 72 h, 50% from **206d**; (2) TBAF (3.0 eq), THF, rt, 15 min; (3) KF (10 eq), 18-crown-6 (10 eq), DMF, rt, 7 h, 37% in 2 steps for **207e**.

Scheme 22. Optimization of an Aryl Group (R^1) of 3-OH Type 15-β-Substituted 16-Membered 11-Azalides[a].

Neutral sugar transformation, including a reaction with ethyl isocyanate, and intramolecular migration of a propionyl group in a sealed tube proceeded in 78-93% yield, and this reaction was not affected by a 7-membered fused silyl hemiacetal. Deprotections of compounds **206a**-**206c** afforded the desired products **207a**-**207c**. A quinolin-3-yl analogue (**207a**), an isoquinolin-4-yl analogue (**207b**), and a 6-aminopyridin-3-yl analogue (**207c**) showed strong antibacterial activities against target pathogens

(Table 20), and their SARs observed for these compounds were similar to those observed during the screening of 3-*O*-COEt-type derivatives. We focused on the strong antibacterial activities of not only compounds **207a** and **207b**, which possess a fused aryl moiety, but also compound **207c** with an amino group introduced even at the single aromatic ring such as pyridine. Therefore, we finally designed a hybrid-type molecule of compounds **207b** and **207c** and synthesized a 1-aminoisoquinolin-4-yl analogue (**207e**) *via* an important intermediate **206d** prepared from the allyl intermediate **205** with 4-bromo-1-[2-(trimethylsilyl)ethoxycarbonylamino]isoquinoline. When we used this arylbromide protected by the Teoc group in Heck reaction with **205**, partial rearrangement of the double bond at the 15-substituent was observed as the first example in 15-substituted derivatives. After 2-step deprotections of the acetyl groups and the silyl hemiacetal, a single double bond isomer could be isolated.

Table 20. Antibacterial Activities of 3-OH Type 15-β-Substituted 16-Membered Azalides (MIC, μg/mL).

Test organisms	Characteristics	**207a**	**207b**	**207c**	**207e**	TEL	CAM	RKM
Streptococcus pneumoniae	standard	<0.008	<0.008	0.03	0.015	<0.008	0.03	0.03
S. pneumoniae	*ermB* methylase (c)	4	2	4	1	0.5	>128	>128
S. pneumoniae	*ermB* methylase + *mefA* (c)	4	4	4	1	1	>128	>128
S. pneumoniae	*ermB* methylase (c)	4	4	4	1	1	>128	>128
S. pneumoniae	*ermB* methylase (i)	0.06	0.06	0.25	0.06	0.06	>128	1
S. pneumoniae	*ermB* methylase (i)	0.06	0.06	0.25	0.03	0.06	>128	1
S. pneumoniae	*mefA* efflux	0.015	0.015	0.06	0.015	0.06	1	0.06
S. pneumoniae	*mefA* efflux	0.015	0.015	0.13	0.015	0.06	1	0.06
Streptococcus pyogenes	standard	0.06	0.03	0.13	0.03	<0.008	0.03	0.06
S. pyogenes	*ermB* methylase (c)	8	32	16	2	16	>128	>128
S. pyogenes	*mefA* efflux	0.06	0.13	0.13	0.03	0.5	8	0.13

(c): constitutive; (i): inducible

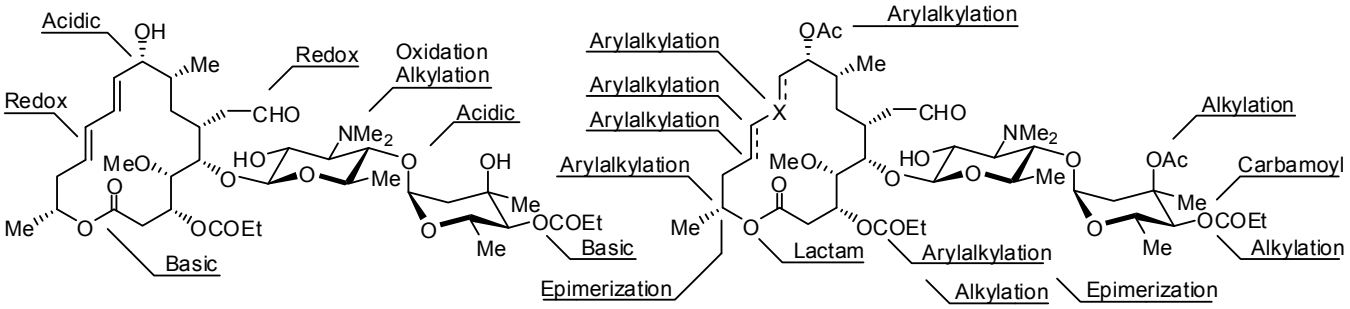
1-Aminoisoquinolin-4-yl analogue (**207e**) showed strong antibacterial activities against constitutive resistant *S. pneumoniae* (Table 20). Antibacterial activities of **207e** against resistant *S. pneumoniae* with an *erm* gene were similar to those of TEL, and its activities against resistant *S. pneumoniae* with an *mef* gene and resistant *S. pyogenes* were clearly stronger than those of TEL. On the other hand, the antibacterial activity of **207e** against *H. influenzae* was slightly weaker than that of TEL, but it was stronger than that of CAM or RKM.[121] To our knowledge, **207e** is the first leucomycin analogue derived from 16-membered macrolides that has optimal and strong activities against clinically important pathogens in respiratory infections.

Metabolic stability of **207e** in mouse liver S9 was qualitatively confirmed to be comparable to that of **198a**. We described that 3-OH-type 16-membered macrolides were relatively unstable than the 3-*O*-acyl-type macrolides because of less hindered lactone linkage in section 1.7. In the case of

15-β-substituted 16-membered 11-azalides, however, chemical or biological instability was not observed at all, probably because a large substituent at the C-15 position would stabilized the lactone linkage. Further studies on **207e**, including evaluation of *in vivo* efficacy, pharmacokinetic studies, and toxicity studies, are currently underway.

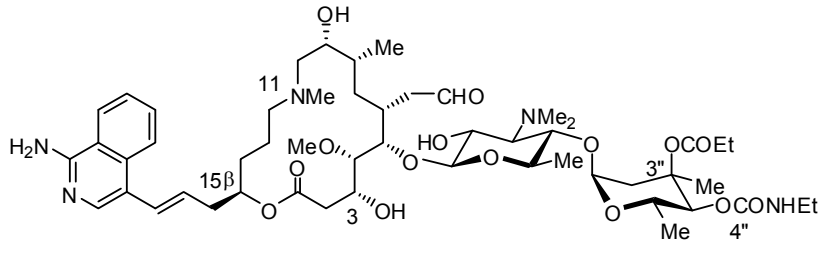
CONCLUSIONS AND PERSPECTIVE

16-Membered macrolides have many sensitive functional groups, including an aldehyde, double bonds, an allylic alcohol, 2-deoxy sugar with less 1,3-diaxial interaction, and less hindered lactone linkage (Figure 34). Moreover, synthesis and analysis of macrolides have some limitations, such as, complicated NMR analyses, a time consuming purification process, and a lack of opportunities for crystallization. Therefore, for a long time, the major chemical modifications of 16-membered macrolides included simple introductions of acyl groups to hydroxyl groups.

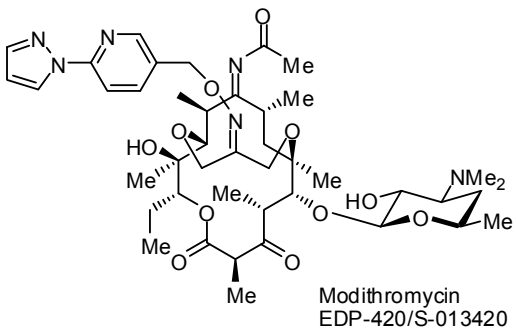


Reactive Molecule, 16-Membered Macrolide

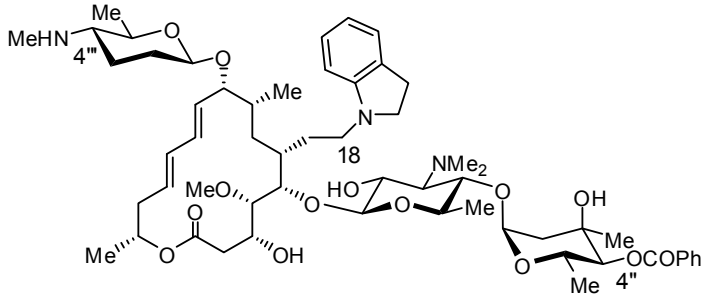
Chemical Modifications of 16-Membered Macrolide by Our Group



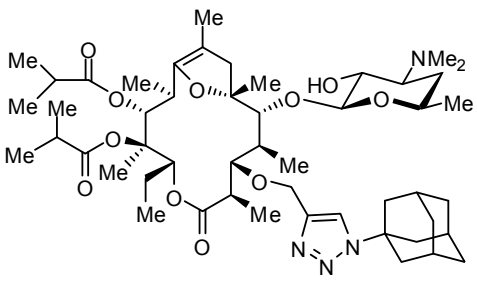
Optimized 15-β-Substituted 16-Membered 11-Azalide (**207e**)



Modithromycin
EDP-420/S-013420



Novel Spiramycin Derivative: Effective against Drug-Resistant Bacteria



Novel EM Derivative: Effective against MRSA and VRE

Figure 34. Conclusions and Perspective.

We referred to the pioneer work about the cladinose analogue in a 16-membered macrolide reported by Tatsuta *et al*.[20] and the alternative original research about 16-membered macrolides, which possess a 4”-*O*-alkyl neutral sugar moiety, reported by Sano *et al*.[42] and Kiyoshima *et al*.[47] We integrated their research concepts and designed and synthesized novel 16-membered macrolides (**34d** and **35d**), which possess a 4-*O*-alkyl cladinose moiety, and proved that these novel macrolides had better pharmacokinetics and *in vivo* potency than the existing 16-membered macrolides did. We performed several novel chemical modifications to address the issue of the emergence of resistant Gram-positive pathogens, including *S. pneumoniae* and *S. pyogenes*. Our major strategies were (i) introduction of an arylalkyl group to the hydroxyl group at the C-3 position or the western hemisphere and (ii) construction of a variety of 11-azalides. Although we introduced an arylalkyl group to the hydroxyl group at the C-3 position or the western hemisphere, we could not generate promising derivatives that showed strong antibacterial activities against constitutive resistant *S. pneumoniae*. However, the introduction of an arylalkyl group to the western hemisphere of a 16-membered macrolide enhanced its antibacterial activities against inducible resistant *S. pneumoniae*. Thus, a more precise three-dimensional molecular design focusing on an arylalkyl group was required to improve antibacterial activities against constitutive resistant *Streptococcus* species. Therefore, we prepared 14-, 15-, and 16-membered azalides (azalactone derivatives) and azalactams, and selected a 16-membered 11-azalide as a novel template for further medicinal chemistry on the basis of its antibacterial activities against target pathogens. A position for the introduction of an arylalkyl group in the western hemisphere was decided as the C-15 position, according to synthetic efficiency (higher yield and no rearrangement of double bond) and in-house fundamental conformational analysis. The final optimization of the 15-β-substituted 16-membered 11-azalide at the C-3 position, an arylalkyl moiety, and a neutral sugar moiety generated compound **207e**.

The antibacterial activity of compound **207e** against constitutive resistant *S. pneumoniae* was similar to that of TEL, but it showed stronger antibacterial activity against resistant *S. pneumoniae* with an *mef* gene (efflux type) than TEL. Moreover, **207e** showed 4 to 8 times stronger antibacterial activities than TEL did against constitutive and efflux-type resistant *S. pyogenes*. In conclusion, we synthesized a novel azalide with the desired profiles in efficiency and metabolic stability, by using a novel transformation of the carbon framework.

The development of a novel oral antibiotic is not very easy. Although ABT-773/cethromycin[74] is in the final stage, its development required a relatively long time. Further, development of EDP-420/modithromycin[125] was reported to be terminated. On the other hand, macrolide drug discovery programs[115c, 126] are ongoing, and they focus on alternative target pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). Although azalide research has been performed for a long time, macrolide chemists have to explore novel approaches to generate a

novel macrolide or a novel azalide with improved clinical efficacy, safety, and taste. We still have many possibilities toward generation of novel macrolides and azalides with or without an arylalkyl group. Recent studies[127] suggest that a novel azalide, which is clinically safe and effective against resistant *Streptococcus* species will be hopefully developed in Japan in the near future.

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