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Synthesis and antibacterial activity of 3-*O*-acyl-6-*O*-carbamoyl erythromycin A derivatives

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Abstract—A series of 3-*O*-acyl-6-*O*-carbamoyl erythromycin A derivatives has been synthesized. Several functional groups were identified as the optimal C3-substituents, and the best compounds in this series possess potent in vitro antibacterial activity against erythromycin-susceptible and erythromycin-resistant bacteria.

Erythromycin A is a well-established macrolide antibiotic that has been used to treat bacterial infections for more than five decades. Erythromycin A derivatives with improved pharmacokinetic properties, such as clarithromycin and azithromycin, were introduced in the early 1990s. These second-generation macrolides are now widely prescribed to treat upper and lower respiratory tract infections.[1] The emergence of bacterial resistance to macrolide antibiotics in recent years, however, has compromised the therapeutic utility of the available macrolides.[2] The most widespread mechanisms of bacterial resistance in the important respiratory pathogen, *Streptococcus pneumoniae*, involve the Erm(B) methyltransferase, which methylates a specific adenine residue in the macrolide binding site of the bacterial ribosome, and the Mef(A) efflux pump.[3]

The need for more potent macrolide antibiotics has prompted renewed interest in the macrolide research area. The recent discovery of ketolides, in which the natural C3-cladinosyl group of erythromycin A is replaced by a keto group, represents a significant breakthrough in the search for macrolide antibiotics with activity against resistant pneumococci.[4] The two most advanced ketolides reported to date are telithromycin[5] and cethromycin[6] (Fig. 1). Both compounds incorporate a C11,C12-cyclic carbamate and an aryl side chain into the macrolide skeleton to achieve potent antibacterial activity against most erythromycin-susceptible and erythromycin-resistant pneumococci.

The C3-cladinose group was long considered to be crucial for the antibacterial activity of erythromycin A. However, recent structural studies have revealed that the C3-substituent of macrolides and ketolides contributes little to the binding interaction with the bacterial ribosome and appears to occupy a region of considerable steric bulk tolerance.[7] In addition, researchers from Taisho have shown that macrolides in which the cladinose sugar is replaced by a C3-*O*-acyl group are active against many bacterial respiratory pathogens, including erythromycin-resistant strains.[8] We have recently identified a series of novel ketolides in which the aryl side

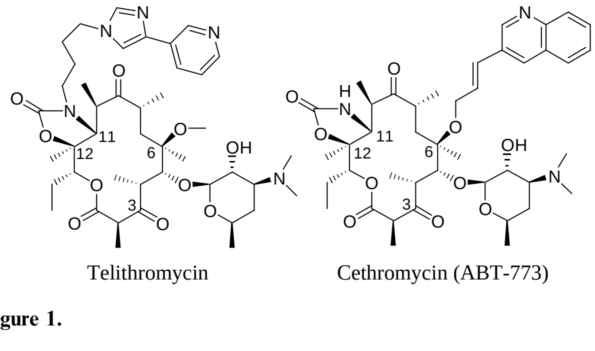

Figure 1.

Keywords: Macrolide; Erythromycin; Antibiotic; Antibacterial.

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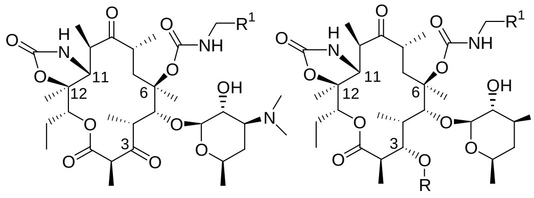
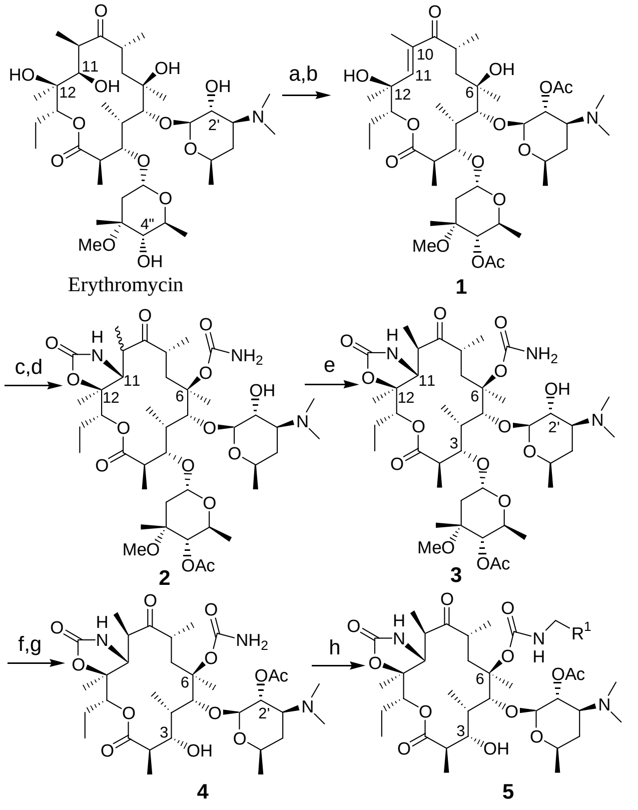


6-*O*-Carbamoyl Ketolides 3-*O*-Acyl-6-*O*-Carbamoyl Macrolides

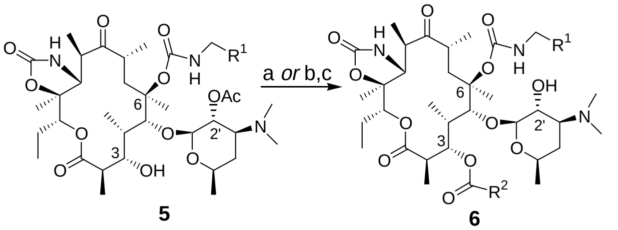
Figure 2.

chain is attached to the macrolide core via a C6-carbamate linkage.[9] To broaden our investigation of this 6-*O*-carbamoyl series, we synthesized 6-*O*-carbamoyl macrolides with various ester and carbamate groups at the C3-position (Fig. 2). Herein we report the chemistry and antibacterial activity of this series of 3-*O*-acyl-6-*O*-carbamoyl macrolides.

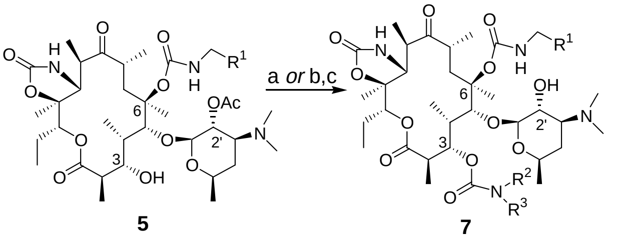
The synthesis of the 3-*O*-acyl-6-*O*-carbamoyl macrolide derivatives is outlined in Schemes 1–3. Intermediate **4** was prepared by methods described previously.[9] Briefly, erythromycin A was reacted with acetic anhydride in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) to give 11,2′,4″-triacetylerythromycin A. Subsequent elimination of the C11-*O*-acetyl group using sodium bis(trimethylsilyl)amide led to 10,11-anhydroerythromycin A derivative **1**. Treatment of compound **1** with trichloroacetyl-isocyanate, followed by base hydrolysis (Et_3N, $MeOH/H_2O$), generated the C6, C12-primary carbamates.



Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, cat DMAP, CH_2Cl_2; (b) $NaN(TMS)_2$, THF, 0 °C; (c) $Cl_3CC(O)NCO$, CH_2Cl_2, 0 °C; (d) Et_3N, MeOH, H_2O, reflux; (e) KO^tBu, THF, 0 °C; (f) Ac_2O, Et_3N, CH_2Cl_2; (g) aq HCl, EtOH rt, 35% for seven steps; (h) R^1CHO, CF_3CO_2H, Et_3SiH, CH_3CN, 65 °C, yields range from 28% to 66%.



Scheme 2. Reagents and conditions: (a) $(R_2CO)_2O$, Et_3N, DMAP, CH_2Cl_2; (b) R_2CO_2H, DCC, DMAP, CH_2Cl_2; (c) MeOH, rt, yields range from 16% to 73% for two steps.

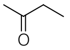
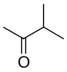
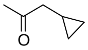
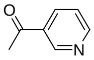
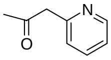
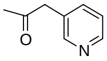
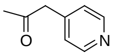
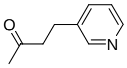
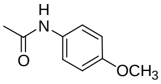
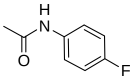
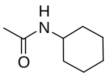
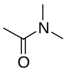
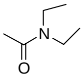
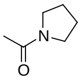
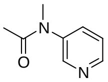
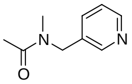
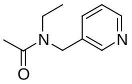


Scheme 3. Reagents and conditions: (a) R_2NCO, DMAP; (b) (I) $Cl_3COC(O)Cl$, Et_3N, DMAP, CH_2Cl_2; (II) HNR^2R^3; (c) MeOH, rt, yields range from 5% to 72% for two steps.

Under the reaction conditions, the C12-primary carbamate underwent spontaneous intramolecular Michael addition to form the C11,12-cyclic carbamate. The Michael addition product **2** was obtained as a mixture of C10-methyl epimers, which could be equilibrated to the desired C10-β-epimer **3** by treatment with potassium *tert*-butoxide. The C2′-hydroxyl group of **3** was reprotected, and the C3-cladinose sugar was then selectively removed under acidic conditions (1 N aq HCl, EtOH) to give 3-descladinosyl derivative **4**. Compound **4** was reacted with various aldehydes under reductive alkylation conditions (trifluoroacetic acid, triethylsilane) to install the desired aryl side chain at the C6-position via a carbamate linkage (Scheme 1). The resulting C3-hydroxy compound **5** was converted to the corresponding C3-ester derivative using standard acylation conditions. Removal of the C2′-hydroxy-protecting group by methanolysis yielded the target C3-ester compounds **6** (Scheme 2).

Two methods were utilized to synthesize the C3-carbamate macrolides **7** (Scheme 3). In the first method, C3-hydroxy compound **5** was reacted with isocyanates in the presence of 4-dimethylaminopyridine (DMAP) to form the C3-secondary carbamate. Removal of the C2′-hydroxy-protecting group gave macrolide compounds **7a**–**c**. The second method involved treatment of compound **5** with diphosgene in the presence of triethylamine and 4-dimethylaminopyridine (DMAP), followed by reaction with various secondary amines. Deprotection of the C2′-hydroxyl group led to the desired C3-tertiary carbamate compounds **7d-l**.

Table 1. In vitro antibacterial activity of 3-*O*-acyl-6-*O*-carbamoyl macrolides **6a**–**i** and **7a**–**i**

Compound	R	MIC (μg/ml)				
		Sta. aureus[a]	*S. pneumoniae*[b]	*S. pneumoniae* [*erm*(B)][c]	*S. pneumoniae* [*mef*(A)][d]	*H. influenzae*[e]
EryA	—	0.5	0.06	>16	4	8
Telith	—	0.25	0.03	0.06	0.25	2
8	—	0.12	0.03	0.06	0.25	4
6a		1	0.03	0.5	0.12	16
6b		0.5	0.03	0.25	0.12	8
6c		0.5	0.06	0.12	0.12	8
6d		1	0.06	0.12	0.12	16
6e		0.5	0.06	4	0.25	16
6f		0.25	0.03	0.12	0.12	4
6g		0.12	0.03	0.12	0.06	4
6h		0.25	0.03	0.25	0.12	4
6i		0.25	0.03	2	0.06	8
7a		1	0.12	4	0.25	16
7b		1	0.12	2	0.25	16
7c		1	0.12	4	0.25	16
7d		0.25	0.03	0.25	0.06	8
7e		0.5	0.06	0.12	0.06	8
7f		0.5	0.06	0.25	0.12	16
7g		1	0.06	0.25	0.25	8
7h		0.25	0.03	0.5	0.06	4
7i		0.5	0.06	0.25	0.12	16

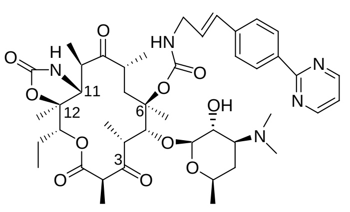
[a] *Staphylococcus aureus* OC4172 (erythromycin-susceptible).

[b] *Streptococcus pneumoniae* OC9132 (erythromycin-susceptible).

[c] *Streptococcus pneumoniae* OC4051 (*erm*(B)-containing, erythromycin-resistant).

[d] *Streptococcus pneumoniae* OC4438 (*mef*(A)-containing, erythromycin-resistant).

[e] *Haemophilus influenzae* OC4882.



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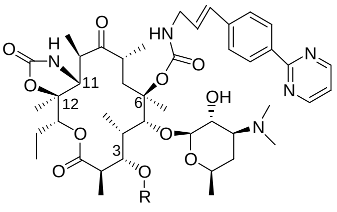
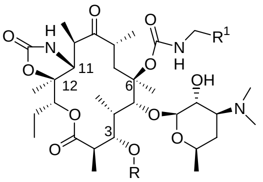


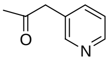
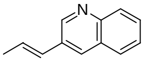
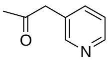
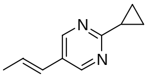
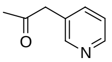
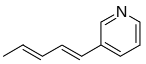
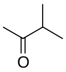
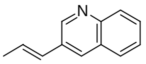
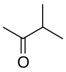
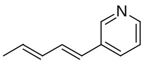
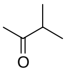
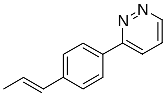
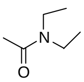
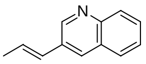
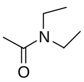
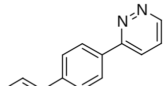
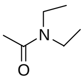
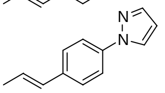
6a-i & 7a-i

Figure 3.

Table 2. In vitro antibacterial activity of 3-*O*-acyl-6-*O*-carbamoyl macrolides **6j–o** and **7j–l**



6j-o & 7j-l

Compound	R	R^1	MIC (μg/ml)				
			Sta. aureus[a]	*S. pneumoniae*[b]	*S. pneumoniae* [*erm*(B)][c]	*S. pneumoniae* [*mef*(A)][d]	*H. influenzae*[e]
Telith	—	—	0.25	0.03	0.06	0.25	2
6j			0.5	0.06	0.12	0.06	4
6k			0.5	0.06	0.25	0.25	2
6l			0.5	0.03	0.12	0.12	2
6m			0.25	0.03	0.06	0.03	4
6n			0.5	0.06	0.25	0.12	4
6o			0.5	0.06	0.12	0.06	2
7j			0.5	0.06	0.12	0.06	4
7k			0.5	0.06	0.25	0.12	4
7l			1	0.12	0.5	0.12	4

[a] *Staphylococcus aureus* OC4172 (erythromycin-susceptible).

[b] *Streptococcus pneumoniae* OC9132 (erythromycin-susceptible).

[c] *Streptococcus pneumoniae* OC4051 (*erm*(B)-containing, erythromycin-resistant).

[d] *Streptococcus pneumoniae* OC4438 (*mef*(A)-containing, erythromycin-resistant).

[e] *Haemophilus influenzae* OC4882.

The in vitro antibacterial activity of the 3-*O*-acyl-6-*O*-carbamoyl macrolide compounds was assessed against both erythromycin-susceptible and erythromycin-resistant bacteria, with particular emphasis on respiratory pathogens. Data are presented in Table 1 as the minimal inhibitory concentration (MIC)[10] against a panel of five selected bacterial strains, including macrolide-susceptible *Staphylococcus aureus* (Smith) OC4172, macrolide-susceptible *S. pneumoniae* OC9132, macrolide-resistant *S. pneumoniae* OC4051 with the *erm*(B) ribosomal methylase gene, macrolide-resistant *S. pneumoniae* OC4438 containing the Mef(A) efflux pump, and *Haemophilus influenzae* OC4882, a Gram-negative respiratory pathogen. Twofold differences in the MIC value are within the error of the method.

To readily interpret the structure–activity relationships of the C3-position substituent, the C6-carbamoyl side chain was initially held constant, while various C3-esters and C3-carbamates were examined. (*E*)-3-[4-(2-Pyrimidinyl)phenyl]prop-2-enyl was chosen as the initial C6-side chain based on the antibacterial profile of the corresponding ketolide analog **8** (Fig. 3).[9]

Among the C3-ester derivatives, compounds with an isopropyl or pyridylmethyl substituent possessed the best antibacterial activity, particularly against erythromycin-resistant *S. pneumoniae* strains and *H. influenzae* (Table 1). Attempts to improve activity by shortening or lengthening the pyridylalkyl chain, as in compounds **6e** and **6i**, had a detrimental effect against *erm*(B)-containing *S. pneumoniae*. Compound **6g**, with a 3-pyridylmethyl substituent, exhibited in vitro activity comparable to those of telithromycin and the C6-carbamate ketolide analog **8**. In the C3-carbamate series, analogs with a tertiary carbamate, such as compounds **7d**–**i**, had an antibacterial profile better than that of compounds with a secondary carbamate (compounds **7a**–**c**). The detrimental effect of the secondary carbamate functionality was particularly apparent in the activity of **7a**–**c** against the *erm*(B)-containing *S. pneumoniae* strain, with MIC values ranging from 2 to 4 μg/ml. *N*,*N*-Diethyl carbamate appeared to be the preferred C3-carbamate substituent, in view of the activity of **7e** against the erythromycin-resistant *S. pneumoniae* strains.

In reviewing the data of Table 1, it is noteworthy that while the C3-substituent had a dramatic effect on activity against the *erm*(B)-containing *S. pneumoniae* strain, activity against the *S. pneumoniae* strain resistant to erythromycin due to efflux was largely unaffected by the structure of the ester or carbamate group. This result is consistent with previous studies which indicate that the cladinose sugar is a key molecular feature for recognition by the Mef efflux pump.[11]

Once the optimal C3-substituents were identified, we combined these preferred substituents with different C6-carbamoyl side chains to search for compounds with further improvements in antibacterial activity, especially against *H. influenzae*. Table 2 provides data for some representative macrolides (**6j**–**o** and **7j**–**l**) containing such combinations. Compound **6m**, with a C6-*O*-(*E*)-3-(3-quinolinyl)prop-2-enyl-carbamoyl group and a C3-isopropyl ester, demonstrated improved activity against erythromycin-resistant *S. pneumoniae* strains compared to **6c**. Compounds with less lipophilic C6-position substituents, such as **6o**, showed improved activity against *H. influenzae* compared to the corresponding C6-*O*-(*E*)-3-[4-(2-pyrimidinyl)phenyl]-prop-2-enyl analog **6c**.

In conclusion, an efficient synthesis of a novel series of 3-*O*-acyl-6-*O*-carbamoyl macrolide compounds has been developed. Compounds with various ester and carbamate groups at the C3-position were studied for their antibacterial activity against erythromycin-susceptible and erythromycin-resistant bacteria. Several functional groups have been identified as the optimal substituents at the C3-position, and the combination of these substituents with a variety of C6-*O*-carbamoyl side chains was examined. The best compounds of this series (**6g**, **6l**, **6m**, and **6o**) possess in vitro antibacterial activity comparable to that of telithromycin. Further studies on this series of macrolide compounds will be reported in the future.

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