

ORIGINAL ARTICLE

Synthesis and antibacterial activity of new 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether

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A series of new 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether were synthesized and evaluated for their *in vitro* antibacterial activity. All the desired compounds demonstrated favorable activity ($0.03 \mu\text{g ml}^{-1}$) against erythromycin-susceptible *Streptococcus pneumoniae* comparable to the references, exhibiting 133-fold higher activity than precursor 2 or 3. Similarly, all of the analogs exhibited improved activity against the erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene and the *erm* and *mef* genes, showing 4–32-fold more effectiveness than erythromycin A.

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INTRODUCTION

The first macrolide, erythromycin A (EMA) (Figure 1), discovered in the early 1950s, was widely prescribed for upper and lower respiratory tract infections, particularly for the patients who are drug resistant or allergic to β -lactam antibiotics.¹ However, EMA readily loses its activity due to degradation under acidic condition, resulting in poor bioavailability and undesirable gastrointestinal side effects.² To overcome the shortcomings of EMA, a wide variety of structural modifications were investigated, which resulted in the discovery of second-generation macrolides such as clarithromycin (CAM)³ and azithromycin (AZM)⁴ (Figure 1). Unfortunately, an increasing resistance of bacteria to various antimicrobials is a pandemic phenomenon, which severely threatens the therapeutic effectiveness of the macrolides as well.⁵ Two of the commonest mechanisms of macrolide resistance are *erm*-encoded methylation of 23S rRNA and *mef*-encoded efflux. The methyltransferase encoded by the *erm* gene can modify the key nucleotide A2058 in domain V of 23S rRNA through methylation, which bears the main responsibility for the resistance to macrolide antibiotics.^{6–8} A member of ketolides, similar to the third-generation macrolides, telithromycin, demonstrated significantly enhanced activity against resistant bacteria encoded by *erm* gene. Its C-11,12 extended carbamate side chain can interact with the nucleotide A752 directly in domain II of the 23S rRNA in addition to the main interaction with the nucleotide A2058, which imparts an increased affinity for the resistant ribosome, resulting in potent activity against the resistant bacteria.⁹

In addition, many other analogs obtained by introducing some side chains with appropriate length and terminal groups to C-11, C-6 or

C-4'' position of the existing macrolides, such as acylides and 4''-carbamates, have been investigated to fight against the increasing resistance to the macrolides.¹⁰ CP-544372 (see ref. 11) (Figure 1), an example of 4''-carbamates, which is characterized by a prolonged anchor group at the C-4'' position, demonstrated good *in vitro* and *in vivo* activity against macrolide-resistant strains. It has been reported that its long C-4'' side chain can reach the chloramphenicol-binding site, displaying an additional affinity to bacterial ribosomes besides the main interaction with A2058.¹² The additional affinity can enhance the activity against resistant bacteria. Recently, we have reported the synthesis of novel 4''-carbamate derivatives of CAM and AZM, which showed improved activity against erythromycin-resistant *Streptococcus pneumoniae*, and the significance of the arylalkyl group attached to the C-4'' position for overcoming MLS_B resistance was described as well.^{13–15}

The 6,9-imino ether of EMA, a key intermediate^{16,17} for synthesis of AZM, is characterized by a novel azalide backbone that is different from those of the 14-membered macrolides and the 15-membered azalides. Accordingly, the 6,9-imino ether of EMA was chosen as the parent nucleus for structural modifications. On the basis of the consideration above, we designed a series of 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether through condensations with various primary amines, which have a different bulk of molecules and different distribution of heteroatoms in the structures. We expect that the introduced 4''-O-carbamoyl side chains containing aromatic or alkyl moieties can interact with the binding sites in the peptidyl transferase-associated region¹⁸ and produce additional affinity for the resistant ribosome via hydrogen bonding, π -stacking and electrostatic interactions.¹³

RESULTS

Chemistry

The desired compounds, 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether (**5a-i**), were synthesized as shown in Scheme 1. Treatment of EMA with hydroxylamine hydrochloride in methanol in the presence of acetic acid and sodium acetate afforded the erythromycin A 9-oxime, which has a high content of the desired (*E*)-isomer (**1**). Erythromycin A 9-oxime was subjected to Beckmann rearrangement in the presence of *p*-toluenesulfonyl chloride in acetone/water with sodium hydrocarbonate as a base, to provide erythromycin A 6,9-imino ether (**2**). Protection of the 2'-hydroxyl group of the 6,9-imino ether (**2**) with acetic anhydride gave 2'-O-acetyl product (**3**), which was treated with 1,1-carbonyldiimidazole (CDI) in toluene at 55 °C to generate the important intermediate 11,12-carbonate 4''-O-acylimidazole (**4**). Finally, 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether (**5a-i**) were obtained by coupling the intermediate (**4**) with corresponding amines catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by deprotection of the acetyl group in methanol.

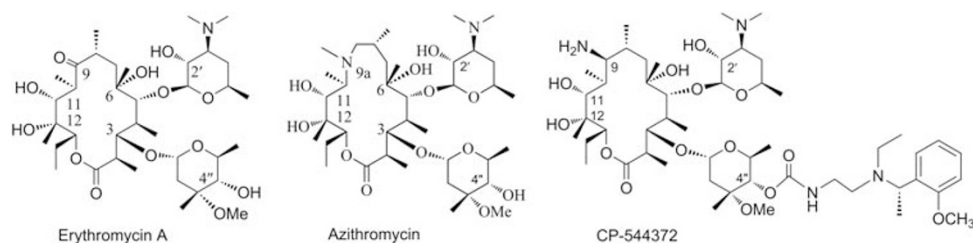
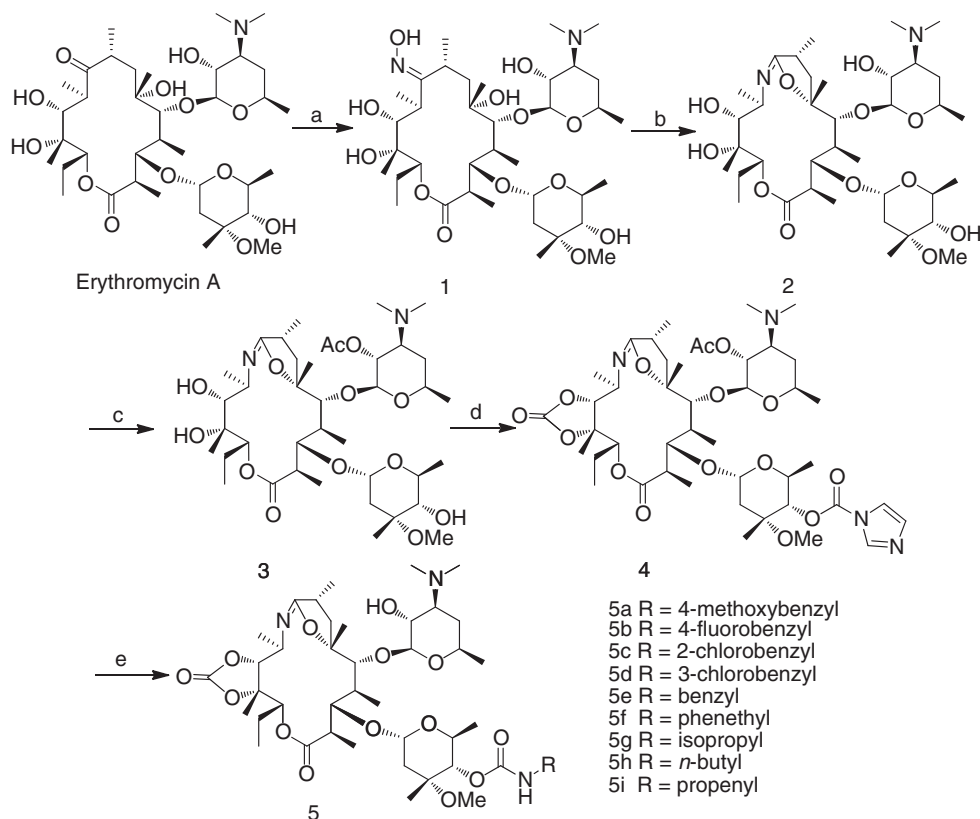


Figure 1 Structures of erythromycin A, azithromycin and CP-544372.



Scheme 1 Reagents and conditions: (a) $\text{NH}_2\text{OH}\cdot\text{HCl}$, AcOH , AcONa , MeOH , 55 °C, 24 h; (b) *p*-TolCl, NaHCO_3 , acetone/water; (c) Ac_2O , CH_2Cl_2 , Et_3N , rt, 4 h; (d) CDI, toluene, 55 °C, 48 h; (e) RNH_2 , DBU, DMF, rt, 24 h; (f) CH_3OH , 55 °C, 24 h.

Antibacterial activity

The 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether (**5a-i**) and intermediates **2** and **3**, as well as the control drugs EMA, CAM and AZM, were tested for *in vitro* antibacterial activity against eight phenotypes of Gram-positive strains. The MICs of these compounds determined using the broth microdilution method are shown in Table 1. *Staphylococcus aureus* ATCC25923 and *Streptococcus pneumoniae* ATCC49619 are erythromycin-susceptible strains. *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11 are three erythromycin-resistant strains, resistances of which are encoded by the *erm* gene, the *mef* gene or both of them, respectively. Other three bacteria are wild strains isolated clinically. *Streptococcus pyogenes* S2 and *S. pyogenes* R2 are erythromycin-susceptible and erythromycin-resistant strains, respectively, while *S. aureus* is a penicillin-resistant strain.

As shown in Table 1, all the 6,9-imino ether derivatives (**5a-i**) demonstrated favorable activity ($0.03 \mu\text{g ml}^{-1}$) against erythromycin-susceptible *S. pneumoniae* comparable to the references, exhibiting 133-fold higher activity than compound **2** or **3** as precursors of the

Table 1 *In vitro* antibacterial activity of 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether

Compound	Strain/MIC ($\mu\text{g ml}^{-1}$)							
	<i>S. pneumoniae</i> ATCC49619 ^a	<i>S. pneumoniae</i> B1 ^b	<i>S. pneumoniae</i> A22072 ^c	<i>S. pneumoniae</i> AB11 ^d	<i>S. pyogenes</i> <i>pyogenes</i> S2 ^e	<i>S. pyogenes</i> R2 ^f	<i>S. aureus</i> ATCC25923 ^g	<i>S. aureus</i> ^h
EMA	0.03	128	8	256	0.03	4	0.06	0.25
CAM	0.03	64	4	128	0.03	4	0.12	0.03
AZM	0.03	128	4	256	0.03	8	0.25	0.125
2	4	64	4	64	0.5	32	4	16
3	4	64	4	64	1	0.25	8	16
5a	0.03	16	2	16	1	64	16	16
5b	0.03	32	4	32	1	64	4	4
5c	0.03	32	4	32	1	64	6	64
5d	0.03	16	4	16	1	64	8	32
5e	0.03	32	4	32	2	64	16	32
5f	0.03	8	4	8	2	64	8	32
5g	0.03	32	4	32	0.5	64	16	64
5h	0.03	32	4	32	1	64	8	8
5i	0.03	32	4	32	1	64	64	64

Abbreviations: AZM, azithromycin; CAM, clarithromycin; EMA, erythromycin A.

^aErythromycin-susceptible strain.

^bErythromycin-resistant strain encoded by the *erm* gene.

^cErythromycin-resistant strain encoded by the *mef* gene.

^dErythromycin-resistant strain encoded by the *erm* and *mef* genes.

^eErythromycin-susceptible strain isolated clinically.

^fErythromycin-resistant strain isolated clinically.

^gErythromycin-susceptible strain.

^hPenicillin-resistant strain isolated clinically, not characterized.

6,9-imino ether derivatives. We speculated that the introduced C-4'' side chains can fit well in the binding pocket of macrolides in the bacterial ribosome and impart additional affinity to the binding sites, which lead to the restored activity against the erythromycin-susceptible *S. pneumoniae*. Among them, compound **5f** possessed the most greatly improved activity against erythromycin-resistant *S. pneumoniae* B1 and *S. pneumoniae* AB11, showing 16- and 32-fold more potent activity than EMA or AZM, respectively. In contrast, all of the desired 6,9-imino ether derivatives except compound **5a** had no changes in activity against erythromycin-resistant *S. pneumoniae* A22072 encoded by the *mef* gene compared with CAM and AZM or compounds **2** and **3**. All of the 6,9-imino ether derivatives (**5a–i**) possessed lower activity against erythromycin-susceptible and penicillin-resistant *S. aureus* than the references. The results described above clearly suggested that the introduction of the arylalkyl or alkyl group to the C-4'' position of the precursor can completely restore the activity against erythromycin-susceptible *S. pneumoniae* and improve the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene, or the *erm* and *mef* genes, compared with precursors **2** and **3**.

DISCUSSION

In our published paper,^{13–15} some 4''-carbamate derivatives were synthesized through introduction of the arylalkyl groups to 11,12-cyclic carbonate AZM, and some of them were found to have potent activity against erythromycin-susceptible and erythromycin-resistant *S. pneumoniae*. In the published paper,¹⁵ all of the 4''-carbamate derivatives of 11,12-cyclic carbonate AZM with the same C-4'' side chains as those of the 6,9-imino ether derivatives demonstrated similar activity to the derivatives **5a–i** against erythromycin-susceptible *S. pneumoniae* or erythromycin-resistant *S. pneumoniae* encoded by *erm* gene or *erm* and *mef* genes. However, some of the 4''-carbamate derivatives of 11,12-cyclic carbonate AZM exhibited high activity, while almost all of derivatives **5a–i** did not show potent activity

against erythromycin-resistant *S. pneumoniae* A22072 encoded by the *mef* gene.

In this paper, the 6,9-imino ether of EMA with weak activity was chosen as the parent nucleus for modification, in order to probe the effect of the C-4'' arylalkyl of alkyl side chains in the improvement of antibacterial activity. As expected, some derivatives of 6,9-imino ether showed improved antibacterial activity, suggesting that the C-4'' arylalkyl or alkyl side chains may produce additional affinity for the bacterial ribosomes and impart improved activity. On the other hand, the introduction of C-4'' side chain to the 6,9-imino ether of EMA with different conformations from EMA, CAM and AZM may result in variation in stretching direction of the C-4'' side chain in the peptidyl transferase-associated region. The variation might further enhance the affinity for the bacterial ribosomes.

In conclusion, new 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether were designed, synthesized and evaluated for their *in vitro* antibiotic activities. All of the target compounds showed potent activity against erythromycin-susceptible *S. pneumoniae* comparable to EMA, CAM and AZM, and exhibited improved activity against erythromycin-resistant *S. pneumoniae* B1 and *S. pneumoniae* AB11 compared with EMA or AZM. The results led us to imagine that the introduction of side chains with appropriate extended length and favorable terminal group to the C-4'' position of the lactone core can restore the activity against erythromycin-susceptible *S. pneumoniae* and improve the activity against erythromycin-resistant *S. pneumoniae* to some extent.

METHODS

General experimental procedures

All necessary solvents were purified before use, unless noted otherwise. Reactions were monitored by TLC using 0.25-mm pre-coated silica gel plates (Qingdong Yumingyuan silica gel reagent factory, Shandong, China, YUYUAN). Flash chromatography was performed with the indicated solvents

using silica gel 60 (particle size 0.040–0.063 mm, Qingdong Yumingyuan silica gel reagent factory, YUYUAN, Shandong, China). IR spectra were recorded on KBr pellets using a Nicolet Nexus 470FT-IR spectrometer (Nicolet, Madison, WI, USA). ¹H NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer at ambient temperature (tetramethylsilane (TMS) as an internal standard of chemical shifts). Mass spectra were recorded on an API 4000 instrument (Applied Biosystems, Foster City, CA, USA), and the high-resolution mass spectra data were obtained using Agilent Q-TOF6510 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Melting points are uncorrected and were determined on an X-6 melting point apparatus (Beijing Tianchengwode Biotech Co. Ltd, Beijing, China). All of the target compounds were no less than 95% pure on the basis of HPLC analysis. HPLC analyses were performed on a Shimadzu HPLC LC-10ATVP instrument (Shimadzu, Tokyo, Japan) equipped with a Diaspher C18 column (4.6×150 mm) (Shimadzu, Shim-pack VP-ODS) and a UV detector set at 226 nm. Elutions were carried out at a flow of 1.00 ml min⁻¹ using a phosphate buffer (pH=5.5)–MeCN mixture (600:400, v/v) at 25 °C.

Erythromycin A 9-oxime (1)

To a solution of erythromycin A (5.0 g, 6.82 mmol) in methanol (20 ml) was added sodium acetate (3.9 g, 47.56 mmol), acetic acid (0.35 ml) and hydroxylamine hydrochloride (2.4 g, 34.78 mmol). The resulting solution was allowed to stir for 24 h at 55 °C. After concentrated in vacuum, the residue was dispersed in ethyl acetate (30 ml) and water (15 ml) and was adjusted to pH 11–12 with 2 M sodium hydroxide. The resultant solution was extracted with ethyl acetate (3×30 ml). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give 5.00 g (98.0%) of **1** as a white solid: *R*_F=0.58 (petroleum ether–ethyl acetate–diethylamine, 7.5:3:2), m.p. 154–157 °C.

Erythromycin A 6,9-imino ether (2)

The solution of **1** (2.0 g, 2.67 mmol) in acetone (14 ml) was added to sodium bicarbonate (0.5 g, 5.95 mmol) in water (10 ml). Then *p*-toluenesulfonyl chloride (2.28 g, 16.02 mmol) in acetone (8 ml) was added dropwise. The resulting solution was stirred at room temperature for 4 h. After adjusting to pH 10–11, the mixture was extracted with dichloromethane (3×15 ml). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give 1.85 g (94.8%) of **2** as a white solid: *R*_F=0.56 (petroleum ether–ethyl acetate–diethylamine, 7.5:3:2), m.p. 126–129 °C.

2-O-acetyl erythromycin A 6,9-imino ether (3)

Compound **2** (1.0 g, 1.37 mmol) was treated with acetic anhydride (0.35 ml) in anhydrous dichloromethane (10 ml) in the presence of triethylamine (1.5 ml). The resulting mixture was washed with saturated solution of sodium bicarbonate and brine, and then was extracted with dichloromethane (3×15 ml). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give 1.0 g (94.6%) of **3** as a white solid: *R*_F=0.48 (dichloromethane–methanol, 10:1), m.p. 108–110 °C.

2-O-acetyl-4''-O-acylimidazolyl erythromycin A 6,9-imino ether 11,12-cyclic carbonate (4)

Compound **4** was prepared from intermediate **3** according to the procedures¹⁷ reported by Ma *et al*. The crude **4** was a white solid: m.p. 112–114 °C; TLC *R*_F=0.50 (dichloromethane–methanol, 10:1).

General methods for 4''-O-carbamates of 11,12-cyclic carbonate erythromycin A 6,9-imino ether (5a–i)

To a solution of **4** (0.9 g, 1.01 mmol) in *N,N*-dimethylformamide (DMF) (5 ml) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (0.29 ml, 2.00 mmol) and the corresponding amine (2.00 mmol). The resulting solution was stirred for 24 h at room temperature. The saturated solution of sodium dihydrogenphosphate (10 ml) was then added to the reaction solution, and the aqueous layer was extracted with ethyl acetate (3×10 ml). The combined organic layers were

washed with brine (3×10 ml), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated *in vacuo* to afford a crude product.

A solution of the above crude product in methanol (15 ml) was heated to 55 °C and stirred for 24 h at the same temperature. After concentrating the reaction solution *in vacuo*, the residue was purified by flash chromatography (dichloromethane–methanol, 30:1) to give compounds **5a–i** in yields ranging from 67.5 to 83.5%. The mass spectrum, IR spectrum and ¹H NMR spectrum of **5a–i** were provided in Supplementary material.

4''-O-((4-methoxybenzyl)carbamoyl)erythromycin A 6,9-imino ether 11,12-cyclic carbonate (5a)

White solid, yield: 70.2%, m.p.: 116–119 °C, TLC *R*_F=0.56 (dichloromethane–methanol, 10:1); IR (KBr): 3368, 2974, 2936, 1809, 1726, 1613, 1586, 1513, 1457, 1382, 1337, 1300, 1246, 1172, 1110, 1073, 1016 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.26–7.23 (m, 2H), 6.86–6.84 (m, 2H), 5.33 (m, 1H), 4.85 (dd, *J*=9.0 Hz, *J*=3.0 Hz, 1H), 4.62–4.60 (m, 1H), 4.38–4.36 (m, 2H), 4.32–4.29 (m, 3H), 4.26–4.23 (m, 2H), 3.80–3.76 (m, 5H), 3.29–3.21 (m, 4H), 2.80–2.68 (m, 3H), 2.51–2.39 (m, 8H), 2.05–1.95 (m, 1H), 1.90–1.90 (m, 1H), 1.65–1.57 (m, 4H), 1.47–1.45 (m, 3H), 1.45–1.44 (m, 1H), 1.37–1.30 (m, 1H), 1.29–1.27 (m, 6H), 1.27–1.21 (m, 3H), 1.20–1.17 (m, 6H), 1.09–1.07 (m, 3H), 1.06–1.00 (m, 9H); ESI-MS *m/z* calculated for C₄₇H₇₃N₃O₁₅ 919.5, found [M+H]⁺ 920.9; HR-MS (ESI) *m/z* calculated for C₄₇H₇₃N₃O₁₅ [M+H]⁺: 920.0937, found 920.1078.

4''-O-((4-fluorobenzyl)carbamoyl)erythromycin A 6,9-imino ether 11,12-cyclic carbonate (5b)

White solid, yield: 75.6%, m.p.: 128–132 °C, TLC *R*_F=0.52 (dichloromethane–methanol, 10:1); IR (KBr): 3350, 2974, 2935, 1806, 1724, 1605, 1510, 1457, 1383, 1223, 1163, 1111, 1048, 1015 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.32–7.20 (m, 4H), 5.11–5.08 (m, 1H), 4.88 (dd, *J*=9.0 Hz, *J*=3.0 Hz, 1H), 4.60–4.50 (m, 1H), 4.40–4.30 (m, 2H), 4.30–4.25 (m, 3H), 4.24–4.20 (m, 2H), 3.90–3.80 (m, 1H), 3.75–3.67 (m, 1H), 3.27–3.13 (m, 4H), 2.96–2.87 (m, 8H), 2.05–1.95 (m, 1H), 1.67–1.55 (m, 5H), 1.54–1.41 (m, 4H), 1.41–1.36 (m, 7H), 1.30–1.25 (m, 6H), 1.21–1.17 (m, 3H), 1.16–1.12 (m, 6H), 1.11–1.07 (m, 3H), 0.99–0.94 (m, 3H), 0.89–0.83 (m, 3H); ESI-MS *m/z* calculated for C₄₆H₇₀FN₃O₁₄ 907.5, found [M+H]⁺ 908.8; HR-MS (ESI) *m/z* calculated for C₄₆H₇₀FN₃O₁₄ [M+H]⁺: 908.0582, found 908.0720.

4''-O-((2-chlorobenzyl)carbamoyl)erythromycin A 6,9-imino ether 11,12-cyclic carbonate (5c)

White solid, yield: 75.6%, m.p.: 130–133 °C; TLC *R*_F=0.52 (dichloromethane–methanol, 10:1); IR (KBr): 3419, 2975, 2937, 1808, 1724, 1655, 1604, 1511, 1463, 1383, 1243, 1165, 1111, 1074, 1034, 1016 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.32–7.25 (m, 2H), 7.01–6.98 (m, 2H), 5.38–5.36 (m, 1H), 4.85 (m, 1H), 4.59–4.57 (m, 1H), 4.39–4.38 (m, 1H), 4.37–4.35 (m, 1H), 4.25–4.21 (m, 2H), 3.64–3.58 (m, 2H), 3.50–3.40 (m, 1H), 3.26–3.23 (m, 3H), 2.76–2.70 (m, 4H), 2.18–2.00 (m, 8H), 1.88–1.86 (m, 2H), 1.77–1.73 (m, 2H), 1.67–1.59 (m, 5H), 1.54–1.50 (m, 2H), 1.46–1.41 (m, 3H), 1.39–1.30 (m, 4H), 1.22–1.17 (m, 12H), 1.09–1.06 (m, 9H); ESI-MS *m/z* calculated for C₄₆H₇₀ClN₃O₁₄ 923.4, found [M+H]⁺ 924.8; HR-MS (ESI) *m/z* calculated for C₄₆H₇₀ClN₃O₁₄ [M+H]⁺: 924.5125, found 924.4603.

4''-O-((3-chlorobenzyl)carbamoyl)erythromycin A 6,9-imino ether 11,12-cyclic carbonate (5d)

White solid, yield: 70.5%, m.p.: 134–136 °C, TLC *R*_F=0.57 (dichloromethane–methanol, 10:1); IR (KBr): 3361, 2975, 2937, 1808, 1724, 1599, 1575, 1521, 1458, 1383, 1351, 1247, 1169, 1110, 1074, 1034, 1016 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.26–7.24 (m, 2H), 6.86–6.84 (m, 2H), 5.35–5.33 (m, 1H), 4.86 (dd, *J*=9.0 Hz, *J*=3.6 Hz, 1H), 4.64–4.62 (m, 1H), 4.38–4.36 (m, 2H), 4.35–4.23 (m, 2H), 3.78–3.76 (m, 2H), 3.62–3.60 (m, 1H), 3.29–3.27 (m, 3H), 2.80–2.70 (m, 3H), 2.52–2.39 (m, 1H), 2.30–2.26 (m, 8H), 1.88–1.83 (m, 2H), 1.75–1.70 (m, 2H), 1.59–1.57 (m, 2H), 1.50–1.49 (m, 2H), 1.47–1.44 (s, 2H), 1.37–1.36 (m, 2H), 1.29–1.25 (m, 6H), 1.23–1.20 (m, 3H), 1.20–1.19 (m, 6H), 1.17–1.14 (m, 3H), 1.10–1.08 (m, 6H), 1.06–1.03 (m, 3H); ESI-MS *m/z* calculated for C₄₆H₇₀ClN₃O₁₄ 923.4, found [M+H]⁺ 924.9; HR-MS (ESI) *m/z* calculated for C₄₆H₇₀ClN₃O₁₄ [M+H]⁺: 924.5125, found 924.4628.

**4''-O-((benzyl)carbamoyl)erythromycin A 6,9-imino ether
11,12-cyclic carbonate (5e)**

White solid, yield: 76.5%, m.p.: 124–127 °C, TLC R_F =0.51 (dichloromethane–methanol, 10:1); IR (KBr): 3265, 2974, 2936, 1805, 1767, 1726, 1657, 1510, 1455, 1383, 1350, 1244, 1167, 1109, 1081, 1015 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 7.28–7.26 (m, 5H), 5.31–5.30 (m, 1H), 4.88 (dd, $J=9.0$ Hz, $J=3.0$ Hz, 1H), 4.59–4.55 (m, 1H), 4.34–4.25 (m, 2H), 4.23–4.20 (m, 2H), 4.19–4.18 (m, 1H), 3.75–3.70 (m, 2H), 3.52–3.51 (m, 1H) 3.43–3.40 (m, 1H), 3.30–3.28 (m, 3H), 2.89–2.88 (m, 1H), 2.88–2.83 (m, 2H), 2.44–2.41 (m, 2H), 2.41–2.31 (m, 6H), 1.95–1.85 (m, 2H), 1.70–1.68 (m, 1H), 1.65–1.61 (m, 1H), 1.56–1.48 (m, 5H), 1.47–1.42 (m, 2H), 1.38–1.33 (m, 1H), 1.31–1.23 (m, 3H), 1.27–1.23 (m, 6H), 1.23–1.17 (m, 6H), 1.15–1.10 (m, 3H), 1.09–0.92 (m, 9H); ESI-MS m/z calculated for $\text{C}_{46}\text{H}_{71}\text{N}_3\text{O}_{14}$ 889.5, found $[\text{M}+\text{H}]^+$ 890.9; HR-MS (ESI) m/z calculated for $\text{C}_{46}\text{H}_{71}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$: 890.0678, found 890.0816.

**4''-O-((phenethyl)carbamoyl)erythromycin A 6,9-imino ether
11,12-cyclic carbonate (5f)**

White solid, yield: 83.5%, m.p.: 120–123 °C, TLC R_F =0.52 (dichloromethane–methanol, 10:1); IR (KBr): 3342, 2974, 2936, 1810, 1727, 1665, 1498, 1455, 1381, 1337, 1245, 1169, 1111, 1073, 1016 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 7.28–7.26 (m, 3H), 7.21–7.18 (m, 2H), 5.31–5.30 (m, 1H), 4.88 (dd, $J=4.0$ Hz, $J=4.8$ Hz, 1H), 4.60–4.59 (m, 1H), 4.34–4.25 (m, 2H), 4.24–4.22 (m, 2H), 4.20–4.18 (m, 1H), 3.78–3.76 (m, 2H), 3.52–3.51 (m, 1H), 3.29–3.28 (m, 3H), 2.97–2.96 (m, 1H), 2.89–2.88 (m, 3H), 2.44–2.41 (m, 1H), 2.31–2.25 (m, 8H), 1.95–1.85 (m, 2H), 1.70–1.67 (m, 2H), 1.61–1.56 (m, 5H), 1.48–1.42 (m, 2H), 1.38–1.33 (m, 1H), 1.33–1.31 (m, 6H), 1.26–1.23 (m, 3H), 1.18–1.13 (m, 6H), 1.11–1.08 (m, 3H), 1.07–0.92 (m, 9H); ESI-MS m/z calculated for $\text{C}_{47}\text{H}_{73}\text{N}_3\text{O}_{14}$: 903.5, found $[\text{M}+\text{H}]^+$ 904.9; HR-MS (ESI) m/z calculated for $\text{C}_{47}\text{H}_{73}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$: 904.0943, found 904.1084.

**4''-O-((isopropyl)carbamoyl)erythromycin A 6,9-imino ether
11,12-cyclic carbonate (5g)**

White solid, yield: 72.7%, m.p.: 121–124 °C, TLC R_F =0.53 (dichloromethane–methanol, 10:1); IR (KBr): 3342, 3026, 2974, 2936, 1810, 1727, 1655, 1598, 1455, 1381, 1337, 1245, 1169, 1111, 1073, 1016 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 5.18–5.16 (m, 1H), 4.88 (dd, $J=9.0$ Hz, $J=3.0$ Hz, 1H), 4.50–4.48 (m, 1H), 4.30–4.26 (m, 3H), 4.18–4.16 (m, 1H), 3.80–3.72 (m, 2H), 3.47–3.41 (m, 4H), 2.88–2.83 (m, 1H), 2.81–2.77 (m, 1H), 2.67–2.54 (m, 2H), 2.52–2.48 (m, 6H), 2.18–2.10 (m, 1H), 1.94–1.90 (m, 2H), 1.79–1.74 (m, 2H), 1.66–1.56 (m, 3H), 1.54–1.50 (m, 2H), 1.47–1.45 (m, 2H), 1.44–1.42 (m, 1H), 1.29–1.27 (m, 6H), 1.25–1.17 (m, 9H), 1.20–1.16 (m, 3H), 1.16–1.09 (m, 3H), 1.07–1.04 (m, 3H), 1.04–0.94 (m, 6H), 0.93–0.92 (m, 3H); ESI-MS m/z calculated for $\text{C}_{42}\text{H}_{71}\text{N}_3\text{O}_{14}$ 841.5, found $[\text{M}+\text{H}]^+$ 843.0; HR-MS (ESI) m/z calculated for $\text{C}_{42}\text{H}_{71}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$: 842.0250, found 842.0532.

**4''-O-((butyl)carbamoyl)erythromycin A 6,9-imino ether
11,12-cyclic carbonate (5h)**

White solid, yield: 81.3%, m.p.: 115–118 °C, TLC R_F =0.53 (dichloromethane–methanol, 10:1); IR (KBr): 3304, 2968, 2934, 2874, 1807, 1727, 1656, 1522, 1459, 1383, 1246, 1168, 1109, 1055, 1014 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 5.15–5.13 (m, 1H), 4.95 (dd, $J=9.0$ Hz, $J=3.6$ Hz, 1H), 4.52–4.50 (m, 1H), 4.30–4.26 (m, 3H), 3.78–3.76 (m, 2H), 3.45–3.44 (m, 1H), 3.30–3.28 (m, 3H), 3.28–3.20 (m, 2H), 2.81–2.75 (m, 3H), 2.38–2.35 (m, 6H), 2.18–2.14 (m, 2H), 1.82–1.80 (m, 2H), 1.73–1.70 (m, 2H), 1.65–1.55 (m, 5H), 1.54–1.48 (m, 4H), 1.40–1.35 (m, 3H), 1.33–1.29 (m, 6H), 1.27–1.22 (m, 9H), 1.19–1.15 (m, 3H), 1.12–1.08 (m, 3H), 1.08–1.06 (m, 3H), 0.96–0.87 (m, 6H); ESI-MS m/z calculated for $\text{C}_{43}\text{H}_{73}\text{N}_3\text{O}_{14}$ 855.5, found $[\text{M}+\text{H}]^+$ 856.8; HR-MS (ESI) m/z calculated for $\text{C}_{43}\text{H}_{73}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$: 856.0515, found 856.0768.

**4''-O-((propenyl)carbamoyl)erythromycin A 6,9-imino ether
11,12-cyclic carbonate (5i)**

White solid, yield: 71.8%, m.p.: 112–115 °C, TLC R_F =0.52 (dichloromethane–methanol, 10:1); IR (KBr): 3319, 2975, 2936, 1805, 1724, 1659, 1522, 1457, 1384, 1245, 1167, 1109, 1075, 1046, 1015 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ 5.85 (s, 1H), 5.26–5.09 (m, 3H), 4.88–4.86 (m, 1H), 4.55–4.50 (m, 2H), 4.45 (d, $J=7.2$ Hz, 1H), 4.40–4.35 (m, 3H), 3.91–3.71 (m, 2H), 3.59–3.57 (m, 1H), 3.36 (s, 3H), 3.05–2.96 (m, 3H), 2.92–2.64 (m, 8H), 2.16–2.00 (m, 2H), 1.79–1.76 (m, 3H), 1.63–1.51 (m, 7H), 1.34–1.28 (m, 6H), 1.28–1.08 (m, 12H), 0.9–0.83 (m, 9H); ESI-MS m/z calculated for $\text{C}_{42}\text{H}_{69}\text{N}_3\text{O}_{14}$ 839.5, found $[\text{M}+\text{H}]^+$ 841.1; HR-MS (ESI) m/z calculated for $\text{C}_{42}\text{H}_{69}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$: 840.0091, found 840.1038.

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