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ORIGINAL ARTICLE

Synthesis and antibacterial activity of novel 11,12-cyclic carbonate azithromycin 4″-*O*-carbamate derivatives

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A series of novel 11,12-cyclic carbonate azithromycin 4″-*O*-carbamate derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activities. Compounds 7b and 7d were the most effective (0.5 and 0.5 μg ml^{-1}) against two strains of erythromycin-resistant *Streptococcus pneumoniae* whose resistance was encoded by the *erm* gene and the *erm* and *mef* genes, respectively. Compounds 7a, 7e and 7g showed significantly potent activity against erythromycin-susceptible strains such as *Staphylococcus aureus* and *S. pyogenes*. These results suggest that the introduction of the prolonged arylalkylcarbamoyl group to the C-4″ position can dramatically enhance the activity against erythromycin-resistant bacteria encoded by the *erm* gene or the *erm* and *mef* genes.
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INTRODUCTION

Macrolides belong to one of the most commonly used families of clinically important antibiotics in treating infections caused by Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *S. pyogenes*. First-generation macrolides such as erythromycin A (EMA) demonstrated a broad spectrum of antimicrobial activity and were used primarily for respiratory, skin and soft tissue infections. However, they readily lose their antibacterial activity under acidic conditions due to degradation. The degraded products are known to be responsible for undesirable gastrointestinal side effects.[1] The drug delivery problems resulting from acid instability prompted the design of newer macrolides. Increased acid stability and an increase in the range of antimicrobial activity characterize second-generation macrolides, such as clarithromycin (CAM) and azithromycin (AZM) (Figure 1), which have been widely prescribed for the infections of the upper and lower respiratory tract.[2] Unfortunately, the therapeutic utility of these macrolides has led to rapid increases in the resistance rates of bacteria isolated clinically.[3] Two of the most important mechanisms of macrolide resistance are mediated by *erm*-encoded methylation of 23S rRNA and *mef*-encoded efflux, respectively. Expression of an *erm*-resistant determinant in bacteria leads to the production of a methyltransferase, which modifies the key nucleotide, A2058, in the macrolide–lincosamide–streptogramin B (MLS_B) binding site, thereby conferring resistance to macrolide antibiotics.[4–6] The newest generation of macrolides, the ketolides (for example, telithromycin), are characterized by improved activity against some of the resistant strains and may offer alternative therapy for Gram-positive infections attributable to resistant pathogens.[7] Their mechanism of action has been reported to interact with nucleotide A752 directly in domain II of the 23S rRNA in addition to the main interaction of the drugs in domain V and inhibit protein synthesis by blocking elongation.[8] This results in tighter binding to ribosomes and imparts some activity against methylated ribosomes in some species.[9,10]

The study of the high-resolution X-ray co-crystal structures has revealed that macrolides bind at the entrance to the peptide tunnel in the 23S rRNA, and the cladinose group in their structures is located at and fits with the cavity formed by G2505, C2610 and C2611 in domain V.[11] On the basis of the results of the X-ray co-crystal structure study, many new derivatives of macrolides for the effective management of erythromycin resistance have been investigated by different research groups.[12] These investigations have led to the discovery of 4″-modified macrolide derivatives such as CP-544372[13] and A-60565[14] (Figure 1). CP-544372 contains a long anchor group at the C-4″ position of the cladinose sugar structure, the length of which is six atoms distant from 4″-oxygen atom to the aromatic ring. It demonstrates good *in vitro* and *in vivo* activity against macrolide-resistant organisms with competitive binding to chloramphenicol, suggesting that the anchor group can reach the peptidyl transferase center region, the chloramphenicol-binding site.[15] A-60565, which has both an 4″-*O*-arylalkylacyl group and a five-membered cyclic carbonate attached to the C-11,12 positions of the 14-membered mother ring, shows antibacterial activities against not only erythromycin-susceptible *S. pyogenes* EES61 and 2707 (0.12 and 0.03 μg ml^{-1}), but also erythromycin-resistant *S. pyogenes* 2548 (0.12 μg ml^{-1}).

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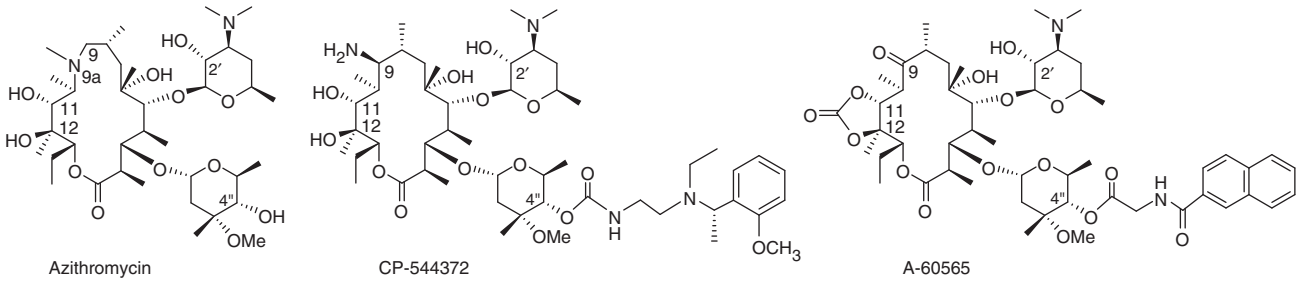
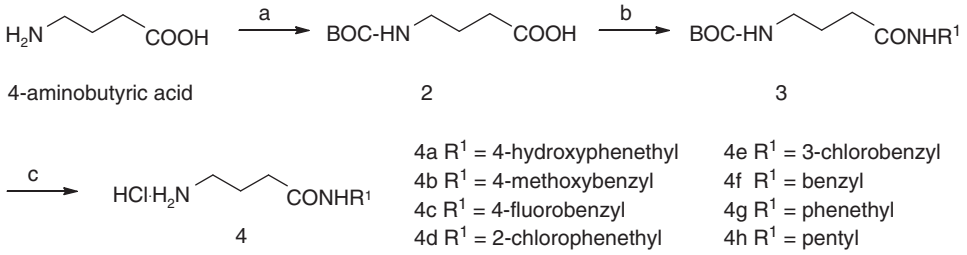


Figure 1 Structures of azithromycin, CP-544372 and A-60565.



Scheme 1 Reagents and conditions: (a) BOC_2O, 1 M NaOH, THF, room temperature, 24 h, 76.0%; (b) HOBt, DCC, THF, 0 °C, 8 h, R^1NH_2, room temperature, 2 h; (c) HCl, AcOEt, room temperature, 2 h, 78.1–85.4% for two steps.

These 4″-modified macrolide derivatives display a higher affinity for forming interactions with bacterial ribosomes. This increased affinity has been shown to be because of the additional interaction mediated by the 4″-*O*-arylalkyl side chain. In addition, the structure–activity relationships of 14-membered macrolide derivatives have been extensively explored. The numerous results have showed that the arylalkyl group attached to the C-4″ position of the cladinose sugar is essential for overcoming MLS_B resistance, whereas the 11,12-cylic carbonate group or the 11,12-cylic carbamate is important for overcoming efflux resistance. The structural modification of existing antibiotics, therefore, remains one of the most effective approaches for overcoming bacterial resistance.

Azithromycin is the first 15-membered erythromycin derivative formed by the addition of methyl-substituted nitrogen at the C-9a position. The addition of this second basic nitrogen atom to form an azalide structure appears not only to prevent degradation of the drug, but also to increase antibacterial activity against *Haemophilus influenzae*, other Gram-negative bacteria and atypical pathogens. This structural alteration also leads to excellent tissue penetration and a significantly prolonged serum half-life.[16] The AZM skeleton is very similar to that of CAM, except that the lactone ring is expanded around the C-9a position.[15] In anticipation of inheriting its beneficial antibacterial and excellent pharmacokinetic profiles, we have recently reported the synthesis of novel 11,12-cyclic carbonate-4″-*O*-carbamate derivatives of AZM, which showed improved activity against erythromycin-resistant *S. pneumoniae* (Supplementary Materials).[17,18] These results prompted us to further explore modifications at the C-4″ position of the cladinose of AZM.

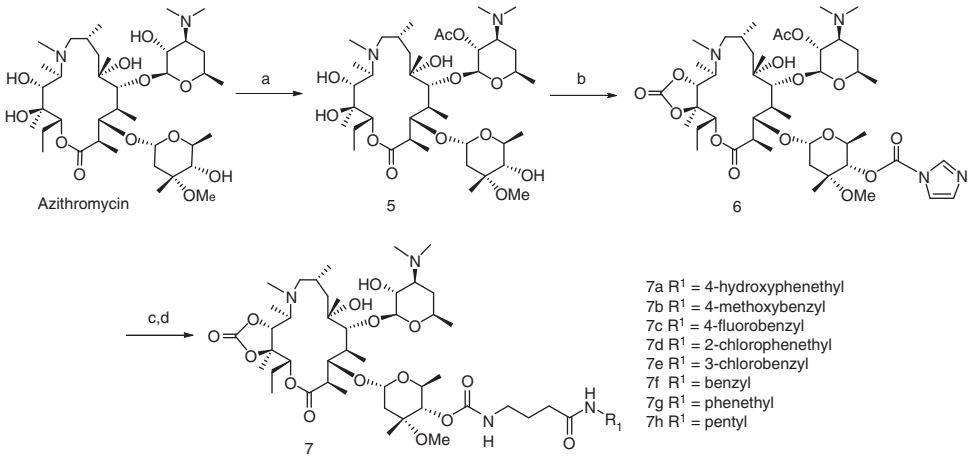
Schlünzen *et al.* reported that the binding sites of macrolides, clindamycin and chloramphenicol differed from each other, but they showed some overlapping nucleotides.[11] The overlapping of the macrolide binding structure with clindamycin and chloramphenicol is very informative for the development of new 4″-modified macrolide derivatives with the activity against erythromycin-resistant strains. Particularly, the high nucleotide content in the peptide tunnel gives rise to a number of possible interactions such as hydrogen bonding, π-stacking, as well as electrostatic interactions. Therefore, a long anchor group at the C-4″ position is helpful for the interaction with the above binding sites of the nucleotides in the peptide tunnel. In order to probe the effect of different lengths of 4″-*O*-carbamoyl groups in antibacterial activity, we designed a series of novel 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives with C-4″ prolonged side chains by condensation of various aryl-substituted primary amines. The aryl-substituted primary amines chosen here were obtained from 4-aminobutyric acid by coupling with corresponding amines. As hydrogen bonding and π-stacking may increase binding affinity, the substituted aromatic moieties in the structures of these amines have different bulk of molecular and different distribution of hetero atoms.

RESULTS

Chemistry

The synthetic method of the aryl-substituted primary amines (**4**) is shown in Scheme 1. Protection of the amino group of 4-aminobutyric acid with di-tert-butyl dicarbonate (BOC_2O) provided BOC-aminobutyric acid (**2**). Condensation of **2** with corresponding amine catalyzed by dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HOBt) afforded an amide product (**3**). Removal of the BOC moiety from **3** with saturated hydrochloric acid (HCl) in ethyl acetate gave the desired primary amines (**4a–h**).

Scheme 2 describes the synthesis of 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives (**7**) starting from AZM. Protection of the 2′-hydroxyl group of AZM with acetic anhydride in the presence of triethylamine (Et_3N) gave 2′-*O*-acetylazithromycin (**5**). Treatment of **5** with 1,1′-carbonyldiimidazole (CDI) in the presence of Et_3N in toluene at 75 °C afforded 11,12-carbonate 4″-*O*-acylimidazolide (**6**) as a common intermediate to introduce various functional groups at the C-4″ position. In contrast, treatment of **5** with CDI in toluene at room temperature provided a 4″-*O*-acylimidazolide product. Finally, 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives (**7a–h**) were obtained by coupling with the corresponding amines **4a–h** in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),



Scheme 2 Reagents and conditions: (a) acetic anhydride, CH_2Cl_2, Et_3N, room temperature, 12 h, 91.2%; (b) CDI, Et_3N, toluene, 75 °C, 24 h, 92.1%; (c) R^1NH_2, DMF, DBU, room temperature, 10 h; (d) CH_3OH, 55 °C, 24 h, 68.5–80.7% for two steps.

Table 1 ***In vitro*** **antibacterial activity of 11,12-cyclic carbonate azithromycin 4″-*O*-carbamate derivatives**

	MICs ($\mu g\,ml^{-1}$)										
Strain/compound	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**EMA**	**CAM**	AZM
S. aureus ATCC25923[a]	0.25	2	4	1	0.25	4	0.5	2	0.12	0.12	0.12
S. pyogenes[b]	1	1	2	1	0.5	2	0.5	2	0.12	0.12	0.12
S. pneumoniae ATCC49619[c]	4	1	1	1	8	2	4	1	0.03	0.03	0.03
S. pneumoniae B1[d]	2	0.5	8	0.5	2	8	4	8	128	64	128
S. pneumoniae A22072[e]	4	4	32	4	8	32	4	16	8	4	4
S. pneumoniae AB11[f]	4	0.5	4	0.5	4	2	8	4	256	128	256

[a]*S. aureus* ATCC25923: erythromycin-susceptible strain.
[b]*S. pyogenes*: erythromycin-susceptible strain.
[c]*S. pneumoniae* ATCC49619: erythromycin-susceptible strain.
[d]*S. pneumoniae* B1: erythromycin-resistant strain encoded by the *erm* gene.
[e]*S. pneumoniae* A22072: erythromycin-resistant strain encoded by the *mef* gene.
[f]*S. pneumoniae* AB11: erythromycin-resistant strain encoded by the *erm* and *mef* genes.

followed by selective deprotection of the 2′-*O*-acetyl group by heating with methanol.

Antibacterial activity

The 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives, as well as EMA, CAM and AZM as references, were tested for *in vitro* antibacterial activity against six phenotypes of Gram-positive strains. The activities are reported in Table 1 as MICs determined using the broth microdilution method. *S. aureus* ATCC25923, *S. pyogenes* and *S. pneumoniae* ATCC49619 are three erythromycin-susceptible strains. *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11 are three erythromycin-resistant strains whose resistance were encoded by the *erm* gene, the *mef* gene, and the *erm* and *mef* genes, respectively.

The values of MIC for the 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives are presented in Table 1. All of the tested compounds showed greatly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene or the *erm* and *mef* genes in comparison with the references. Among them, compounds **7b** and 7**d** possessed the most potent activity ($0.5\,\mu g\,ml^{-1}$) against erythromycin-resistant *S. pneumoniae* B1 encoded by the *erm* gene, showing 256- and 256-fold better activity than AZM or EMA, respectively. Similarly, compounds **7b** and 7**d** were the most effective ($0.5\,\mu g\,ml^{-1}$) against erythromycin-resistant *S. pneumoniae* AB11 encoded by the *erm* and *mef* genes, showing 512- and 512-fold higher activity than AZM or EMA, respectively. In contrast, all of the tested compounds did not show improved activity against erythromycin-resistant *S. pneumoniae* A22072 encoded by the *mef* gene compared with AZM or CAM. These results described above clearly indicated that the introduction of the prolonged arylalkylcarbamoyl group to the C-4″ position and a cyclic carbonate into the 11,12-position of 15-membered azalides remarkably enhance the activity against erythromycin-resistant bacteria encoded by the *erm* gene or the *erm* and *mef* gene. However, only a few of the tested compounds showed antibacterial activity against erythromycin-susceptible strains. Among them, compounds **7a**, **7e** and **7g** possessed potent activity against *S. aureus*, and compounds **7e** and **7g** had potent activity against *S. pyogenes*, but they showed lower antibacterial activity than EMA, CAM or AZM. These results led us to presume that the chemical modification that affects the conformation might affect their ability to bind to bacterial ribosomes, resulting in the variation of antibacterial activity.

DISCUSSION

As part of our continuous synthetic efforts focused on the preparation of 4″-substituted azalide derivatives, we investigated the synthesis of novel 4″-*O*-carbamate derivatives of AZM with the C-4″ short arylalkyl side chain in previous work.[17,18] In the published paper,[18] we reported that all of the tested compounds showed significantly potent activity ($0.03–0.12\,\mu g\,ml^{-1}$) against erythromycin-susceptible bacteria, and some of them showed potent activity ($0.06–0.25\,\mu g\,ml^{-1}$) against erythromycin-resistant *S. pneumoniae* A22072 encoded by the *mef* gene, compared with AZM or CAM. Particularly, the compounds with 4-fluorobenzyl, phenethyl or 3,4-methylenedioxyphenethyl groups at the C-4″ position had the most potent activity against erythromycin-resistant *S. pneumoniae* A22072 encoded by the *mef* gene ($0.06\,\mu g\,ml^{-1}$). In the case of the most effective compounds, the length of arylalkyl side chain was three or four atoms distant from oxygen atom at the C-4″ position to the aromatic ring.

Modeling studies suggest that six to eight amino acids can potentially fit between the peptidyl transferase active site and the macrolide roadblock.[11] Accordingly, we hoped that a prolonged anchor group at the C-4″ position might increase the affinity for the new binding sites of the nucleotides in the peptide tunnel, further enhancing antibacterial activity against resistant strains. On the basis of the result

of the high-resolution X-ray co-crystal structure study,[11,19] which showed the overlapping of the macrolide binding structure with clindamycin and chloramphenicol, we designed a series of novel 15-membered AZM derivatives with the C-4″ prolonged side chains, the length of which was eight or nine atoms distant from oxygen atom at the C-4″ position to the aromatic ring. These compounds with prolonged side chains showed remarkably improved activity against strains encoded by the *erm* gene or the *erm* and *mef* genes, compared with the corresponding compounds with the C-4″ short arylalkyl side chain reported by previously by us.[18] Compound **7b** and **7d**, especially, showed significant potent activity against erythromycin-resistant strains encoded by the *erm* gene and the *erm* and *mef* genes, respectively. In contrast, almost all of the compounds with the C-4″ prolonged side chains were less active against erythromycin-susceptible strains than the corresponding compounds with the C-4″ short arylalkyl side chain in the published paper.[18] These results described above suggest that the prolonged side chain may further enhance the activity against erythromycin-resistant strains encoded by the *erm* gene or the *erm* and *mef* genes, but lose their activity against erythromycin-susceptible strains.

In conclusion, novel 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activities. All of the tested compounds showed greatly improved activity against two strains of erythromycin-resistant *S. pneumoniae* whose resistance was encoded by the *erm* gene and the *erm* and *mef* genes. Among them, compounds **7b** and 7**d** were the most effective against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene and the *erm* and *mef* genes, respectively. In contrast, only compounds **7a**, **7e** and **7g** showed potent activity against erythromycin-susceptible strains such as *S. aureus* and *S. pyogenes*. These results suggest that the introduction of the prolonged arylalkylcarbamoyl group to the C-4″ position can dramatically enhance the activity against erythromycin-resistant bacteria encoded by the *erm* gene or the *erm* and *mef* genes. It is worthy of notice that 4″-modified derivatives of 14-membered macrolides are not the only class of new macrolides for the effective management of macrolide resistance, and the 4″-modified derivatives of 15-membered azalides have an important function in the development of new macrolide derivatives overcoming MLS_B resistance as well.

METHODS

General experimental procedures

All necessary solvents were purified before use, unless noted otherwise. Reactions were monitored by TLC using 0.25-mm precoated 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). IR spectra were recorded on KBr pellets using a Nicolet Nexus 470FT-IR spectrometer (Nicolet Nexus, Madison, WI, USA). ^{1}H NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer at ambient temperature (TMS as internal standard of chemical shifts) (Bruker, Fällanden, Switzerland). Mass spectra were recorded on an API 4000 instrument (Applied Biosystems, Middletown, CT, USA). The C, H, N analyses were carried out on a PE-2400 elemental analyzer (Perkin-Elmer, Waltham, MA, USA). Melting points are uncorrected and were determined on an X-6 melting point apparatus (Beijing Tianchengwode Biotech Co. Ltd, Beijing, China). AZM was used as starting material from Nexchem Pharmaceutical (Zhejiang, China).

BOC-aminobutyric acid (2)

To a solution of 4-aminobutyric acid (2.0 g, 15.27 mmol) in 1 M NaOH (15 ml) was added dropwise BOC_2O (3.7 g, 16.97 mmol) in THF (5 ml). The resulting solution was allowed to stir for 24 h at room temperature. After being concentrated *in vacuo*, the reaction solution was adjusted to pH 2–3 with 1 M citric acid and extracted with ethyl acetate (3×15 ml). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give 2.98 g (76.0%) of **2** as white solid: R_F=0.32 (dichloromethane–methanol, 10:1).

General methods for 4-aminobutyryl arylalkylamines hydrochloride (4a–h)

To a 0 °C solution of **2** (1.5 g, 7.35 mmol) and HOBt (1.13 g, 8.09 mmol) in THF (10 ml) was added dropwise DCC (1.67 g, 8.09 mmol) in THF (5 ml). The resulting solution was stirred at 0 °C for 8 h. After the addition of corresponding amine (7.5 mmol), the reaction mixture was stirred for 2 h at the same temperature and filtered. The filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in ethyl acetate (20 ml) and the resulting solution was washed with saturated $NaHCO_3$ solution, 1 M citric acid and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give crude product **3**.

Crude product **3** (6.0 mmol) was dissolved in saturated HCl in ethyl acetate (10 ml) and the mixture was stirred for 12 h at room temperature. The precipitate was collected by filtration and washed with cold ethyl acetate to give the desired product. The yields were within the range of 78.1–85.4%.

2′-*O*-Acetyl-4″-*O*-acylimidazolyl AZM 11,12-cyclic carbonate (6)

Compound **6** was prepared from intermediate **5**, according to the procedures reported by Ma *et al*.[17] The crude **6** was purified by flash chromatography (dichloromethane–methanol, 20:1) to give **6** (92.1%) as a white solid: m.p. 117–120 °C; TLC R_F=0.62 (dichloromethane–methanol, 10:1); ESI-MS *m/z* calculated for $C_{45}H_{74}N_4O_{15}$ 911.1; found $(M+H)^+$ 912.4.

General methods for 11,12-cyclic carbonate AZM 4″-*O*-carbamate derivatives (7a–h)

To a solution of **6** (1.33 g, 1.50 mmol) in DMF (15 ml) was added DBU (0.33 ml, 2.25 mmol) and corresponding amine (2.25 mmol). The resulting solution was stirred for 10 h at the room temperature. The reaction was quenched with water (30 ml) and the aqueous layer was extracted with ethyl acetate (3×15 ml). The combined organic layers were washed with brine (3×15 ml), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo* to give 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, 20:1) to give compounds **7a–h** in yields ranging from 68.5 to 80.7%.

4″-*O*-(((4-Hydroxyphenethyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7a)

White solid, yield 68.5%, m.p.: 146–150°C; TLC R_F=0.518 (dichlormethane–methanol, 10:1); IR (KBr): 3410, 2972, 2936, 1812, 1727, 1652, 1615, 1594, 1515, 1456, 1379, 1238, 1169, 1109, 1074, 1046, 1015 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.03 (d, *J*=8.4 Hz, 2H), 6.79 (d, *J*=8.4 Hz, 2H), 5.06 (d, *J*=4.8 Hz, 1H), 4.95 (m, 1H), 4.50 (m, 1H), 4.45 (m, 1H), 4.40–4.35 (m, 2H), 3.66–3.62 (m, 2H), 3.49–3.47 (m, 1H), 3.37–3.30 (m, 4H), 3.23–3.18 (m, 3H), 3.17–3.10 (m, 2H), 2.87–2.84 (m, 3H), 2.74 (t, 2H), 2.44 (m, 1H), 2.39–2.37 (m, 9H), 2.18 (m, 4H), 2.11 (t, 2H), 1.91 (m, 2H), 1.87–1.81 (m, 2H), 1.74–1.69 (m, 2H), 1.67–1.57 (m, 3H), 1.44 (m, 4H), 1.30 (m, 4H), 1.25–1.20 (m, 6H), 1.17–1.15 (m, 6H), 0.95–0.87 (m, 9H); ESI-MS *m/z* calculated for $C_{52}H_{86}N_4O_{16}$ 1022.6; found $(M+H)^+$ 1024.0; analysis calculated for $C_{52}H_{86}N_4O_{16}$: C 61.04, H 8.47, N 5.48. Found: C 61.17, H 8.50, N 5.46.

4″-*O*-(((4-Methoxybenzyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7b)

White solid, yield 74.5%, m.p.: 126–130 °C, TLC R_F=0.574 (dichlormethane–methanol, 10:1); IR (KBr): 3426, 2971, 2936, 2835, 1812, 1724, 1655, 1613, 1513, 1457, 1379, 1353, 1301, 1247, 1170, 1110, 1074, 1045, 1015 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (d, *J*=9.0 Hz, 2H), 6.87–6.81 (m, 2H), 5.10–5.07 (m, 1H), 4.88 (dd, *J*=9.0 Hz, *J*=3.6 Hz, 1H), 4.54–4.52 (d, *J*=9.6 Hz, 1H), 4.44–4.35 (m, 7H), 3.80–3.78 (m, 3H), 3.68–3.63 (m, 1H), 3.60 (m, 1H),

3.33–3.31 (m, 3H), 3.28–3.20 (m, 4H), 2.96–2.86 (m, 3H), 2.43–2.41 (m, 2H), 2.37–2.33 (m, 6H), 2.28–2.22 (m, 3H), 2.20 (m, 3H), 1.99 (m, 1H), 1.92 (m, 1H), 1.91–1.80 (m, 4H), 1.75 (m, 1H), 1.65–1.61 (m, 2H), 1.60–1.58 (m, 2H), 1.55–1.53 (m, 2H), 1.47–1.44 (s, 4H), 1.28–1.25 (m, 5H), 1.24–1.17 (m, 9H), 1.17–1.11 (m, 4H), 1.07–1.01 (m, 6H), 0.98–0.87 (m, 6H); ESI-MS *m/z* calculated for $C_{52}H_{86}N_4O_{16}$ 1022.6; found $(M+H)^+$ 1024.3; analysis calculated for $C_{52}H_{86}N_4O_{16}$: C 61.04, H 8.47, N 5.48. Found: C 60.97, H 8.44, N 5.49.

4″-*O*-(((4-Fluorobenzyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7c)

White solid, yield 72.0%, m.p.: 126–130 °C, TLC R_F=0.56 (dichlormethane–methanol, 10:1); IR (KBr): 3431, 2970, 2932, 1813, 1734, 1647, 1510, 1457, 1379, 1353, 1298, 1222, 1167, 1110, 1074, 1046, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.27–7.25 (m, 2H), 7.03–7.00 (m, 2H), 5.08 (m, 1H), 4.89 (dd, *J*=9.0 Hz, *J*=3.0 Hz, 1H), 4.54–4.52 (m, 1H), 4.47–4.44 (m, 1H), 4.41–4.35 (m, 2H), 4.31–4.28 (m, 1H), 4.16–4.13 (m, 1H), 3.80 (m, 1H), 3.66–3.59 (m, 2H), 3.37–3.29 (m, 3H), 3.27–3.13 (m, 3H), 2.89–2.85 (m, 3H), 2.66 (m, 1H), 2.44–2.42 (m, 1H), 2.40–2.35 (m, 6H), 2.33–2.29 (m, 3H), 2.27–2.24 (m, 1H), 2.22–2.20 (m, 1H), 2.07–2.00 (m, 3H), 1.92 (m, 2H), 1.89–1.80 (m, 2H), 1.68–1.53 (m, 4H), 1.46–1.44 (m, 4H), 1.33–1.29 (m, 4H), 1.27–1.23 (m, 3H), 1.24–1.19 (m, 7H), 1.15 (m, 3H), 1.11–1.05 (m, 6H), 0.98–0.84 (m, 6H); ESI-MS *m/z* calculated for $C_{51}H_{83}FN_4O_{15}$ 1010.6; found $(M+H)^+$ 1011.9; analysis calculated for $C_{51}H_{83}FN_4O_{15}$: C 60.57, H 8.27, N 5.54. Found: C 60.44, H 8.30, N 5.50.

4″-*O*-(((2-Chlorophenethyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7d)

White solid, yield: 77.5%, m.p.: 145–148 °C, TLC R_F=0.53 (dichlormethane–methanol, 10:1); IR (KBr): 3428, 2972, 2936, 1812, 1723, 1656, 1517, 1456, 1379, 1353, 1335, 1298, 1237, 1167, 1109, 1074, 1046, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.36 (m, 1H), 7.24–7.16 (m, 3H), 5.11–5.08 (m, 2H), 4.88 (dd, *J*=9.0 Hz, *J*=3.0 Hz, 1H), 4.54–4.50 (m, 1H), 4.45–4.35 (m, 3H), 3.66–3.59 (m, 1H), 3.56–3.53 (m, 2H), 3.31 (m, 3H), 3.29–3.26 (m, 2H), 3.23–3.19 (m, 3H), 2.98–2.96 (t, 3H), 2.89–2.84 (m, 3H), 2.64 (m, 2H), 2.43–2.41 (m, 1H,), 2.38–2.31 (m, 6H), 2.26–2.20 (m, 3H), 2.18–2.16 (m, 2H), 2.07–2.00 (m, 2H), 1.91 (m, 1H), 1.86–1.76 (m, 2H), 1.65–1.59 (m, 2H), 1.45 (m, 4H), 1.31–1.29 (m, 4H), 1.29–1.25 (m, 2H), 1.22–1.19 (m, 7H), 1.15 (m, 3H), 1.07 (m, 6H), 0.93–0.87 (m, 6H); ESI-MS *m/z* calculated for $C_{52}H_{85}ClN_4O_{15}$ 1040.6; found $(M+H)^+$ 1041.9; analysis calculated for $C_{52}H_{85}ClN_4O_{15}$: C 59.96, H 8.22, N 5.38. Found: C 60.06, H 8.20, N 5.40.

4″-*O*-(((3-Chlorobenzyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7e)

White solid, yield 76.5%, m.p.: 145–148 °C, TLC R_F=0.52 (dichlormethane–methanol, 10:1); IR (KBr): 3427, 2972, 2935, 1812, 1723, 1656, 1599, 1575, 1521, 1459, 1380, 1353, 1238, 1167, 1109, 1076, 1045, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.31 (m, 1H), 7.27–7.22 (m, 2H), 7.20–7.18 (m, 1H), 5.04 (d, *J*=4.2 Hz, 1H), 4.88 (dd, *J*=9.0 Hz, *J*=3.0 Hz, 1H), 4.58–4.53 (m, 2H), 4.47–4.39 (m, 3H), 4.30 (m, 2H), 4.07 (t, 1H), 3.60–3.59 (d, *J*=6.0 Hz, 1H), 3.45–3.42 (m, 2H) 3.30–3.28 (m, 3H), 3.28–3.18 (m, 2H), 2.88–2.78 (m, 3H), 2.69 (m, 6H), 2.46–2.44 (m, 1H), 2.37–2.35 (m, 2H), 2.31–2.28 (m, 2H), 2.23–2.21 (m, 3H), 2.06–2.02 (m, 1H), 1.93–1.81 (m, 4H), 1.65–1.60 (m, 3H), 1.51 (m, 1H), 1.45 (m, 3H), 1.42–1.34 (m, 3H), 1.28 (m, 3H), 1.25–1.19 (m, 8H), 1.17 (m, 3H), 1.11–1.07 (m, 3H), 1.02–0.87 (m, 9H); ESI-MS *m/z* calculated for $C_{51}H_{83}ClN_4O_{15}$ 1026.6; found $(M+H)^+$ 1028.0; analysis calculated for $C_{51}H_{83}ClN_4O_{15}$: C 59.60, H 8.14, N 5.45. Found: C 59.50, H 8.18, N 5.41.

4″-*O*-(((Benzyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7f)

White solid, yield 73.5%, m.p.: 120–122 °C, TLC R_F=0.53 (dichlormethane–methanol, 10:1); IR (KBr): 3423, 2972, 1813, 1725, 1659, 1514, 1455, 1379, 1353, 1299, 1238, 1167, 1110, 1074, 1046, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.34–7.27 (m, 5H), 5.07 (d, *J*=4.8 Hz, 1H), 4.88 (dd, *J*=4.0 Hz, *J*=4.8 Hz, 1H), 4.53 (d, *J*=9.6 Hz, 1H), 4.45–4.34 (m, 4H), 3.61–3.59 (m, 3H), 3.32 (m, 3H), 3.29–3.16 (m, 3H), 2.88 (m, 3H), 2.44 (m, 2H), 2.44–2.33 (m, 8H), 2.20 (m, 4H), 2.18 (m, 1H), 2.02 (m, 2H), 1.98 (m, 2H), 1.92–1.77 (m, 4H), 1.59–1.56 (m, 1H), 1.45 (m, 4H), 1.30–1.29 (m, 4H), 1.22–1.19 (m, 9H), 1.16–1.14 (m, 3H), 1.07–1.05 (m, 6H), 0.93–0.89 (m, 6H); ESI-MS *m/z* calculated for $C_{51}H_{84}N_4O_{15}$ 992.6; found $(M+H)^+$ 994.0; analysis calculated for $C_{51}H_{84}N_4O_{15}$: C 61.67, H 8.52, N 5.64. Found: C 61.58, H 8.57, N 5.69.

4″-*O*-(((Phenethyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7g)

White solid, yield 76.5%, m.p.: 158–164 °C, TLC R_F=0.55 (dichlormethane–methanol, 10:1); IR (KBr): 3411, 3063, 2971, 2933, 1812, 1721, 1653, 1524, 1456, 1379, 1353, 1239, 1167, 1109, 1044, 1014 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.31–7.28 (m, 2H), 7.27–7.20 (m, 3H), 5.03 (d, *J*=4.2 Hz, 1H), 4.88 (dd, *J*=9.0 Hz, *J* =3 .0 Hz, 1H), 4.58–4.52 (m, 1H), 4.40–4.28 (m, 3H), 3.76 (m, 2H), 3.65–3.53 (m, 4H), 3.35–3.30 (m, 3H), 3.22–3.19 (m, 3H), 2.85–2.83 (m, 3H), 2.78 (m, 5H), 2.46–2.42 (m, 1H), 2.38–2.31 (m, 3H), 2.22–2.02 (m, 9H), 1.92 (m, 3H), 1.87–1.73 (m, 3H), 1.70–1.56 (m, 3H), 1.45 (m, 4H), 1.41–1.35 (m, 3H), 1.28 (m, 3H), 1.23 (m, 4H), 1.20–1.18 (m, 3H), 1.16 (m, 3H), 1.01–0.96 (m, 3H), 0.96–0.86 (m, 6H); ESI-MS *m/z* calculated for $C_{52}H_{86}N_4O_{15}$ 1006.6; found $(M+H)^+$ 1007.9; analysis calculated for $C_{52}H_{86}N_4O_{15}$: C 62.01, H 8.61, N 5.56. Found: C 61.96, H 8.57, N 5.59.

4″-*O*-(((Pentyl)amino)-4-oxobutyl)carbamoylazithromycin 11,12-cyclic carbonate (7h)

White solid, yield 80.7%, m.p. 132–136 °C, TLC R_F=0.54 (dichlormethane–methanol, 10:1); IR (KBr): 3419, 2934, 2872, 1814, 1724, 1653, 1518, 1457, 1379, 1238, 1167, 1110, 1074, 1046, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 5.08 (d, *J*=4.2 Hz, 1H), 4.88 (dd, *J*=9.0 Hz, *J*=3.6 Hz, 1H), 4.55 (m, 1H), 4.45 (d, *J*=7.2 Hz, 1H), 4.40–4.35 (m, 2H), 3.66–3.64 (m, 1H), 3.60–3.59 (m, 1H), 3.34–3.29 (m, 4H), 3.29–3.20 (m, 5H), 2.87–2.86 (m, 3H), 2.43–2.41 (m, 1H), 2.38–2.35 (m, 6H), 2.41–2.35 (m, 6H), 2.26 (m, 1H), 2.22–2.18 (m, 6H), 1.92 (m, 1H), 1.87–1.79 (m, 4H), 1.66–1.55 (m, 4H), 1.53–1.46 (m, 4H), 1.48–1.41 (m, 2H), 1.36–1.27 (m, 5H), 1.22–1.20 (m, 9H), 1.16 (m, 3H), 1.07 (m, 3H), 0.93–0.86 (m, 9H); ESI-MS *m/z* calculated for $C_{49}H_{88}N_4O_{15}$ 972.6; found $(M+H)^+$ 974.0; analysis calculated for $C_{49}H_{88}N_4O_{15}$: C 60.47, H 9.11, N 5.76. Found: C 60.57, H 9.08, N 5.71.

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