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3-*O*-Acyl Derivatives of Bridged-15-Membered Azalides: Synthesis, Structural Determination and Antibacterial Activity

Andrea Fajdetić,* Gabrijela Kobrehel, Gorjana Lazarevski, Vlado Štimac, and Stjepan Mutak

PLIVA – Research Institute, Ltd., Prilaz baruna Filipovića 25, 10000 Zagreb, Croatia

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The synthesis, structural determination and biological evaluation of 15-membered azalides acylated at the C-3 position are described. 3-Descladinosyl-9a,11-cyclic carbamate of the 9a-aza-9a-homoerythromycin A and their 12-*O*-alkyl derivatives were synthesized *via* acidic hydrolysis of adequate 3-cladinosyl analogues. Protections of 2'-hydroxyl group were performed to furnish starting compounds for acylation of the C-3-hydroxyl group. After deprotection various 3-*O*-acyl derivatives were obtained and their structures confirmed by spectroscopic methods (IR, MS, NMR). The new compounds were evaluated *in vitro* against a panel of Gram-positive and Gram-negative bacteria and their activities compared with those of parent derivatives. The 3-*O*-acyl derivatives exhibited improved antibacterial activity, but it was lower than by standard macrolides.

INTRODUCTION

Macrolides are a well-known class of antibacterial agents that have been used in the treatment of bacterial infections for many years.[1] They are of natural origin, consisting of 12-, 14- and 16-membered lacton ring systems linked by glycoside bonds with an amino-sugar and neutral sugars. Macrolide antibiotics are lipophilic molecules that inhibit the biosynthesis of proteins binding to the bacterial ribosome.[2]

Since the discovery of erythromycin in 1952, many semisynthetic macrolides have been prepared to improve antibacterial profiles, acid stability and oral bioavailability. From the clinical point of view, the most successful among them are clarithromycin[3–6] and azithromycin.[7,8] Azithromycin, a 15-membered macrolide, is characterized by its enhanced antibacterial profile and improved pharmacokinetic properties compared to the 14-membered macrolides, erythromycin and clarithromycin (Figure 1).[9] Unfortunately, like erythromycin, both clarithromycin and azithromycin have poor efficacy against some resistant bacteria.

Cladinose sugar at position 3 was for many years considered to be an essential component for antibacterial activity. However, some newly discovered derivatives without L-cladinose have overcome the known mechanisms of resistance. The most successful analogues are ketolides, macrolides that have a keto functionality at position 3.[10–13] Some other 3-descladinosyl derivatives (3-*O*-acyl,[14,15] 2,3-anhydro[16,17] and 3-deoxy derivatives[18]) have also shown very high activity against macrolide-re-

* Author to whom correspondence should be addressed. (E-mail: Andrea.Fajdetic@pliva.hr)

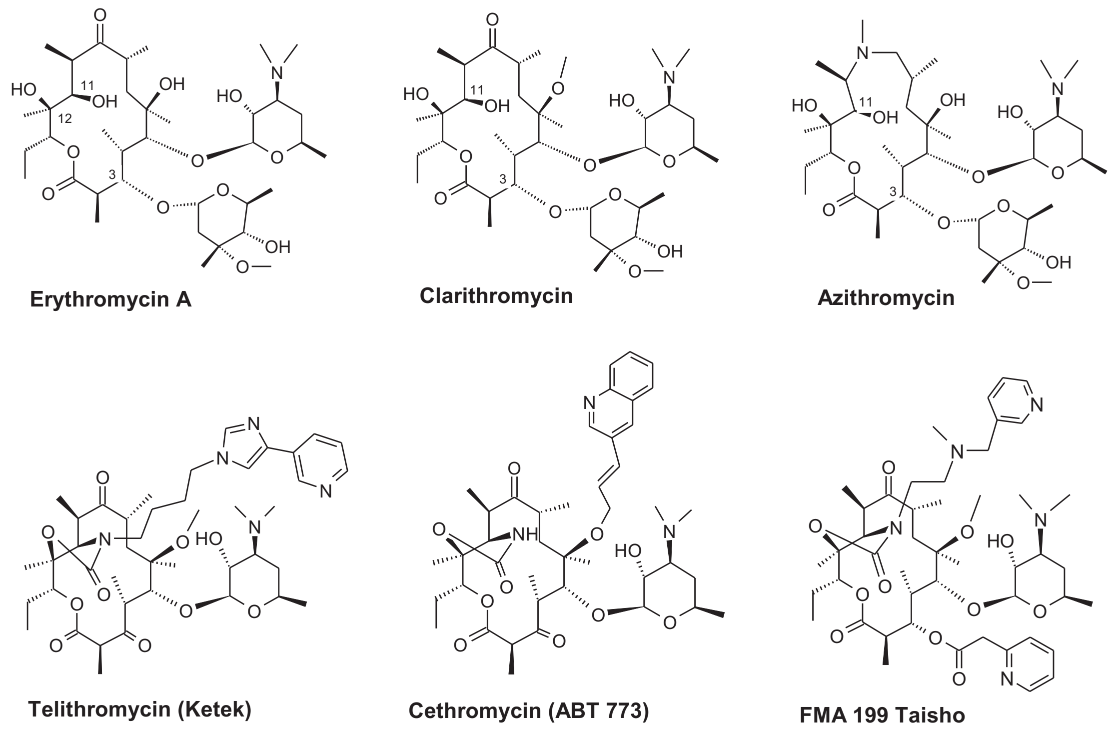


Figure 1. Chemical structures of 11,12-cyclic carbamates of 14-membered macrolides.

sistant strains. Also, introduction of a cyclic carbamate group to the 11,12-position of the 14-membered ring has been successfully used to enhance the molecule's ability to bind to the ribosome (Figure 1).[19,20]

EXPERIMENTAL

IR spectra were recorded in KBr pastilles on a Nicolet Magna-IR 760 FT-IR spectrometer. Mass spectra were obtained on a Varian-Mat 311A for FAB-MS or Platform LCZ and LCQ Deca for ESI-MS. ^{1}H and ^{13}C-NMR spectra were measured with a Varian Unity Inova 600, Bruker Advance DRX 500 and Bruker Advance DPX 300 spectrometers in $CDCl_3$ using trimethylsilan as internal standard.

Synthesis of 3-Descladinosyl Derivatives

3-Descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate 4

To a solution of 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **1** (5.18 g) in 96 % ethanol (150 ml), 0.25 M hydrochloric acid (50 ml) was added and the reaction mixture was stirred for 48 hours at room temperature. Ethanol was evaporated, and $CHCl_3$ (150 ml) was added. The pH-value of the mixture was about 1.2. The layers were separated and the water phase was extracted two more times with $CHCl_3$. The pH value of water layer was adjusted to pH 9.5 and then extracted three times with $CHCl_3$. Combined organic extracts at pH 9.5 were rinsed with brine, dried over K_2CO_3 and evaporated, yielding the product **4** (3.96 g, 93 %).

Under the same conditions, starting from 12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **2** and 12-*O*-ethyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **3**, the corresponding 3-descladinosyl-12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **5** and 3-descladinosyl-12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **6** were prepared. Results and physicochemical data of 3-decladonosyl derivatives are given in Table I.

*Synthesis of 2'-*O*-Acetyl-3-Descladinosyl Derivatives*

*2'-*O*-Acetyl-3-descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate 7*

To a solution of 3-descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **4** (3.0 g, 4.98 mmol) in CH_2Cl_2 (100 ml), $NaHCO_3$ (1.09 g, 13.0 mmol) and acetic acid anhydride (0.62 ml, 6.57 mmol) were added. The mixture was stirred for 4 hours at room temperature. Saturated $NaHCO_3$ solution was added into the reaction mixture, the layers were separated and the aqueous phase was extracted two more times with CH_2Cl_2. Combined organic extracts were rinsed with saturated $NaHCO_3$ solution and water and evaporated, yielding the product **7** (2.93 g, 91 %)

Applying the same conditions, starting from 3-descladinosyl-12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **5** and 3-descladinosyl-12-*O*-ethyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **6**, the corresponding 2'-*O*-acetyl-3-descladinosyl-12-*O*-methyl-9-deoxo-9-dihydro-

TABLE I. Results and physical-chemical data of 3-descladinosyl derivatives **4–6**

Comp. No.	R_1	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}	^1H NMR (500 MHz, $CDCl_3$) δ/ppm	^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm
4	H	93	$C_{30}H_{54}N_2O_{10}$ (602.77)	603.7	3442, 2973, 2937, 2879, 2789, 1743, 1638, 1459, 1417, 1380, 1166, 1113, 1078, 1049, 1001, 947, 915, 897, 770, 670	5.14 (H-13), 4.45 (H-1'), 4.26 (H-11), 3.78 (H-3), 3.60 (H-5'), 3.56 (H-5), 3.53 (H-9a), 3.49 (H-10), 3.26 (H-2'), 2.58 (H-2), 2.51 (H-3'), 2.35 (H-8), 2.34 (H-9b), 2.26 /3'N$(CH_3)_2$/, 2.15 (H-4), 1.91 (H-14a), 1.68 (H-4'a), 1.52 (H-14b), 1.50 (H-7a), 1.33 (6-CH_3), 1.31 (10-CH_3), 1.30 (2-CH_3), 1.26 (5'-CH_3), 1.25 (H-4'b), 1.22 (12-CH_3), 1.16 (H-7b), 1.01 (4-CH_3), 1.01 (8-CH_3), 0.88 (14-CH_3)	174.9 (C-1), 156.4 (9a,11 C=O), 106.0 (C-1'), 93.6 (C-5), 78.2 (C-11), 77.8 (C-3), 75.6 (C-13), 73.7 (C-12), 71.5 (C-6), 70.9 (C-9), 70.2 (C-2'), 69.7 (C-5'), 65.1 (C-3'), 58.4 (C-10), 49.5 (C-9), 44.3 (C-2), 39.9 /3'N$(CH_3)_2$/, 36.6 (C-7), 36.5 (C-4), 27.9 (C-4'), 25.4 (6-CH_3), 25.3 (C-8), 20.9 (5'-CH_3), 20.2 (8-CH_3), 20.2 (C-14), 15.7 (2-CH_3), 15.0 (12-CH_3), 13.8 (10-CH_3), 10.1 (14-CH_3), 7.5 (4-CH_3)
5	CH_3	87	$C_{31}H_{56}N_2O_{10}$ (616.80)	617.3	3451, 2972, 2938, 2879, 2787, 1744, 1638, 1458, 1414, 1381, 1163, 1113, 1078, 1050, 1002, 949, 896, 835, 781, 670	5.59 (H-13), 4.46 (H-1'), 4.26 (H-11), 3.59 (H-5), 3.59 (H-5'), 3.58 (H-9a), 3.55 (H-10), 3.49 (12-*O*-CH_3), 3.27 (H-2'), 2.58 (H-2), 2.53 (H-3'), 2.38 (H-8), 2.36 (H-9b), 2.27 /3'N$(CH_3)_2$/, 2.09 (H-4), 1.75 (H-14a), 1.69 (H-4'a), 1.59 (H-14b), 1.47 (H-7a), 1.33 (6-CH_3), 1.32 (10-CH_3), 1.32 (2-CH_3), 1.30 (H-4'b), 1.25 (5'-CH_3), 1.22 (H-7b), 1.16 (12-CH_3), 1.03 (8-CH_3), 1.01 (4-CH_3), 0.93 (14-CH_3)	174.5 (C-1), 156.6 (9a,11 C=O), 106.1 (C-1'), 93.6 (C-5), 79.3 (C-11), 77.6 (C-3), 73.0 (C-13), 75.3 (C-12), 73.8 (C-6), 70.1 (C-2'), 69.9 (C-5'), 65.2 (C-3'), 58.1 (C-10), 53.2 (12-*O*-CH_3), 49.6 (C-9), 44.4 (C-2), 40.0 /3'N$(CH_3)_2$/, 36.9 (C-7), 36.9 (C-4), 27.9 (C-4'), 25.2 (6-CH_3), 25.6 (C-8), 20.9 (5'-CH_3), 20.5 (8-CH_3), 20.6 (C-14), 15.8 (2-CH_3), 16.1 (12-CH_3), 13.6 (10-CH_3), 10.1 (14-CH_3), 7.6 (4-CH_3)
6	CH_2CH_3	89	$C_{32}H_{58}N_2O_{10}$ (630.83)	631.8	3449, 2973, 2936, 2785, 1745, 1638, 1459, 1414, 1381, 1320, 1251, 1218, 1163, 1113, 1051, 1003, 948, 895, 836, 768, 689	5.59 (H-13), 4.47 (H-1'), 4.25 (H-11), 3.94 (12-*O*-CH_2a/Et), 3.77 (H-3), 3.58 (H-5'), 3.57 (H-5), 3.56 (12-*O*-CH_2b/Et), 3.52 (H-9a), 3.50 (H-10), 3.05 (H-2'), 2.57 (H-2), 2.51 (H-3'), 2.47 (H-8), 2.36 (H-9b), 2.24 /3'N$(CH_3)_2$/, 2.08 (H-4), 1.68 (H-14a), 1.67 (H-4'a), 1.55 (H-14b), 1.29 (10-CH_3), 1.25 (6-CH_3), 1.21 (4'-Hb), 1.19 (2-CH_3), 1.17 (5'-CH_3), 1.14 (7-Ha), 1.09 (12-CH_3), 1.09 (12-O-CH_3/Et), 1.00 (4-CH_3), 1.00 (8-CH_3), 0.90 (14-CH_3)	174.6 (C-1), 156.9 (9a,11C=O), 106.7 (C-1'), 94.3 (C-5), 79.7 (C-11), 78.1 (C-3), 76.1 (C-12), 75.7 (C-13), 73.6 (C-6), 70.5 (C-2'), 70.4 (C-5'), 65.8 (C-3'), 60.8 (12-*O*-CH_2/Et), 58.6 (C-10), 50.1 (C-9), 44.8 (C-2), 40.5 /3'N$(CH_3)_2$/, 37.4 (C-7), 37.1 (C-4), 28.3 (C-4'), 26.1 (C-8), 25.8 (6-CH_3), 21.1 (8-CH_3), 21.1 (14-C), 20.9 (5'-CH_3), 17.2 (2-CH_3), 16.3 (12-*O*-CH_3/Et), 15.8 (12-CH_3), 14.1 (10-CH_3), 10.6 (14-CH_3), 8.0 (4-CH_3)

TABLE II. Results and physical-chemical data of 2'-O-acetyl-3-descladinosyl derivatives **7**–**9**

Comp. No.	R_1	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}
7	H	91	$C_{32}H_{56}N_2O_{11}$ (644.81)	645.7	3485, 2973, 2879, 2786, 1747, 1579, 1461, 1417, 1377, 1249, 1168, 1113, 1049, 1006, 947, 899, 810, 770
8	CH_3	85	$C_{33}H_{58}N_2O_{11}$ (658.84)	659.4	3442, 2973, 2938, 2879, 2786, 1744, 1460, 1417, 1381, 1250, 1166, 1113, 1050, 1004, 949
9	CH_2CH_3	98	$C_{34}H_{60}N_2O_{11}$ (672.86)	673.7	3483, 2973, 2878, 2789, 1747, 1578, 1459, 1413, 1380, 1249, 1166, 1113, 1050, 1006, 947, 897, 770

9a-aza-9a-homoerithromycin A 9a,11-cyclic carbamate **8** and 2'-*O*-acetyl-3-descladinosyl-12-*O*-ethyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **9** were prepared respectively. Results and physicochemical data are given in Table II.

*Synthesis of 3-*O*-(2-Aryl-acetyl)-3-descladinosyl Derivatives*

*3-Descladinosyl-3-*O*-acetyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate* ***10a***

To a solution of 2'-*O*-acetyl-3-descladinosyl-9-deoxo-9--dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate (**7**) (0.215 g, 0.321 mmol) in pyridine (6.0 ml, 79.0 mmol), acetic acid anhydride (3.0 ml, 31.6 mmol) was added and the reaction mixture was stirred at 60 °C for 10 hours. The reaction mixture was poured into ice water (50 ml), CH_2Cl_2 (50 ml) was added and the layers were separated. The water layer was extracted two more times with CH_2Cl_2. Combined organic extracts were rinsed with saturated aqueous solution of $NaHCO_3$, brine, dried over K_2CO_3 and evaporated. The product obtained was dissolved in MeOH (50 ml) and the solution was stirred for 24 hours at room temperature. The solvent was evaporated and the crude product was purified by crystallization from CH_2Cl_2 – diethyl ether – n-hexane, yielding product **10a** (0.12 g, 59 %)

*3-Descladinosyl-3-*O*-(4-nitrophenyl)acetyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate* ***10b***

To a solution of 4-nitrophenylacetic acid (0.644 g, 3.55 mmol) in dry CH_2Cl_2 (15 ml) TEA (0.504 ml, 3.55 mmol) was added and the reaction mixture was cooled to 0 °C. Pivaloyl chloride (0.469 ml, 3.55 mmol) was added and the reaction mixture was stirred at the same temperature for 30 minutes. Pyridine (0.966 ml, 11.94 mmol) and 2'-*O*-acetyl--3-descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **7** (0.70 g, 1.08 mmol) solution in dry CH_2Cl_2 (5 ml) were added and the reaction mixture was stirred at 0 °C for 4 hours. Saturated aqueous solution of $NaHCO_3$ (30 ml) was added and the layers were separated. The water layer was extracted two more times with CH_2Cl_2. Combined organic extracts were rinsed with brine, dried over K_2CO_3 and evaporated, yielding 0.70 g of an oily product. The product obtained was dissolved in MeOH (50 ml) and the solution was stirred for 24 hours at room temperature. The solvent was evaporated and the crude product was purified by chromatography on a silica gel column using the system CH_2Cl_2 – MeOH – NH_4OH (90 : 3 : 0.5). Combining and evaporating chromatographically homogenous fractions gave the title product, which was crystallized from CH_2Cl_2 – diethyl ether – n-hexane, yielding product **10b** (0.30 g, 36 %):

Under the same reaction conditions, starting with compound **7**, applying 4-fluorophenylacetic acid, 4-methoxyphenylacetic acid, phenylpropionic acid and 4-pyridyltioacetic acid, the corresponding 3-descladinosyl--3-*O*-(4-fluorophenyl)acetyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10c**, 3-descladinosyl-3-*O*-(4-methoxyphenyl)acetyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10d**, 3-descladinosyl-3-*O*-phenylpropionyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10e** and 3-descladinosyl-3-*O*-(pyridylthio)acetyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a, 11-cyclic carbamate **10f,** were prepared, respectively. Similarly, starting with compound **8** or **9,** applying 4-nitrophenylacetic acid, the corresponding 3-descladinosyl-3-*O*-(4-nitrophenyl)acetyl-12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10g** and 3-descladinosyl-3-*O*-(4-nitrophenyl)acetyl-12-*O*-ethyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10h** were prepared. Results and physicochemical data are given in Table III.

*3-Descladinosyl-3-*O*-(4-aminophenyl)acetyl-9-deoxo--9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate* ***10i***

To a solution of 3-descladinosyl-3-*O*-(4-nitrophenyl)acyl-9--deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11--cyclic carbamate **10b** (0.20 g, 0.26 mmol) in conc. acetic acid (25 ml), $PtO_2 \cdot H_2O$ (0.12 g, 0.52 mmol) was added and the reaction mixture was stirred for 2 hours at room temperature under H_2 pressure of about 2.25×10^4 torr. The catalyst was filtered, washed and the liquor was evaporated. The residue was dissolved in CH_2Cl_2 (30 ml), water (30 ml) was added and the pH value of the mixture was adjust-

TABLE III. Results and physical-chemical data of 3-O-(2-aryl-acetyl)-3-descladinosyl derivatives **10a–10h**

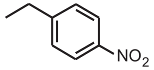
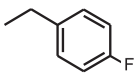
Comp. No.	R_1	R_2	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}	^1H NMR (500 MHz, $CDCl_3$) δ/ppm	^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm
10a	H	CH_3	59	$C_{32}H_{56}N_2O_{11}$ (644.81)	645.6	3478, 2973, 2933, 1739, 1464, 1416, 1380, 1316, 1244, 1169, 1114, 1077, 1041, 1003, 968, 946, 904, 832, 774, 674	5.24 (H-3), 5.16 (H-13), 4.34 (H-11), 4.09 (H-1'), 3.84 (H-1''a), 3.80 (H-1''b), 3.52 (H-10), 3.50 (H-5), 3.48 (H-9a), 3.40 (H-5'), 3.26 (H-2'), 2.81 (H-2), 2.61 (H-3'), 2.51 (H-4), 2.39 /3'$N(CH_3)_2$/, 2.32 (H-9b), 2.30 (H-8), 2.15 (1''-CH_3), 1.93 (H-14a), 1.75 (H-4'a), 1.52 (H-14b), 1.32 (H-7a and 7b), 1.31 (10-CH_3), 1.27 (6-CH_3), 1.27 (H-4'b), 1.22 (5'-CH_3), 1.21 (12-CH_3), 1.12 (4-CH_3), 1.09 (8-CH_3), 0.96 (2-CH_3), 0,87 (14-CH_3)	172.6 (C-1), 170.4 (1''-C=O), 156.1 (9a,11-C=O), 102.5 (C-1'), 84.5 (C-5), 78.7 (C-3), 78.1 (C-11), 75.9 (C-13), 74.4 (C-6), 71.6 (C-12), 70.3 (C-2'), 69.3 (C-5'), 65.9 (C-3'), 58.6 (C-10), 49.7 (C-9), 42.6 (C-2), 40.3 /3'$N(CH_3)_2$/, 35.9 (C-4), 35.8 (C-7), 28.9 (C-4'), 26.4 (6-CH_3), 25.1 (C-8), 21.3 (5'-CH_3), 20.9 (8-CH_3), 20.5 (1''-CH_3), 20.3 (C-14), 15.8 (2-CH_3), 14.9 (12-CH_3), 13.9 (10-CH_3), 10.3 (14-CH_3), 8.6 (4-CH_3)
10b	H		36	$C_{38}H_{59}N_3O_{13}$ (765.91)	766.3	3459, 2974, 2939, 1747, 1606, 1524, 1456, 1415, 1380, 1347, 1251, 1216, 1164, 1112, 1076, 1045, 1000, 966, 947, 904, 856, 768, 731, 673	8.20 (H-4'', H-6''), 7.55 (H-3'', H-7''), 5.28 (H-3), 5.13 (H-13), 4.25 (H-11), 4.04 (H-1'), 3.86 (H-1''a), 3.81 (H-1''b), 3.50 (H-5), 3.48 (H-10), 3.46 (H-9a), 3.27 (H-5'), 3.23 (H-2'), 2.76 (H-2), 2.45 (H-4), 2.38 (H-3'), 2.34 (H-9b), 2.34 (H-8), 2.30 /3'$N(CH_3)_2$/, 1.74 (H-14a), 1.62 (H-4'a), 1.53 (H-14b), 1.37 (H-7a), 1.34 (H-7b), 1.30 (10-CH_3), 1.28 (6-CH_3), 1.24 (H-4'b), 1.20 (12-CH_3), 1.18 (5'-CH_3), 1.10 (4-CH_3), 0.97 (8-CH_3), 0.90 (2-CH_3), 0.88 (14-CH_3)	172.3 (C-1), 169.7 (1''-C=O), 156.2 (9a,11-C=O), 147.2 (C-5''), 141.1 (C-2''), 130.4 (C-4'', C-6''), 123.7 (C-3'', C-7''), 103.7 (C-1'), 84.0 (C-5), 80.2 (C-3), 78.2 (C-11), 76.1 (C-13), 74.6 (C-6), 71.7 (C-12), 70.7 (C-2'), 68.6 (C-5'), 66.0 (C-3'), 58.8 (C-10), 49.8 (C-9), 42.7 (C-2), 41.1 (C-1''), 40.3 /3'$N(CH_3)_2$/, 36.2 (C-7), 36.1 (C-4), 28.3 (C-4'), 26.4 (6-CH_3), 25.1 (C-8), 21.0 (5'-CH_3), 20.5 (8-CH_3), 20.4 (C-14), 15.7 (2-CH_3), 15.0 (12-CH_3), 14.0 (10-CH_3), 10.3 (14-CH_3), 8.7 (4-CH_3)
10c	H		46	$C_{38}H_{59}FN_2O_{11}$ (738.90)	739.3	3445, 2973, 2938, 1747, 1609, 1511, 1457, 1416, 1380, 1251, 1223, 1164, 1077, 1045, 1001, 967, 945, 905, 834, 768, 690, 673	7.35 (H-4'', H-6''), 7.03 (H-3'', H-7''), 5.27 (H-3), 5.14 (H-13), 4.27 (H-11), 4.03 (H-1'), 3.72 (H-1''a), 3.66 (H-1''b), 3.52 (H-5), 3.50 (H-10), 3.45 (H-9a), 3.23 (H-2'), 3.21 (H-5'), 2.76 (H-2), 2.50 (H-4), 2.40 (H-3'), 2.34 (H-9b), 2.33 (H-8), 2.32 /3'$N(CH_3)_2$/, 1.90 (H-14a), 1.62 (H-4'a), 1.50 (H-14b), 1.37 (H-7a and 7b), 1.29 (10-CH_3), 1.29 (6-CH_3), 1.24 (H-4'b), 1.22 (12-CH_3), 1.18 (5'-CH_3), 1.11 (4-CH_3), 0.98 (8-CH_3), 0.89 (2-CH_3), 0,85 (14-CH_3)	172.3 (C-1), 169.9 (1''-C=O), 156.5 (9a,11-C=O), 149.5 (C-5''), 140.2 (C-2''), 130.7 (C-4'', C-6''), 128.1 (C-3'', C-7''), 103.6 (C-1'), 84.9 (C-5), 80.0 (C-3), 78.6 (C-11), 76.3 (C-13), 75.9 (C-6), 71.7 (C-12), 70.3 (C-2'), 69.7 (C-5'), 66.2 (C-3'), 58.7 (C-10), 49.7 (C-9), 43.0 (C-2), 41.8 (C-1''), 40.3 /3'$N(CH_3)_2$/, 36.3 (C-4), 36.0 (C-7), 28.8 (C-4'), 26.5 (6-CH_3), 24.9 (C-8), 20.9 (5'-CH_3), 20.8 (C-14), 20.8 (8-CH_3), 15.6 (2-CH_3), 15.8 (12-CH_3), 13.9 (10-CH_3), 10.1 (14-CH_3), 8.8 (4-CH_3)

TABLE III. continued

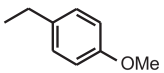
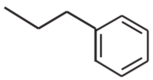
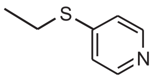
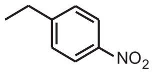
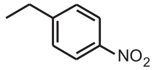
Comp. No.	R_1	R_2	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}	^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm	^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm
10d	H		69	$C_{39}H_{62}N_2O_{12}$ (750.94)	751.4	3459, 2973, 2938, 1747, 1614, 1514, 1456, 1416, 1380, 1300, 1250, 1216, 1164, 1077, 1040, 969, 904, 821, 769, 674	7.27 (H-4'', H-6''), 6.87 (H-3'', H-7''), 5.24 (H-3), 5.14 (H-13), 4.26 (H-11), 4.04 (H-1'), 3.80 (5''-OMe), 3.66 (H-1''a), 3.60 (H-1''b), 3.50 (H-5), 3.44 (H-10), 3.43 (H-9a), 3.21 (H-2'), 3.18 (H-5'), 2.76 (H-2), 2.48 (H-4), 2.41 (H-3'), 2.35 (H-9b), 2.32 (H-8), 2.30 /3'N(CH$_3$)$_2$/, 1.90 (H-14a), 1.60 (H-4'a), 1.49 (H-14b), 1.33 (H-7a and b), 1.29 (10-CH$_3$), 1.24 (6-CH$_3$), 1.24 (H-4'b), 1.21 (12-CH$_3$), 1.17 (5′-CH$_3$), 1.10 (4-CH$_3$), 0.96 (8-CH$_3$), 0.89 (2-CH$_3$), 0,84 (14-CH$_3$)	172.6 (C-1), 171.3 (1''-C=O), 158.8 (C-5''), 156.8 (9a,11-C=O), 130.3 (C-4'', C-6''), 125.6 (C-2''), 113.9 (C-3'', C-7''), 103.0 (C-1'), 84.9 (C-5), 79.2 (C-3), 78.2 (C-11), 76.3 (C-13), 74.5 (C-6), 71.7 (C-12), 70.5 (C-2'), 69.4 (C-5'), 65.8 (C-3'), 58.8 (C-10), 55.2 (5''-OMe), 49.8 (C-9), 42.7 (C-2), 40.9 (C-1''), 40.3 /3'N(CH$_3$)$_2$/, 36.1 (C-4), 36.0 (C-7), 28.4 (C-4'), 26.5 (6-CH$_3$), 25.1 (C-8), 21.1 (5'-CH$_3$), 20.6 (C-14), 20.4 (8-CH$_3$), 15.6 (2-CH$_3$), 15.0 (12-CH$_3$), 14.0 (10-CH$_3$), 10.3 (14-CH$_3$), 8.7 (4-CH$_3$)
10e	H		44	$C_{39}H_{62}N_2O_{11}$ (734.94)	735.6	3444, 2927, 2927, 1743, 1640, 1456, 1417, 1380, 1260, 1215, 1165, 1107, 1079, 1047, 1002, 965, 938, 810, 769, 702, 675	7.19-7.13 (H-Ar), 5.24 (H-3), 5.14 (H-13), 4.24 (H-11), 4.02 (H-1'), 3.50 (H-10), 3.45 (H-5), 3.39 (H-9a), 3.23 (H-2'), 3.23 (H-5'), 2.99 (H-2''a and 2''b), 2.77 (H-1''a and b), 2.76 (H-2), 2.53 (H-3'), 2.50 (H-4), 2.45 /3'N(CH$_3$)$_2$/, 2.30 (H-9b), 2.30 (H-8), 1.93 (H-14a), 1.66 (H-4'a), 1.50 (H-14b), 1.34 (H-7a and b), 1.29 (10-CH$_3$), 1.28 (H-4'b), 1.25 (6-CH$_3$), 1.21 (12-CH$_3$), 1.21 (5′-CH$_3$), 1.06 (4-CH$_3$), 0.97 (8-CH$_3$), 0.96 (2-CH$_3$), 0,86 (14-CH$_3$)	172.6 (C-1), 172.3 (1''-C=O), 156.1 (9a,11-C=O), 140.2 (C-3''), 128.5 (C-4'', C-8''), 128.2 (C-5'', C-7''), 126.3 (C-6''), 102.8 (C-1'), 84.4 (C-5), 78.9 (C-3), 78.1 (C-11), 75.9 (C-13), 74.4 (C-6), 71.6 (C-12), 70.1 (C-5'), 69.0 (C-2'), 66.0 (C-3'), 58.7 (C-10), 49.7 (C-9), 42.6 (C-2), 40.3 /3'N(CH$_3$)$_2$/, 36.1 (C-1''), 36.1 (C-7), 35.9 (C-4), 30.6 (C-2''), 29.1 (C-4'), 26.4 (6-CH$_3$), 25.0 (C-8), 20.9 (5'-CH$_3$), 20.5 (8-CH$_3$), 20.3 (C-14), 15.8 (2-CH$_3$), 14.9 (12-CH$_3$), 13.9 (10-CH$_3$), 10.3 (14-CH$_3$), 8.7 (4-CH$_3$)
10f	H		39	$C_{37}H_{59}N_3O_{11}S$ (753.96)	754.6	3434, 2972, 2934, 1743, 1649, 1574, 1460, 1411, 1375, 1240, 1167, 1062, 999, 946, 806, 769, 708	8.47 (H-4'', H-6''), 7.29 (H-3'', H-7''), 5.30 (H-3), 5.15 (H-13), 4.24 (H-11), 4.12 (H-1'), 3.98 (H-1''a), 3.95 (H-1''b), 3.50 (H-10), 3.49 (H-5), 3.44 (H-9a), 3.44 (H-2'), 3.34 (H-5'), 2.96 (H-2), 2.52 (H-3'), 2.50 (H-4), 2.33 (H-9b), 2.33 (H-8), 2.32 /3'N(CH$_3$)$_2$/, 1.93 (H-14a), 1.78 (H-4'a), 1.53 (H-14b), 1.37 (H-7a), 1.33 (H-7b), 1.31 (10-CH$_3$), 1.30 (6-CH$_3$), 1.24 (H-4'b), 1.23 (5′-CH$_3$), 1.22 (12-CH$_3$), 1.11 (4-CH$_3$), 1.09 (8-CH$_3$), 0.98 (2-CH$_3$), 0,85 (14-CH$_3$)	172.5 (C-1), 168.8 (1''-C=O), 156.4 (9a, 11-C=O), 149.5 (C-4'', C-6''), 147.3 (C-2''), 120.9 (C-3'', C-7''), 103.6 (C-1'), 87.0 (C-5), 81.1 (C-3), 78.4 (C-11), 76.3 (C-13), 74.6 (C-6), 71.7 (C-12), 70.1 (C-2'), 69.1 (C-5'), 66.4 (C-3'), 59.0 (C-10), 49.9 (C-9), 42.8 (C-2), 40.3 /3'N(CH$_3$)$_2$/, 36.3 (C-4), 36.1 (C-7), 33.7 (C-1''), 29.5 (C-4'), 26.4 (6-CH$_3$), 25.1 (C-8), 21.0 (5'-CH$_3$), 20.6 (8-CH$_3$), 20.4 (C-14), 15.8 (2-CH$_3$), 15.2 (12-CH$_3$), 14.1 (10-CH$_3$), 10.4 (14-CH$_3$), 8.8 (4-CH$_3$)

TABLE III. continued

Comp. No.	R_1	R_2	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}	^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm	^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm
10g	CH_3		54	$C_{39}H_{61}N_3O_{13}$ (779,93)	780.3	3460, 2974, 1747, 1606, 1523, 1457, 1414, 1347, 1251, 1163, 1077, 1049, 1002, 949, 855, 782, 731, 674	8.21 (H-4'', H-6''), 7.55 (H-3'', H-7''), 5.59 (H-13), 5.30 (H-3), 4.25 (H-11), 4.04 (H-1'), 3.86 (H-1''a), 3.81 (H-1''b), 3.50 (H-5), 3.48 (H-10), 3.48 (12-*O*-Me), 3.46 (H-9a), 3.27 (H-5'), 3.23 (H-2'), 2.76 (H-2), 2.45 (H-4), 2.38 (H-3'), 2.34 (H-9b), 2.34 (H-8), 2.30 /3'N$(CH_3)_2$/, 1.74 (H-14a), 1.62 (H-4'a), 1.53 (H-14b), 1.37 (H-7a and 7b), 1.30 (10-CH_3), 1.28 (6-CH_3), 1.24 (H-4'b), 1.20 (12-CH_3), 1.18 (5'-CH_3), 1.10 (4-CH_3), 0.97 (8-CH_3), 0.90 (2-CH_3), 0.88 (14-CH_3)	172.0 (C-1), 169.8 (1''-C=O), 156.5 (9a,11-C=O), 147.3 (C-5''), 141.2 (C-2''), 130.6 (C-4'', C-6''), 123.7 (C-3'', C-7''), 103.8 (C-1'), 85.6 (C-5), 80.0 (C-3), 79.4 (C-11), 76.5 (C-13), 74.6 (C-6), 73.6 (C-12), 70.4 (C-2'), 69.4 (C-5'), 66.2 (C-3'), 58.7 (C-10), 53.4 (12-*O*-Me), 49.9 (C-9), 42.8 (C-2), 41.4 (C-1''), 40.4 /3'N$(CH_3)_2$/, 36.3 (C-4), 36.1 (C-7), 28.7 (C-4'), 26.5 (6-CH_3), 24.9 (C-8), 21.0 (5'-CH_3), 20.8 (8-CH_3), 20.8 (C-14), 16.3 (2-CH_3), 15.8 (12-CH_3), 13.8 (10-CH_3), 10.3 (14-CH_3), 8.9 (4-CH_3)
10h	CH_2CH_3		43	$C_{40}H_{63}N_3O_{13}$ (793.96)	794.7	3459, 2974, 2936, 1747, 1606, 1523, 1456, 1414, 1380, 1347, 1250, 1218, 1163, 1111, 1077, 1048, 1002, 949, 855, 767, 731, 687	8.18 (H-4'', H-6''), 7.55 (H-3'', H-7''), 5.56 (H-13), 5.26 (H-3), 4.21 (H-11), 4.05 (H-1'), 3.94 (12-*O*-CH_2a and b/Et), 3.84 (H-1''a), 3.80 (H-1''b), 3.54 (H-5), 3.47 (H-10), 3.42 (H-9a), 3.27 (H-5'), 3.23 (H-2'), 2.73 (H-2), 2.54 (H-3'), 2.45 (H-4), 2.38 (H-8), 2.38 /3'N$(CH_3)_2$/, 2.30 (H-9b), 1.70 (H-14a), 1.66 (H-4'a), 1.54 (H-14b), 1.31 (H-7a and 7b), 1.29 (10-CH_3), 1.24 (6-CH_3), 1.24 (H-4'b), 1.16 (12-CH_3), 1.13 (5'-CH_3), 1.13 (4-CH_3), 1.07 (12-*O*-CH_3/Et), 0.95 (8-CH_3), 0.87 (2-CH_3), 0.84 (14-CH_3)	172.0 (C-1), 169.8 (1''-C=O), 156.6 (9a,11-C=O), 147.3 (C-5''), 141.2 (C-2''), 130.5 (C-4'', C-6''), 123.8 (C-3'', C-7''), 103.6 (C-1'), 85.8 (C-5), 80.0 (C-3), 79.43 (C-11), 75.3 (C-13), 74.6 (C-6), 73.8 (C-12), 70.3 (C-2'), 69.3 (C-5'), 67.7 (C-3'), 60.5 (12-*O*-CH_2/Et), 58.2 (C-10), 49.8 (C-9), 42.8 (C-2), 41.4 (C-1''), 40.3 /3'N$(CH_3)_2$/, 36.2 (C-7), 36.0 (C-4), 28.8 (C-4'), 26.9 (6-CH_3), 24.8 (C-8), 21.1 (C-14), 21.0 (5'-CH_3), 20.7 (8-CH_3), 16.7 (12-*O*—CH_3/Et), 16.0 (2-CH_3), 15.8 (12-CH_3), 13.9 (10-CH_3), 10.3 (14-CH_3), 8.8 (4-CH_3)

ed to pH 9.5. The layers were separated and the organic layer was extracted twice with a saturated aqueous solution of the $NaHCO_3$. Organic layer was dried over K_2CO_3 and evaporated, yielding a crude product, which was purified by chromatography on a silica gel column using the system CH_2Cl_2 – MeOH – NH_4OH (90 : 3 : 0.5). Combining and evaporating chromatographically homogenous fractions gave the title product, which was crystallized from CH_2Cl_2 – diethyl ether – n-hexane, yielding product **10i** (0.13 g, 68 %).

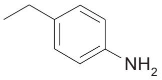
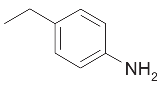
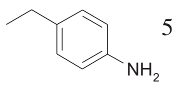
Applying the same reduction procedure to nitro compounds **10g** or **10h**, the corresponding 3-descladinosyl-3-*O*-(4-aminophenyl)acetyl-12-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10j** and 3-descladinosyl-3-*O*-(4-aminophenyl)acetyl-12-*O*-ethyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate **10k** were prepared. Results and physicochemical data are given in Table IV.

RESULTS AND DISCUSSION

Keeping the structures of new biologically active 14-membered macrolides with 11,12-carbamate groups, we assumed that the combination of 3-*O*-acyl functionality, the 15-membered scaffold and a 9a,11-cyclic carbamate group would provide molecules with high antibacterial activity and the desired pharmacokinetic profile. As mentioned in the Introduction, those are the structural functionalities responsible for the high efficacy against resistant bacteria and improved pharmacokinetic properties.

In this paper we describe the synthesis, structural determination and antibacterial activity of novel 3-*O*-acyl derivatives of 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A 9a,11-cyclic carbamate (Figure 2).

TABLE IV. Results and physical-chemical data of 3-(2-aminoaryl-acetyl)-3-descladinosyl derivatives **10i**–**10k**

Comp. No.	R_1	R_2	Yield %	Molecular formula (M_r)	FAB-MS m/z $(M+H)^+$	IR (KBr) ν_{max}/cm^{-1}	^1H NMR (500 MHz, $CDCl_3$) δ/ppm
10i	H		68	$C_{39}H_{62}N_2O_{11}$ (734.94)	736.3	3445, 2973, 2936, 1747, 1633, 1519, 1456, 1415, 1380, 1252, 11654, 1077, 1044, 1001, 967, 946, 903, 834, 768, 734, 690, 673	7.14 (H-4", H-6"), 6.65 (H-3", H-7"), 5.22 (H-3), 5.14 (H-13), 4.26 (H-11), 4.01 (H-1'), 3.58 (H-5), 3.58 (H-1"a), 3.50 (H-1"b), 3.50 (H-10), 3.43 (H-9a), 3.24 (H-2'), 3.14 (H-5'), 2.77 (H-2), 2.56 (H-3'), 2.47 (H-4), 2.39 /3'N(CH_3)$_2$/, 2.37 (H-9b), 2.32 (H-8), 1.91 (H-14a), 1.64 (H-4'a), 1.49 (H-14b), 1.33 (H-7a and 7b), 1.29 (10-CH_3), 1.27 (6-CH_3), 1.23 (H-4'b), 1.20 (12-CH_3), 1.16 (5'-CH_3), 1.10 (4-CH_3), 0.97 (8-CH_3), 0.91 (2-CH_3), 0.84 (14-CH_3)
10j	CH_3		60	$C_{40}H_{64}N_2O_{11}$ (748.96)	750.7	3439, 2973, 2931, 1744, 1631, 1518, 1463, 1416, 1382, 1252, 1165, 1079, 1054, 1001, 944, 901, 799, 690, 674	7.18 (H-4", H-6"), 6.72 (H-3", H-7"), 5.57 (H-13), 5.22 (H-3), 4.24 (H-11), 4.02 (H-1'), 3.58 (H-1"a), 3.56 (H-5), 3.48 (H-10), 3.48 (12-*O*-Me), 3.44 (H-1"b), 3.44 (H-9a), 3.24 (H-2'), 3.14 (H-5'), 2.73 (H-2), 2.58 (H-3'), 2.45 (H-4), 2.42 (H-9b), 2.40 /3'N(CH_3)$_2$/, 2.34 (H-8), 1.75 (H-14a), 1.64 (H-4'a), 1.52 (H-14b), 1.33 (H-7a and 7b), 1.30 (10-CH_3), 1.27 (6-CH_3), 1.24 (H-4'b), 1.22 (12-CH_3), 1.17 (5'-CH_3), 1.13 (4-CH_3), 1.05 (8-CH_3), 0.96 (2-CH_3), 0.92 (14-CH_3)
10k	CH_2CH_3		58	$C_{41}H_{67}N_2O_{11}$ (764.00)	764.7	3425, 2973, 2933, 1742, 1638, 1518, 1466, 1417, 1382, 1253, 1219, 1166, 1103, 1080, 1052, 1003, 968, 948, 903, 803, 799, 690, 673	7.19 (H-4", H-6"), 6.74 (H-3", H-7"), 5.58 (H-13), 5.23 (H-3), 4.23 (H-11), 4.03 (H-1'), 3.98 (12-*O*-CH_2a and CH_2b/Et), 3.72 (H-1"a), 3.66 (H-1"b), 3.55 (H-5), 3.50 (H-10), 3.42 (H-9a), 3.24 (H-2'), 3.17 (H-5'), 2.75 (H-2), 2.58 (H-3'), 2.47 (H-4), 2.40 /3'N(CH_3)$_2$/, 2.36 (H-8), 2.33 (H-9b), 1.75 (H-14a), 1.65 (H-4'a), 1.53 (H-14b), 1.34 (H-7a and b), 1.29 (10-CH_3), 1.26 (6-CH_3), 1.24 (H-4'b), 1.20 (12-CH_3), 1.18 (5'-CH_3), 1.13 (4-CH_3), 1.11 (12-*O*-CH_3/Et), 1.05 (8-CH_3), 0.95 (2-CH_3), 0.90 (14-CH_3)

Chemistry

Starting from 3-*O*-cladinosyl derivatives **1**, **2** and **3** that had earlier been synthesized in PLIVA,[21,22] 3-*O*-descladinosyl derivatives **4**, **5** and **6** were prepared by acidic hydrolysis as shown in Scheme 1. For acylation reactions at position 3, it was necessary to protect the hydroxyl group at position C-2'. Treatment of **4**, **5** and **6** with acetic anhydride and $NaHCO_3$ in dichloromethane furnished adequate, selectively protected compounds **7**, **8** and **9** (Scheme 1).

Compound **10a** was obtained using acetic anhydride in pyridine at elevated temperature,[23] while the 3-*O*-acyl derivatives **10b–10h** were prepared by acylation of protected macrolide intermediates **7**, **8** and **9** with mixed anhydrides (Scheme 2). Applying a similar synthetic route, some 3-*O*-acyl-3-*O*-decladinosyl-8a-aza-8a-homoerythromycins (8a-lactams)[24] were previously prepared in our laboratories.

The reaction conditions depended on the reactivity of the reagents applied. In some reactions 4-(dimethylamino)pyridine was used as a catalyst. Also, some reactions required higher temperature. Substituents on the phenyl core influenced the reaction rate as well. In the case of an electron acceptor group (*e.g.,* nitro group – **10b** derivative), the conversion was completed within 4 hours at 0 °C. In contrast, in the presence of an electron donor group (*e.g.*, fluorine atom – **10c** derivative or methoxy group – derivative **10d**), complete conversion occurred at room temperature within 20 to 44 hours.

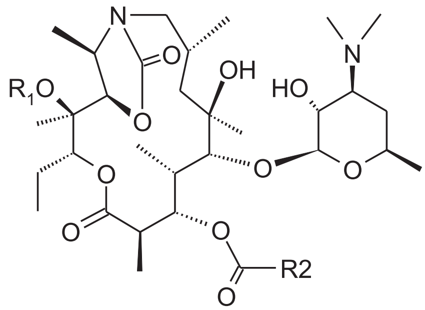
After removal of the acetyl group at the C-2' position, the desired 3-*O*-acyl analogues **10a–10h** were obtained.

Amino phenyl derivatives **10i–10k** were synthesized in the next step, starting with the nitro phenyl analogues **10b**, **10g** and **10h**, by hydrogenation in acetic acid using Pt(IV) oxide as catalyst (Scheme 3).

Structures of the 3-*O*-descladinosyl derivatives **4–6** were confirmed by the disappearance of the L-cladinose signals and also by the upfield shifts of the 3-C-atom signals in the NMR spectra.[25]

Watching the ^{1}H NMR spectra of the 3-*O*-acyl derivatives **10a–10k** for the 3-H signals, a downfield shift from ~3.8 to ~5.2 ppm was observed. The acylation was supported by a new carbonyl signal at ~170 ppm in ^{13}C NMR spectra. The new signal at 20.5 ppm in the spectrum of 3-*O*-acetyl ester **10a** was attributed to the Me part of the acetyl group. Aromatic derivatives **10b–10k** have characteristic signals in the ^{1}H NMR spectra at ~7.5±1.0 ppm and at ~3.5±0.5 ppm. The ^{13}C NMR spectra also showed new signals, at ~150 to ~120 ppm (aromatic carbon atoms) and at ~40 ppm (methylene groups).

The IR spectra of aromatic substituted compounds **10b–10k** have two bonds at 1630–1610 cm^{-1} and 1530 ~ 1510 cm^{-1}, which are characteristic of the stretching vibration of the carbon-carbon bond of the aromatic ring. Nitrophenyl derivatives **10b**, **10g** and **10h** have two additional bonds at 1523 and 1347 cm^{-1} characteristic of asymmetric and symmetric stretching vibrations of the nitro group. The IR spectrum of compound **10a** has one strong bond at 1244 cm^{-1}, which is characteristic of



10a–10k

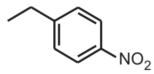
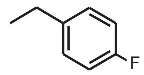
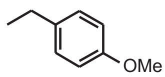
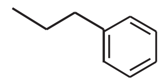
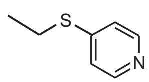
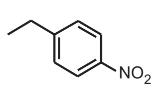
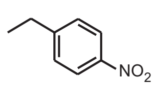
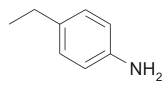
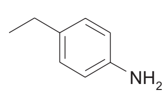
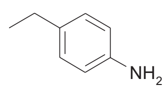
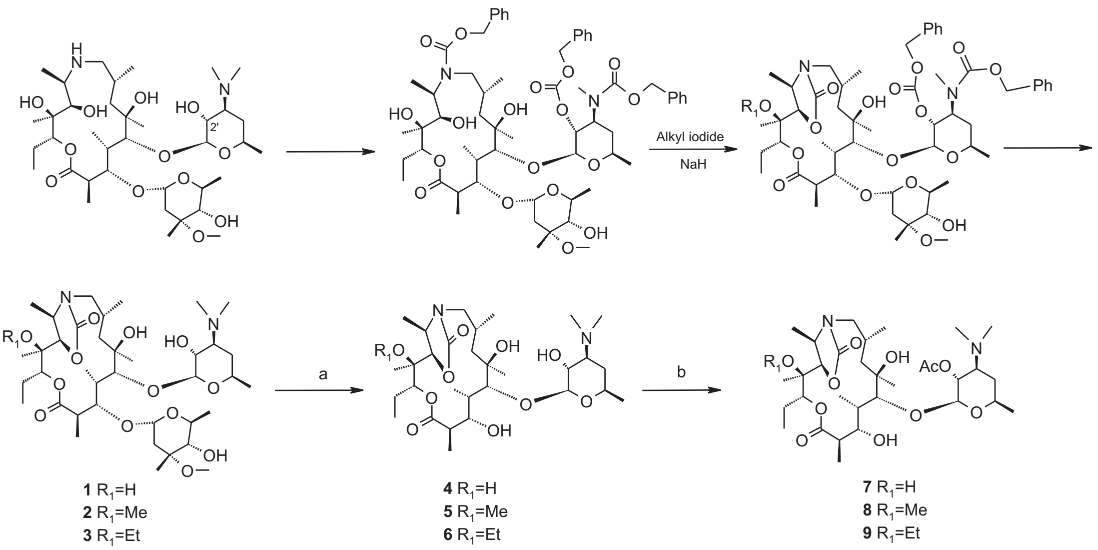
Compound	R_1	R_2
10a	H	CH_3
10b	H	
10c	H	
10d	H	
10e	H	
10f	H	
10g	Me	
10h	Et	
10i	H	
10j	Me	
10k	Et	

Figure 2. Structures of the novel 3-O-acyl derivatives of 15-membered azalides.



Scheme 1. a) HCl, MeOH-H_2O (4:1), r.t., overnight; b) Ac_2O, $NaHCO_3$, CH_2Cl_2, r.t., 4 h.

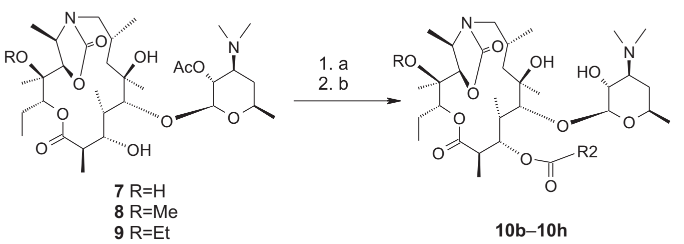
acetyl esters and is associated with the asymmetric stretching vibration of the carbon-oxygen bond.

Microbiology

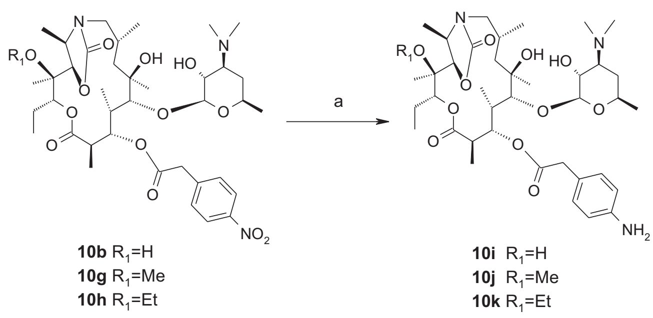
The antibacterial activity of the 3-*O*-acyl derivatives of the 15-membered 9a,11-cyclic carbamates **10a–10k** and the reference compounds **1–6** were evaluated against a panel of representative pathogens selected from PLIVA's strain collection. Various macrolide-resistant strains were included in the tests in order to evaluate their potential for overcoming macrolide resistance. The results are given in Table V.

The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MIC's), which were determined by the microdilution method using the NCCLS recommended methods for dilution antibacterial tests M7-A5.

Some 3-*O*-acyl derivatives showed lower activity against sensitive and efflux resistant *S. pneumoniae* and *S. pyogenes* compared to reference compounds.



Scheme 2. a) R_2COOH, $(CH_3)_3CCOCl$, Et_3N, pyridine, 0 °C–45 °C, 4 h–44 h; b) MeOH, r.t., overnight.



Scheme 3. a) $PtO_2 \cdot H_2O$, H_2, 2.25×10^{4} torr, 2 h.

TABLE V. Antibacterial activities of 3-O-acyl derivatives **10a**–**10k** and the reference compounds **1**–**6** against selected pathogens

	S. aureus *B 0329* ATCC 13709	*S. aureus* *B 0331*	*S. pneumoniae* *B 0541*	*S. pneumoniae* *B 0326*	*S. pyogenes* B 0542	*S. pyogenes* B 0545	*E. fecalis* B 0004 ATCC 29212
	MIC (μg/ml)						
1	4	>64	2	>64	>64	>64	4
2	4	>64	1	>64	>64	>64	4
3	32	>64	8	64	8	16	32
4	>64	>64	64	>64	64	>64	>64
5	>64	>64	64	>64	64	>64	>64
6	>64	>64	64	>64	64	>64	>64
10a	>64	>64	>64	>64	>64	>64	>64
10b	64	>64	8	8	8	16	8
10c	>64	>64	32	32	>64	>64	>64
10d	>64	>64	64	>64	>64	>64	>64
10e	>64	>64	>64	32	16	>64	>64
10f	>64	>64	16	16	32	32	32
10g	>64	>64	32	16	32	32	16
10h	64	64	64	64	64	>64	64
10i	>64	64	>64	>64	>64	>64	>64
10j	>64	>64	>64	>64	>64	>64	>64
10k	>64	>64	64	>64	>64	>64	>64

CONCLUSION

A series of 3-*O*-acyl derivatives of 15-membered macrolides were synthesized and evaluated for antibacterial activity. As assumed, cleavage of L-cladinose abolished the antibacterial activity. Unfortunately, substitution of L-cladinose with 3-*O*-acyl substituents did not improve activity as we had expected.

To explore new aspects of macrolide antibiotics, other modifications of 9a,11-cyclic carbamates of 15-membered macrolides are currently under investigation.

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SAŽETAK

3-*O*-Acilni derivati premoštenih 15-članih azalida: priprava, određivanje strukture i antibakterijska aktivnost

Andrea Fajdetić, Gabrijela Kobrehel, Gorjana Lazarevski, Vlado Štimac i Stjepan Mutak

Opisani su priprava, određivanje strukture i biološka ispitivanja 15-članih azalida aciliranih na položaju C-3. Kiselom hidrolizom pripadajućih 3-kladinozil analoga pripravljeni su 3-dekladinozil-9a,11-ciklički karbamat 9a-aza-9a-homoeritromicina A i njegovi 12-*O*-alkil derivati. Zaštićivanjem 2'-hidroksilne skupine pripravljeni su početni spojevi za aciliranje C-3-hidroksilne skupine. Nakon deprotekcije dobiveni su razni 3-*O*-acil derivati čije su strukture dokazane spektroskopskim metodama (IR, MS, NMR). Novi spojevi ispitani su *in vitro* na panelu Gram-pozitivnih i Gram-negativnih bakterija i njihove su aktivnosti uspoređene s aktivnostima derivata osnovne supstance. 3-*O*-Acil derivati pokazuju poboljšanu antibakterijsku aktivnost, ali slabiju od aktivnosti standardnih makrolida.