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Synthesis and biological activity of 4″-*O*-acyl derivatives of 14- and 15-membered macrolides linked to ω-quinolone-carboxylic unit

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ABSTRACT

The synthesis and antimicrobial activity of a new class of macrolide antibiotics which consist of a macrolide scaffold and a quinolone unit covalently connected by an appropriate linker are described. Optimization of several synthetic steps and structural properties of lead compound **26** are discussed. Promising antibacterial properties of this compound and some of its analogues are reported.

1. Introduction

Macrolide antibiotics represent an established class of antibacterial compounds, in particular effective in a treatment of respiratory infections.[1–3] Among them, 9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (Azithromycin) is characterized by its enhanced chemical stability and exhibited characteristic pharmacokinetic properties in a comparison to erythromycin and clarithromycin, two closely related antibiotics.[4–6]

However, continuous increase in number of infections caused by bacteria resistant to one or multiple antibiotic classes is of great concern.[7,8] In fact, spread of resistance among common respiratory pathogens including *Streptococcus pneumoniae* is recognized by Infectious Diseases Society of America as one of the three major areas of concern that creates a need for new antibiotics.[8] There are several drug discovery strategies aiming at overcoming this issue by intensive search for alternative approach, targeting bacterial virulence factors, cell division mechanisms, or targets specific for narrow range of bacterial species.[9] Nevertheless, the clinical phase 2 or 3 current development pipelines consist almost entirely of new compounds derived from or belonging to old, well established antibiotic classes, such as β-lactams, quinolones, or macrolides.[10]

Recent papers report on the synthesis of new types of macrolide derivatives, in particular those derived from erythromycin and clarithromycin, and their in vitro antibacterial acitivty.[11–13]

Our intensive work on azithromycin[1,14,15] and its derivatives,[16–18] have revealed new chemical spaces for further derivatizations. In this paper we disclose the synthesis of a new class of azythromycin derivatives, exemplified to some detail on the present lead compound, and their in vitro antimicrobial activity.

2. Results and discussion

2.1. Retrosynthetic considerations

For the last few years our team has been investigating a new class of antimicrobial compounds which consist of a macrolide scaffold and a quinolone unit, covalently connected by a suitable linker.[19,20] For this class of molecules, we introduced a term 'macrolones'. Among recently designed compounds, compound **26** became a lead within its series characterized by an adequate length and number of heteroatoms in the linker, as well as selected positions of its attachment to azithromycin and the quinolone units. Novel macrolones from this series are shown in Figure 1.

General procedure for target macrolones **26**–**34** is presented in Scheme 1.

Macrolone **26**, as well as its congeners **27**–**28**, and analogues with macrocyclic scaffolds **29**–**34** are characterized by the structure comprising of a linker connecting the quinolone 3-carboxylic

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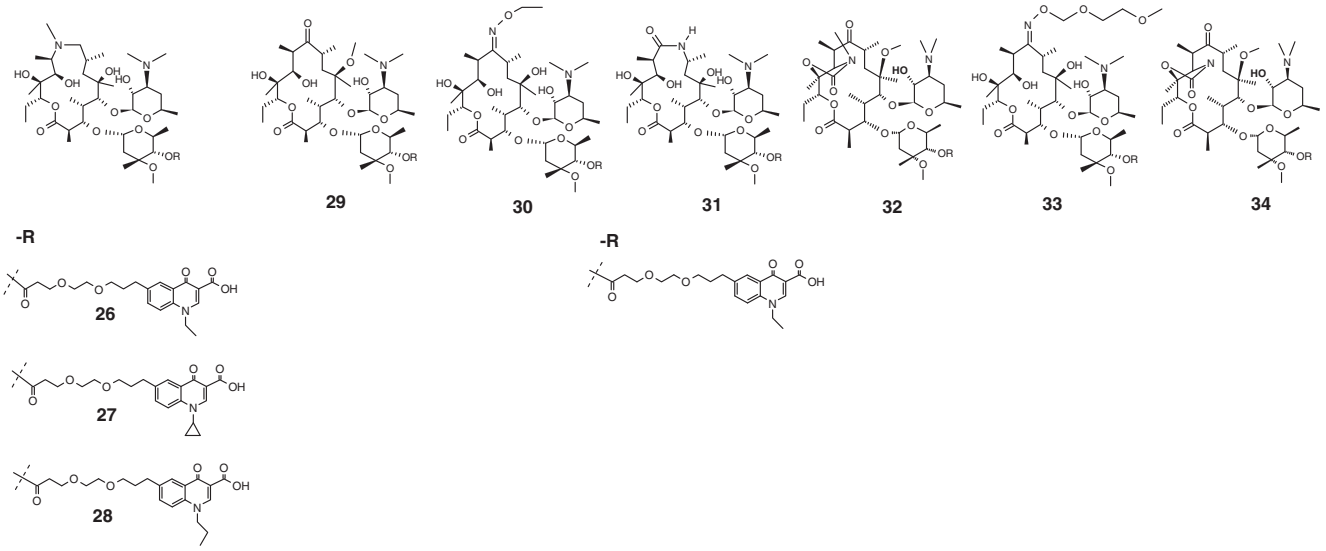
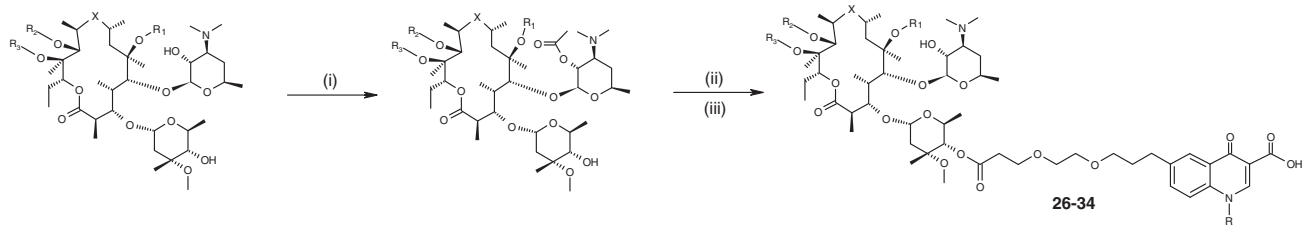


Figure 1. Macrolone derivatives **26**–**34**.



X = -C(=O)-NH-, -C(=O), -N(-CH_3)-CH_2-, -C(=N-OCH_2CH_3)-, -C(=N-$OCH_2OCH_2CH_2OCH_3$)-

R_1 = H, CH_3

R_2 = R_3 = H or R_2R_3 = -CON(H or CH_3)-

X = -C(=O)-NH-, -C(=O), -N(-CH_3)-CH_2-, -C(=N-OCH_2CH_3)-, -C(=N-$OCH_2OCH_2CH_2OCH_3$)-

R_1 = H, CH_3

R_2 = R_3 = H or R_2R_3 = -CON(H or CH_3)-

R = alkyl, cycloalkyl

Scheme 1. General procedure for target macrolones **26**–**34**. Reagents and conditions: (i) Ac_2O, $NaHCO_3$, DCM, rt, 4 h; (ii) EDACxHCl, DMAP, DCM, 0 °C to rt, 4–24 h; (iii) MeOH, 55 °C, 24 h.

acid unit and the macrolide scaffold at the C4″-*O* position of the cladinose sugar. All linkers contain the terminal C–C6 bond to quinolone unit, two ether C–O–C bonds in the linker, and a weaker C4″–O–CO terminal ester bond to the cladinose.

This structural aspect of the macrolone **26**, along with the higher complexity of macrolide scaffold versus quinolone carboxylic acid unit, suggested convergent synthetic approach to target compounds, as outlined in Scheme 1.

The syntheses of 2′-*O*-acetil-protected macrolides[15] and derivatives of quinolone carboxylic acid[21–23] are repeatedly reported in the literature, although the later needed notable modifications to improve workability and the overall yield. Due to the sensitivity of macrolide unit, minimal transformations were envisaged after linking of quinolone unit to macrolide precursor. This concept suggested formation of the C4″-*O* ester bond in the last step, after one C–C bond and the two ether C–O–C bonds were formed on the linker-quinolone unit.

A more detailed synthesis of macrolones **26**–**34** will be described hereafter. Many improvements and modifications of the separate steps on the route to these target compounds cumulated with synthetic protocol for the preparation of the lead compound **26**.

2.2. Synthesis

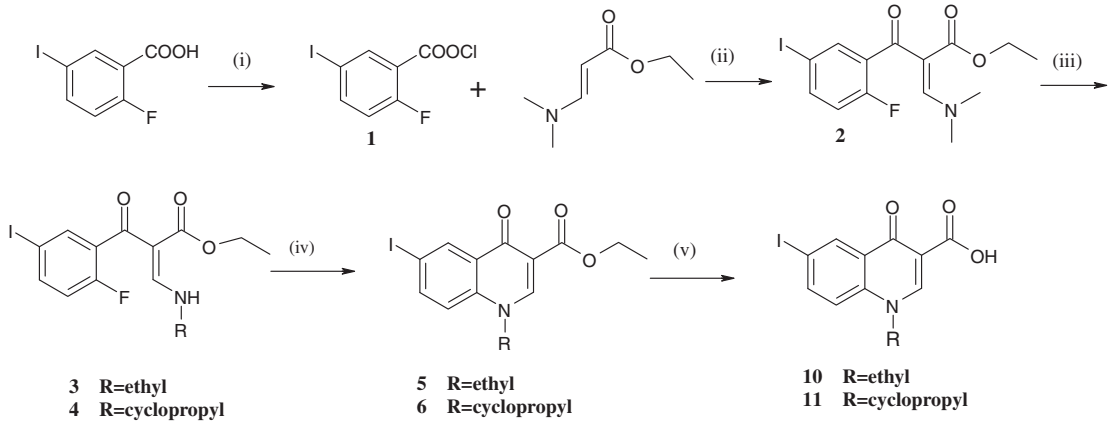
Azithromycin contains three secondary hydroxyl groups whose reactivity, in esterification reaction, is approximately in the order: 2′-OH ≫ 4″-OH > 11-OH.[24,25] Nevertheless, under standard conditions, a mixture of various mono-, di- and tri-ester derivatives is generally obtained. In a view of this order of reactivity, protection of 2′-OH is essential, and can be affected under properly selected mild acylation condition. Based on previous experience of our team,[15] and of the others,[26] the 2′-*O*-acetyl was selected as the protecting group on all scaffolds.

Different synthetic approaches are known for a preparation of the quinolone 3-carboxylic acid derivatives.[21,22] Using the commercially available 2-fluoro-5-iodobenzoic acid as a starting material, iodoquinolinic acids are prepared as outlined in Scheme 2.

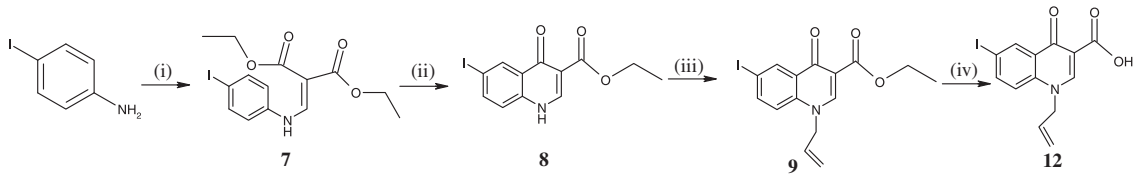
N-Allyl derivative **12** was prepared by an alternative approach[23] presented in Scheme 3.

Synthesis of intermediary dicarboxylic acids **23**–**25** were next target for the final linkage of N1-substituted quinolonic acid derivatives **10**–**12** to the selected macrolide scaffolds.

Construction of the linker in these compounds started from the commercially available *O*-benzyl-protected 1,2-ethanediol, which



Scheme 2. Synthesis of 1-*N*-ethyl (**10**) and 1-*N*-cyclopropyl (**11**) 6-iodoquinolonic acid. Reagents and conditions: (i) $(COCl)_2$, THF/DCM (1:50), rt, 4 h; (ii) TEA, toluene, 90 °C, 6 h; (iii) R-NH_2, THF/EtOH (1:1.5), rt, 3 h; (iv) K_2CO_3, DMF, 140 °C, 1 h; (v) NaOH, THF/H_2O (1:1), 80 °C, 3 h.



Scheme 3. Synthesis of 1-*N*-allyl-6-iodoquinolonic acid (**12**). Reagents and conditions: (i) 2-ethoxymethylene-malonic acid diethyl ester, 105 °C, 10 min; (ii) polyphosphoric acid, 110 °C, 2 h; (iii) allyl bromide, K_2CO_3, DMF, 65 °C, 1 h; (iv) NaOH, THF/H_2O (1:1), 80 °C, 3 h.

was quantitatively propargylated to give **13**, and submitted to Sonogashira-type[27] C–C coupling with 6-iodoquinolone-3-carboxylic acids **10**–**12**, Scheme 4. This reaction proved more effective with acids **10**–**12** than with the esters analogue **5**, **6** and **9**, and was completed on 20 g scale with 78–82% yield. Initially the isolation required column chromatography on silica-gel with an eluent system CH_2Cl_2/MeOH/concd NH_3 (90:15:1.5), with yield of about 50%. The isolation was substantially improved by addition of water to the reaction mixture and controlled acid–base extraction. Extraction of the product by di-*iso*-propyl ether at pH 12 eliminates PPh_3O, while excess of carboxylic acids **14**–**16** remains in the basic aqueous solution. Treatment of this solution with charcoal, followed by filtering through Celite eliminates traces of the metal. Upon acidification to pH 6 pure **14**–**16** were isolated.

Deprotection of terminal hydroxyl group by hydrogenation with concomitant reduction of the triple bond in **14**–**16** was completed by Pd/C/H_2 in MeOH/DCM affording **17**–**19** in 85–91% yield.

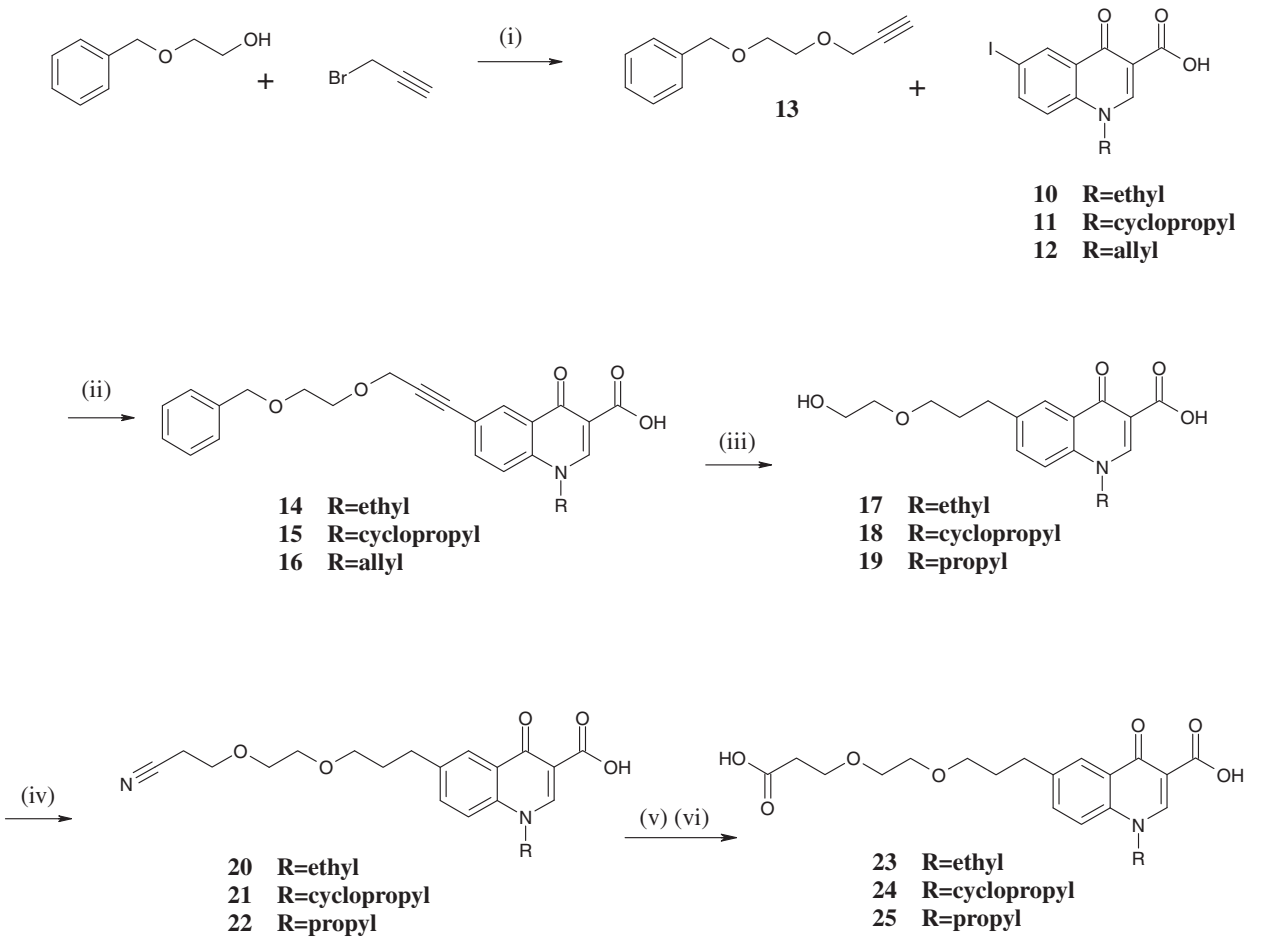
Michael addition of alcohols **17**–**19** to acrylonitrile was substantially improved when DBU in acrylonitrile as the solvent was replaced by 10% aq NaOH. Specifically, polymerization of acrylonitrile was suppressed by lowering the reaction temperature from 80 °C with DBU to 5–10 °C with 10% aq NaOH, and diminishing molar ratio of acrylonitrile from ca. 60:1 to ca. 5:1. Under these conditions the retro-Michael reaction decreased from approx. 20% to less than 4%. The intermediates **20**–**22** were selectively precipitated from aqueous solution at pH 6.3 and gave products with high purity. Hydrolysis of nitriles generally requires harsh conditions and often a mixture of amide and carboxylic acid is formed.[28,29] Since compounds **20**–**22** comprise β-keto carboxylic acid unit, under such conditions 3-carboxylic group can decarboxylate. In the first experiments intensive decarboxylation was actually observed, accompanied by the retro-Michael reaction of β-cyanoethoxy unit back to the alcohols **17**–**19** and acrylonitrile. Proper modification of hydrolytic conditions avoided both difficulties. In the first step hydrolysis of cyano to amido group was completed in concd sulfuric acid at 20–22 °C over 24 h. HPLC control revealed complete and selective transformation of compounds **20**–**22** into amide, which was not isolated. This two-step one-pot procedure was successfully completed by water addition of concd H_2SO_4/H_2O to the ratio 1.5:1. Hydrolysis was completed at 70 °C over 24 h. Diacids **23**–**25** was achieved in high over all yield, while retro-Michael reaction was completely prevented.

The most challenging aspect was the requirement for site-selective acylation at 4″-OH of 2′-*O*-protected macrolide by dicarboxylic acid **23**–**25** in the last step of macrolone synthesis. The three hydroxyl groups in 2′-*O*-protected macrolide and two carboxylic groups in **23** imply the risk of the formation of six structurally isomeric monoesters, beside possible formation of some diesters. Extensive experimentation with **23** has shown that less acidic 3-carboxylic group is not activated with EDAC × HCl coupling reagent as effectively as the terminal one. The pK_a of β-keto-carboxylic group in **23** is estimated to 5.5–6.5,[30,31] respectively, while pK_a of β-alkoxy-carboxylic group is found between 3 and 3.5.[28] EDAC is known to activate the carboxylic function by a mechanism that includes formation of an acyl-guanidinium intermediate.[32,33] In our case selective activation of the terminal, more acidic carboxylic group in **23** resulted in the formation of guanidinium-like species which enter ulterior activation by DMAP before acyl transfer to C4″-OH group, Scheme 5.

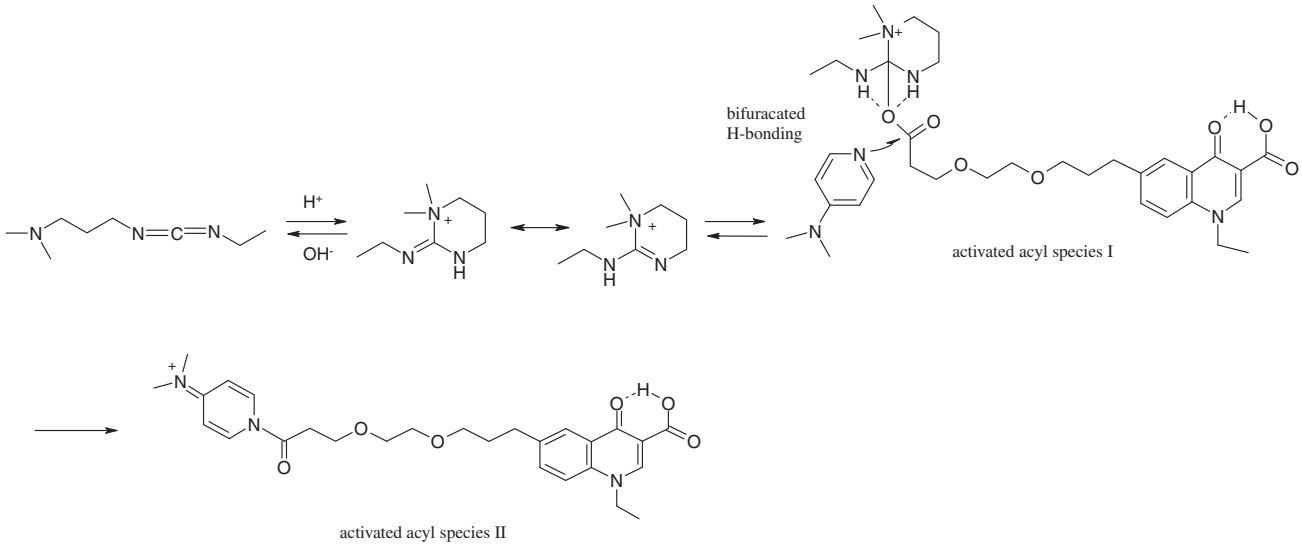
The elaborated synthetic route to compound **26**, was applied with minor modifications to prepare target macrolones **27** and **28** with azithromycin as the scaffold and macrolones **29**–**34** with other macrolide scaffolds, Table 1.

The mass spectra for all new compounds were in agreement with the proposed structure. These results were further confirmed by the NMR spectra of all compounds, which revealed two sets of signals in the aliphatic and aromatic regions arising from the protons and carbons of macrolide and quinolone moieties, respectively, as well as signals reflecting the number of methylene groups in the linker.

The newly formed ester carbonyl resonates in the ^{13}C NMR spectrum around 170 ppm in **26**–**34**. The significant deshielding



Scheme 4. Synthesis of dicarboxylic acid derivatives **23**–**25**. Reagents and conditions: (i) THF, NaH, 0–35 °C, 24 h; (ii) CH_3CN/TEA (1:1), CuI, $Pd(PPh_3)_2Cl_2$, 50 °C, 24 h; (iii) MeOH/DCM (3:1), Pd/C/H_2, rt, 5 bar, 24 h; (iv) CH_3CN, 10%NaOH, 15–20 °C, 2 h; (v) H_2SO_4 concd, 20–22 °C, 24 h; (vi) H_2O, 75 °C, 24 h.



Scheme 5. Mechanism of site-selective activation of dicarboxylic acid derivatives by EDAC.

of the 4″-H signal in the ^{1}H NMR, together with its long-range coupling to the new ester carbonyl signal, provided evidence that the esterification occurred at the 4″-OH group of the macrolide scaffold.

All target macrolones proved chemically stable and the lead compound **26** tended to crystallize from some organic solvents as solvates. High-quality crystals were obtained from di-*iso*-propyl ether. Single-crystal X-ray analysis was completed and packing of

Table 1
Antibacterial activity of macrolones **26**–**34**, given as minimum inhibitory concentration (MIC) in units of μg/mL

Phenotype	Organism & strain										
	S. aureus ATCC13709 eryS	*S. aureus* PK1 M	*S. pneumoniae* Ci137 M	*S. pyogenes* 2 Finland M	*S. aureus* 90256 iMLS	*S. pneumoniae* 134 GR M iMcLS	*S. pyogenes* Finland 11 iMLS	*S. pneumoniae* 58 Spain cMLS	*S. pyogenes* 166 GR-Micro cMLS	*H. influenzae* ATCC 49247	*M. catarrhalis* ATCC 23246
AZM	0.5	>64	8	8	>64	>64	>64	>64	>64	1	⩽0.125
TEL	⩽0.125	⩽0.125	0.25	0.5	⩽0.125	0.25	⩽0.125	0.25	16	2	⩽0.125
26	⩽0.125	0.25	⩽0.125	⩽0.125	0.25	0.25	⩽0.125	⩽0.125	⩽0.125	1	⩽0.125
27	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	⩽0.125	⩽0.125	0.5	2	0.5
28	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	1	0.5
29	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.5	1	0.25
30	1	1	⩽0.125	⩽0.125	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2	-
31	⩽0.125	0.25	⩽0.125	⩽0.125	4	0.25	⩽0.125	1	1	2	⩽0.125
32	⩽0.125	0.5	⩽0.125	⩽0.125	0.25	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1	⩽0.125
33	0.5	1	⩽0.125	⩽0.125	8	0.5	⩽0.125	1	1	4	1
34	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1	1

AZM = azithromycin; TEL = telithromycin; iMLS = inducible resistance to macrolide, licosamide and streptogramin (MLS) antibiotics; iMcL = inducible resistance to macrolide and constitutive resistance to lincosamide antibiotics; cMLS = constitutive MLS resistance; M = efflux mediated macrolide resistance.

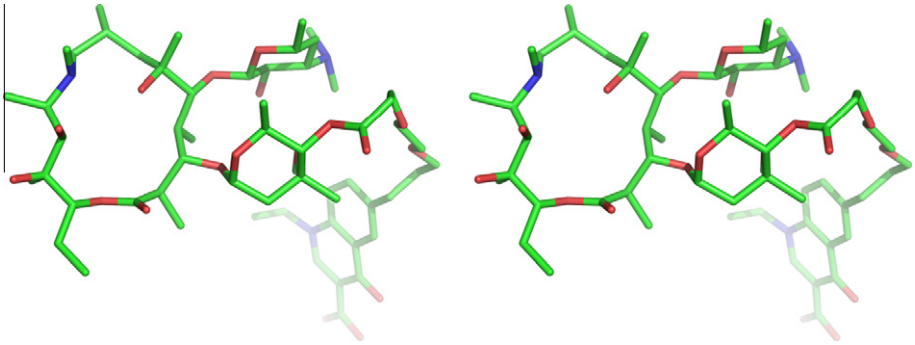


Figure 2. PyMOL[39] stereo drawing of **26**. Hydrogen atoms are omitted for clarity.

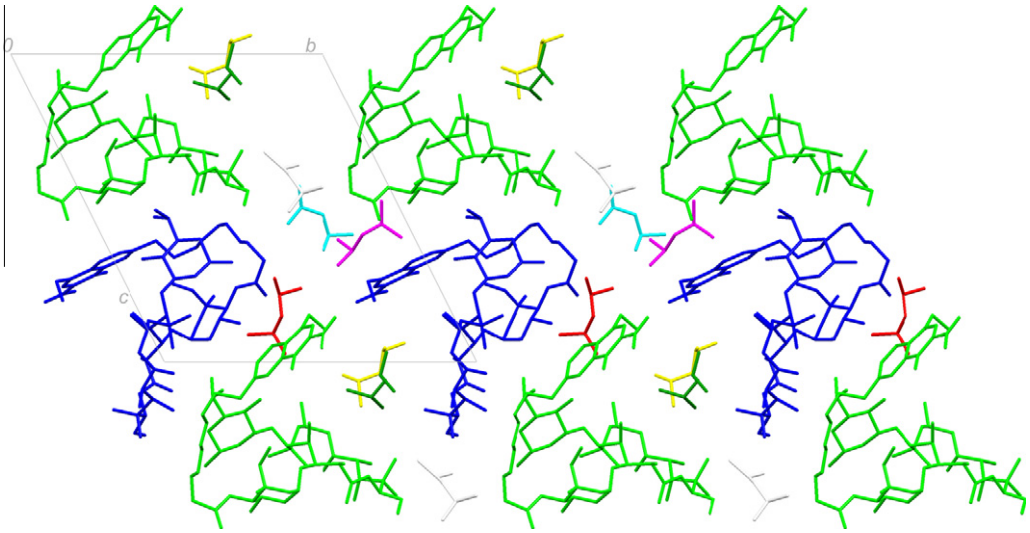


Figure 3. The crystal packing of **26** projected down *a* axis. Hydrogen atoms have been omitted for clarity. Molecules are colored according to symmetry equivalence.

the molecules in the unit cell is shown in Figures 2 and 3. The asymmetric unit (space group *P*1) contains two symmetrically independent molecules of compound **26** and six molecules of di-*iso*-propyl ether. There are only very week hydrogen bonds of the type C–H···O between molecules. These very week intermolecular bonds are the reason for quick loss of the solvent molecules and fast decomposition of the crystals.

2.3. Biological results

Antibacterial activity of compounds **26**–**34** was determined by a standard broth microdilution method.[34] Azithromycin and telithromycin were used as controls and results are shown in Table 1 and are expressed as minimum inhibitory concentrations (MICs) in units of μg/mL. The organisms tested represented relevant Gram-positive (*S.*

pneumoniae, *Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative (*Haemophilus influenzae* and *Moraxella catarrhalis*) respiratory tract pathogens, and were either sensitive or resistant to macrolide antibiotics. Macrolide resistance was due to two major mechanisms—production of efflux pumps (M), or ribosome modification by methylation. Methylase expression was inducible (iMLS) or constitutive (cMLS).

All analogues showed MIC $\leqslant$ 0.125 μg/mL against erythromycin sensitive *S. pneumoniae* and *S. pyogenes*.

Azithromycin derivatives **26**–**28** had very high antibacterial potency against all tested organisms. It is noteworthy that *S. pyogenes* strains are fully covered, regardless the resistance mechanism, and activity against cMLS *S. pyogenes* clearly exceeds that of marketed macrolide or ketolide antibiotics, as telithromycin has MIC 16 μg/mL and **26**–**28** of $\leqslant$ 0.125 μg/mL. As **26** was the most potent compound, in order to investigate influence of a macrolide scaffold, dicarboxylic acid **23** was attached to six different macrolides. Compounds **29**, **32** and **34** retained high overall potency. However, activity of compounds **30**, **31** and **33** against iMLS *S. aureus* strain was impaired, and potency against cMLS *S. pneumoniae* and *S. pyogenes* strains was reduced for **31** and **33**. All derivatives had good activity against *H. influenzae*, comparable to that of azithromycin. Therefore, **26**–**28** and **34** were additionally tested against the wide range (n = 43) of recent clinical isolates of *H. influenzae* and summary of the obtained MIC data is shown in Table 2, given as MIC_{50} (concentration needed to inhibit growth 50% of tested isolates), MIC_{90} (concentration needed to inhibit growth 90% of tested isolates) and range of MIC values.

Pharmacokinetic properties of two selected compounds (**26** and **27**) where determined in rats and are summarized in Table 3. Both compounds are characterized by moderate blood clearance (35% and 43% of liver blood flow, respectively), large volume of distribution (10.4 and 10.5 L/kg, respectively) and long half-life (9.4 and 6.7 h, respectively) supporting once daily dosing. The mean oral bioavailability from a solution formulation was 22% for compound **26** and 16% for compound **27**.

Considering these results, we can conclude that the current lead molecule **26** has the most favorable antibacterial profile, other new macrolones showing comparable or lower activity. Further in vivo experiments with these compounds could confirm their improved in vitro antibacterial activity. Additional studies of correlation of their physico-chemical and biological properties are envisaged.

3. Conclusion

We have presented the synthesis of macrolones **26**–**34** and have shown that only protection of the 2′-OH group of macrolide unit is required to reach complete site-selective acylation at 4″-OH of cladinose unit. Besides, only one of the two carboxylic groups of quinolone-linker precursors was activated by EDAC × HCl.

All novel compounds exhibited improved antibacterial activity against the tested macrolide resistant bacteria (superior to telithromycin), and selected compounds had good potency against the wide panel of recent clinical isolates of *H. influenzae* (comparable to azithromycin). This new class of macrolide derivatives represents a promising approach for the delivery of novel antimicrobials with improved spectrum of activity for the treatment of infections caused by respiratory pathogens.

Within the investigates series, compound **26** exhibited the most promising biological profile. Further experiments in animal models would indicate the potential use of these compounds as new antibacterial agents.

Table 2
Antimicrobial profiling of selected compounds against *H. influenzae* (n = 43)

	26	27	28	34	AZM
MIC 50	1	1	1	2	1
MIC 90	2	2	2	4	2
Range	0.5–2	0.5–2	0.5–2	1–8	0.25–2

MIC in units of μg/mL.
AZM = azithromycin.

Table 3
Summary of the pharmacokinetics of **26** and **27** in rats

Compound	CL[a] % LBF	V_{dss}[b] L/kg	$T_{1/2}$ hours	Oral F[c] %
26	35	10.4	9.4	22
27	43	10.5	6.7	16

[a] CL: moderate blood clearance.
[b] V_{dss}: volume of distribution.
[c] Oral F: oral bioavailability.

4. Experimental

4.1. General

All commercial reagents (Merck, Sigma–Aldrich) were used as provided unless otherwise indicated, and all solvents are of high purity unless otherwise noted.

1D and 2D NMR spectra (^{1}H, APT, COSY, HSQC, HMBC) were recorded at 25 °C in DMSO-d_6 with TMS as the internal standard on Bruker Avance DRX500 spectrometer using QNI probe and Bruker Avance DPX300 spectrometer using dual ^{1}H/^{13}C probe.

Mass spectra were obtained on a Waters Micromass ZQmass spectrometer for ES+-MS. Electrospray positive ion mass spectra were acquired using a Micromass Q-Tof2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 100–2000 Da, with a scan time of 1.5 s and an interscan delay of 0.1 s in a continuum mode. Reserpine was used as the external mass calibrant lock mass ($[M+H]^+$ = 609.2812 Da). The elemental composition was calculated using a MassLynx v4.1 for the $[M+H]^+$ and the mass error quoted within ±5 ppm range.

All 2′-*O*-acetyl-macrolides were prepared according to published literature.[15]

All final compounds were isolated as amorphous solid except by compound **26**.

Reaction flow and purity of products were monitored by thin layer chromatography (TLC) on Merck Kieselgel 60 (230–400 mesh) using specifically solvent systems indicated in the protocol. I_2, UV-light (254 nm) and H_2SO_4, followed by heating to >120 °C were used for detection.

4.2. Biological methods and materials

4.2.1. In vitro antibacterial activity assays

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method as described by CLSI guidelines,[34] except that for *Streptococcus* medium, lysed blood was substituted with 5% horse serum. Dilutions of tested compounds were prepared using TECAN Genesis 150. Bacteria were grown on appropriate agar plates (by Becton Dickinson, USA)—Columbia agar with 5% sheep blood for Streptococci, and *M. catarrhalis*, chocolate agar for *H. influenzae* and Mueller-Hinton agar for Staphylococci.

4.2.2. In vivo rat pharmacokinetic studies

PK po/iv crossover studies were performed in male Sprague-Dawley rats. Animals were dosed at target doses of 30 mg/kg orally (po) and 5 mg/kg intravenously (iv). Compounds were administered as a solution in 1.0% DMSO, 20% Encapsin (w/v) and saline (iv) or water (po), pH 3.5–4.0. Animals where fed for iv and fasted for po administration. Blood was collected at several timepoints up to 1800 min after administration, hemolyzed with water in a 1:1

ratio and stored at −20 °C until analysis. Samples were extracted by deproteinization and the supernatants were analyzed for parent compound by LC/MS.

4.3. Crystal structure elucidation

Single crystal structure determination was done in Department of Chemistry, Faculty of Science, University of Zagreb, Croatia. Data were collected on the Oxford Diffraction Xcalibur diffractometer with Saphire 3 CCD detector. Data reduction was done with the CRYSALIS program package.[35] Program package WINGX[36]was used for structure solution refinement. The structure was solved by direct methods (SIR92)[37] and refined by least squares method based on F^2 by using all reflexes. It was refined in overlapping blocks (SHELXL-97).[38] All non-hydrogen atoms, except for the solvate molecule atoms were refined anisotropically. Hydrogen atoms were generated on geometrical basis (C–H = 0.95 Å, $U_{iso}(H) = 1.2\ U_{eq}$ of the corresponding C atom; $U_{iso}(H) = 1.5\ U_{eq}(C)$ for the methyl group. The reading model was used for refinement of H atoms.

4.4. Synthetic procedures

4.4.1. 2-Fluoro-5-iodo-benzyl chloride (1)

Into a solution of 2-fluoro-5-iodo-benzoic acid (50 g, 0.188 mol) in DCM (250 mL) and DMF (3 mL) at rt oxalyl chloride (19.9 mL, 0.223 mol) was added dropwise. Reaction mixture was stirred at rt for 4 h. The reaction was monitored by TLC in a solvent system EtOAc/*n*-hexane = 1:1. The excess of oxalyl chloride was removed by evaporation under reduced pressure to give the title compound **1** as oily residue (51.93 g, 97%). The residue was used in the next step as is.

4.4.2. Ethyl 3-(dimethylamino)-2-[(2-fluoro-5-iodophenyl)carbonyl]-2-propenoate (2)

A solution of compound **1** (51.93 g, 0.18 mol) in dry toluene (430 mL) was added to the stirring solution of ethyl 3-(dimethylamino)acrylate (26.9 g, 0.19 mol) in dry TEA (33.9 mL, 0.24 mol) at rt The solution was stirred at 90 °C for 6 h. The reaction was monitored by TLC in a solvent system EtOAc/*n*-hexane = 1:1. After cooling to rt insoluble material was filtered off and the solvent was evaporated to dryness. The residue was triturated in *n*-hexane (200 mL), the precipitate was collected on filter, dried in vacuum and then precipitated from EtOH/Et_2O = 1:2, affording 47.17 g (67%) of the title compound **2** as yellow solid.

HRMS (ES) calcd for $C_{14}H_{15}FINO_3$ $[M+H]^+$ 392.0159 found 392.0137.

^{1}H NMR (500 MHz, DMSO) δ: 7.75–7.78 (ov, 2H), 7.66 (dd, 1H), 7.03 (dd, 1H), 3.87 (q, 2H), 2.78 (s, 6H), 0.88 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 184.3, 166.6, 159.8, 158.0, 157.8, 139.6, 132.9, 132.8, 137.3, 118.1, 117.9, 101.1, 87.7, 59.0, 42.1, 13.6.

4.4.3. Ethyl 3-(ethylamino)-2-[(2-fluoro-5-iodophenyl)carbonyl]-2-propenoate (3)

A stirred solution of compound **2** (95.43 g, 0.24 mol) in a mixture EtOH/THF = 1.5:1 (298 mL) was cooled in an ice-bath to 5–10 °C. Into it cold ethylamine (2 M solution in THF, 242.85 mL, 0.49 mol) was added dropwise. The reaction was stirred at rt for 3 h and monitored by TLC in a solvent system EtOAc/cyclohexane = 1:1. Into the mixture *n*-hexane was added. The resulting precipitate was filtered off, washed with *n*-hexane and dried affording 75.26 g (79%) of the title compound **3** as a yellow solid. The product was used in the next step without further purification.

HRMS (ES) calcd for $C_{14}H_{15}FINO_3$ $[M+H]^+$ 392.0159 found 392.0165.

^{1}H NMR (500 MHz, DMSO) δ: 7.77–7.71 (m, 1H), 7.60 (br s, 1H), 7.55 (dd, 1H), 7.02 (dd, 1H), 3.89 (q, 2H), 3.50 (q, 2H), 1.21 (t, 3H), 0.94 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 186.9, 166.0, 159.8, 159.5, 156.3, 138.7, 138.6, 136.76, 136.3, 136.2, 134.0, 133.8, 117.8, 117.6, 99.4, 87.5, 58.8, 44.5, 15.7, 13.7.

4.4.4. Ethyl 3-(cyclopropylamino)-2-[(2-fluoro-5-iodophenyl)-carbonyl]-2-propenoate (4)

The title compound **4** as a yellow solid was prepared in 72% yield starting from compound **2** (23.8 g, 0.06 mol) and cyclopropylamine (6.7 mL, 0.096 mol) according to the procedure used to prepare compound **3**.

MS (ES) *m*/*z* 403.86 $[M+H]^+$.

This compound was used in the next step without further purification.

4.4.5. Ethyl 1-ethyl-6-iodo-4-oxo-1,4-dihydro-3-quinolinecarboxylate (5)

Compound **3** (130.79 g, 0.33 mol) was dissolved in dry DMF (787 mL) at rt K_2CO_3 (94.42 g, 0.67 mol) was added and the mixture was stirred at 140 °C for 1 h. The reaction was monitored by TLC in a solvent system EtOAc/cyclohexane = 1:1. The reaction mixture was filtered and the filtrate evaporated under reduced pressure. To the oily residue THF (300 mL) was added under stirring until a precipitate was formed. The precipitate was filtered off yielding 93.35 g (76%) of the title compound **5** as a yellow solid.

HRMS (ES) calcd for $C_{14}H_{14}INO_3$ $[M+H]^+$ 372.0097 found 372.0096.

^{1}H NMR (500 MHz, DMSO) δ: 8.71 (s, 1H), 8.50 (d, 1H), 8.05 (dd, 1H), 7.64 (d, 1H), 4.39 (q, 2H), 4.23 (q, 2H), 1.36 (t, 3H), 1.29 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 171.3, 164.3, 149.2, 140.6, 138.0, 134.8, 129.9, 119.6, 110.5, 90.0, 59.7, 47.9, 14.2, 14.1.

4.4.6. Ethyl 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydro-3-quinolinecarboxylate (6)

The title compound **6** as a yellow solid was prepared in 60% yield starting from compound **4** according to the procedure used to prepare compound **5**.

HRMS (ES) calcd for $C_{15}H_{14}INO_3$ $[M+H]^+$ 384.0097 found 384.0105.

^{1}H NMR (500 MHz, DMSO) δ: 8.48 (s, 1H), 8.44 (d, 1H), 8.11 (dd, 1H), 7.87 (d, 1H), 4.22 (q, 2H), 3.64 (m, 1H), 1.28 (t, 3H), 1.23 (m, 2H), 1.11 (m, 2H).

^{13}C NMR (125 MHz, DMSO) δ: 171.4, 164.1, 148.6, 140.6, 139.9, 134.4, 129.2, 120.0, 110.3, 90.3, 59.8, 34.63, 14.2, 7.5.

4.4.7. Diethyl {[(4-iodophenyl)amino]methylidene}propanedioate (7)

Iodoaniline (10.00 g, 0.046 mol) and 2-ethoxymethylenemalonic acid diethyl ester (9.22 mL, 0.046 mol) were stirred at 105 °C for 10 min and raw product precipitated from the reaction mixture. The mixture was cooled to rt and *n*-hexane (100 mL) was added. The suspension was filtered yielding 16.37 g (92%) of the title compound **7** as a yellow solid.

HRMS (ES) calcd for $C_{14}H_{16}INO_4$ $[M+H]^+$ 390.0202 found 390.0215.

^{1}H NMR (500 MHz, DMSO) δ: 8.94 (s, 1H), 8.56 (d, 1H), 8.11 (dd, 1H), 7.29 (d, 1H), 4.23 (q, 2H), 4.16 (q, 2H), 1.27 (t, 3H), 1.24 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 166.6, 164.7, 153.6, 150.0, 147.0, 146.6, 115.1, 95.0, 86.84, 59.9, 59.7, 14.3, 14.1.

4.4.8. Ethyl 6-iodo-4-oxo-1,4-dihydro-3-quinolinecarboxylate (8)

A mixture of compound **7** (16.00 g, 0.04 mol) and polyphosphoric acid (45 g) was stirred at 110 °C for 2 h. The reaction was mon-

itored by TLC in a solvent system EtOAc/cyclohexane = 1:1. Water (1 L) was added, the precipitate filtered off and washed with water. The cake was suspended in DMF (15 mL), stirred for 10 min, filtered off and dried at 60 °C in vacuum yielding 8.5 g (62%) of the title compound **8**.

HRMS (ES) calcd for $C_{12}H_{10}INO_3$ $[M+H]^+$ 343.9784 found 343.9807.

^{1}H NMR (500 MHz, DMSO) δ: 8.57 (s, 1H), 8.40 (d, 1H), 7.97 (dd, 1H), 7.43 (d, 1H), 4.22 (q, 2H), 1.27 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 171.5, 164.3, 149.8, 140.6, 134.7, 129.7, 120.3, 109.3, 90.4, 60.0, 14.2.

4.4.9. Ethyl 6-iodo-4-oxo-1-(2-propen-1-yl)-1,4-dihydro-3-quinolinecarboxylate (9)

To the solution of compound **8** (4 g, 0.012 mol) in DMF (40 mL) K_2CO_3 (3.22 g, 0.023 mol) and allyl bromide (1.48 mL, 0.018 mol) were added. The mixture was stirred at 65 °C for 1 h. The reaction was monitored by TLC in a solvent system EtOAc/cyclohexane = 1:1. Reaction mixture was diluted with water (50 mL) and the resulting suspension filtered. The cake was dried at 50 °C in vacuum for 4 h yielding 3.30 g (74%) of the title compound **9** as a yellowish solid.

HRMS (ES) calcd for $C_{15}H_{14}INO_3$ $[M+H]^+$ 384.0097 found 384.0099.

^{1}H NMR (300 MHz, DMSO) δ: 8.71 (s, 1H), 8.50 (d, 1H), 8.04 (dd, 1H), 7.52 (d, 1H), 6.04 (m, 1H), 5.25 (dd, 1H), 5.11 (dd, 1H), 5.03 (d, 2H), 4.23 (q, 2H), 1.29 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 171.7, 164.4, 149.9, 140.7, 138.7, 134.9, 132.6, 129.9, 120.3, 118.1, 110.8, 90.4, 60.0, 54.7, 14.4.

4.4.10. 1-Ethyl-6-iodo-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (10)

Compound **5** (93.35 g, 0.25 mol) and NaOH (46.26 mg, 1.16 mmol) were dissolved in a mixture THF/H_2O =1:1 (900 mL). Reaction mixture was stirred for 3 h at 80 °C. The reaction was monitored by TLC in a solvent system DCM/MeOH/NH_4OH = 90:15:1.5. THF was evaporated under reduced pressure. The residual solution was diluted with water and pH adjusted to 4.5 using 6 M aq HCl. The resulting suspension was filtered, the cake washed with water and dried at 50 °C for 4 h yielding 84.19 g (98%) of the title compound **10** as a white solid.

HRMS (ES) calcd for $C_{12}H_{10}INO_3$ $[M+H]^+$ 343.9784 found 343.9774.

^{1}H NMR (300 MHz, DMSO) δ: 9.06 (s, 1H), 8.57 (d, 1H), 8.21 (dd, 1H), 7.84 (d, 1H), 4.58 (q, 2H), 1.42 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 176.4, 165.8, 149.6, 142.3, 138.5, 134.3, 127.2, 120.4, 108.4, 92.1, 49.2, 14.6.

4.4.11. 1-Cyclopropyl-6-iodo-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (11)

The title compound **11** as a white solid was prepared in 96% yield starting from compound **6**, according to the procedure used to prepare compound **10**.

HRMS (ES) calcd for $C_{13}H_{10}INO_3$ $[M+H]^+$ 355.9784 found 355.9777.

^{1}H NMR (300 MHz, DMSO) δ: 8.75 (s, 1H), 8.58 (d, 1H), 8.28 (dd, 1H), 8.07 (d, 1H), 3.84 (m, 1H), 1.30 (m, 2H), 1.19 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ: 176.7, 165.6, 149.1, 142.3, 140.6, 133.9, 126.8, 120.8, 108.0, 92.4, 36.0, 7.7.

4.4.12. 6-Iodo-4-oxo-1-(2-propen-1-yl)-1,4-dihydro-3-quinolinecarboxylic acid (12)

The title compound **12** was prepared in 93% yield starting from compound **9** according to the procedure used to prepare compound **10**.

HRMS (ES) calcd for $C_{13}H_{10}INO_3$ $[M+H]^+$ 355.9784 found 355.9773.

^{1}H NMR (300 MHz, DMSO) δ: 9.08 (s, 1H), 8.62 (d, 1H), 8.21 (dd, 1H), 7.73 (d, 1H), 6.06 (m, 1H), 5.28 (dd, 1H), 5.22 (d, 2H), 5.16 (dd, 1H).

^{13}C NMR (75 MHz, DMSO) δ: 176.9, 166.0, 150.4, 142.4, 139.2, 134.5, 132.6, 127.5, 121.2, 118.9, 108.8, 92.4, 55.8.

4.4.13. ({[2-(2-Propyn-1-yloxy)ethyl]oxy}methyl)benzene (13)

2-Benzyloxyethanol (107 g, 5 mL, 0.7 mol) was dissolved in THF (500 mL), stirred under N_2 atmosphere for 20 min and then cooled to 0–5 °C. Into this solution NaH (33.8 g, 60% in min. oil, 0.84 mol) was added in small portions. The reaction mixture was stirred at 35 °C for 30 min and then cooled to 0–5 °C. Propargyl bromide (80% solution in toluene, 100.4 g, 94 mL, 0.84 mol) was added dropwise and the stirring continued at rt under N_2 atmosphere overnight. The reaction was monitored by TLC in a system EtOAc/*n*-hexane = 1:4. THF was evaporated under reduced pressure. To the residue water (150 mL) was added and the mixture extracted with EtOAc (3 × 100 mL). Organic phase was dried over Na_2SO_4 and evaporated under reduced pressure yielding 131.12 g (98%) of the title compound **13** as yellow oil. The product was used in the next step without further purification.

MS (ES) *m*/*z* 190.92 $[M+H]^+$.

^{1}H NMR (300 MHz, DMSO) δ: 7.33–7.35 (m, 5H), 4.57 (s, 2H), 4.21 (d, 2H), 3.72 (m, 2H), 3.65 (m, 2H), 2.43 (t, 1H).

^{13}C NMR (75 MHz, DMSO) δ: 138.4, 128.5, 128.4, 127.8, 127.7, 73.3, 69.2, 61.2, 58.5, 19.8.

4.4.14. 1-Ethyl-4-oxo-6-[3-({2-[(phenylmethyl)oxy]ethyl}oxy)-1-propyn-1-yl]-1,4-dihydro-3-quinolinecarboxylic acid (14)

Into a solution of compound **10** (21 g, 0.06 mol) in a mixture MeCN/TEA = 1:1 (568 mL) at rt CuI (1.17 mg, 0.0061 mol) was added. The mixture was stirred for 20 min at rt and then warmed to 50 °C. Into that solution (2-prop-2-ynyloxy-ethoxymethyl)-benzene (**13**) (29 g, 0.15 mol) and $Pd(OPh_3)_2Cl_2$ (2.15 g, 0.003 mol) were added and the mixture was stirred at 50 °C overnight. The reaction was monitored by TLC in a solvent system DCM/MeOH/NH_4OH = 90:15:1.5. After 24 h starting compound was consumed. To the reaction mixture water (800 mL) and *i*-Pr_2O (600 mL) were added, pH was adjusted to 12 using 40% aq NaOH and the layers were separated. Aqueous layer was extracted with *i*-Pr_2O (2 × 400 mL). Aqueous layer was treated with charcoal at 80 °C over 10 min and filtered over Celite. After cooling to 25–35 °C pH was adjusted to 6 using 5% aq HCl. The resulting suspension was filtered and the cake dried at 50 °C in vacuum yielding 20.26 g (82%) of the title compound **14** as yellow solid.

HRMS (ES) calcd for $C_{24}H_{23}NO_5$ $[M+H]^+$ 406.1654 found 406.1639.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.57 (d, 1H), 7.83 (dd, 1H), 7.57 (d, 1H), 7.38–7.27 (m, 5H), 4.60 (s, 2H), 4.48 (s, 2H), 4.39 (q, 2H), 3.81 (m, 2H), 3.71 (m, 2H), 1.59 (t, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.8, 166.8, 148.1, 138.5, 138.0, 136.7, 130.7, 128.4–127.7, 126.5, 121.0, 116.5, 109.4, 87.9, 84.3, 73.4, 69.5, 69.3, 59.1, 49.8, 14.9.

4.4.15. 1-Cyclopropyl-4-oxo-6-[3-({2-[(phenylmethyl)oxy]ethyl}oxy)-1-propyn-1-yl]-1,4-dihydro-3-quinolinecarboxylic acid (15)

The title compound **15** as a yellowish solid was prepared in 78% yield starting from compound **11** according to the procedure used to prepare compound **14**.

HRMS (ES) calcd for $C_{25}H_{23}NO_5$ $[M+H]^+$ 418.1654 found 418.1666.

^{1}H NMR (300 MHz, DMSO) δ: 8.42 (s, 1H), 8.27 (d, 1H), 8.02 (d, 1H), 7.63 (dd, 1H), 7.27 (m, 5H), 4.54–4.52 (ov, 4H), 3.85 (m, 1H), 3.72 (t, 2H), 3.63 (t, 2H), 1.31 (m, 2H), 1.19 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ: 177.1, 165.4, 149.0, 140.6, 138.3, 136.2, 134.4, 127.9–126.6, 125.0, 119.8, 119.2, 107.8, 88.2, 83.9, 71.9, 68.7, 68.9, 58.1, 35.9, 7.5.

4.4.16. 4-Oxo-6-[3-({2-[(phenylmethyl)oxy]ethyl}oxy)-1-propyn-1-yl]-1-(2-propen-1-yl)-1,4-dihydro-3-quinolinecarboxylic acid (16)

The title compound **16** as a yellowish solid was prepared in 80% yield starting from compound **12** according to the procedure used to prepare compound **14**.

HRMS (ES) calcd for $C_{25}H_{24}NO_5$ $[M+H]^+$ 418.1654 found 418.1651.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.32 (d, 1H), 7.87 (dd, 1H), 7.50 (d, 1H), 7.36–7.19 (m, 5H), 6.07 (m, 1H), 5.27 (d, 1H), 5.23 (d, 2H), 5.20 (d, 1H), 4.53 (s, 2H), 4.47 (s, 2H), 3.74 (m, 2H), 3.63 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.8, 166.6, 150.4, 138.7, 136.5, 136.3, 132.6, 129.6, 128.9–127.8, 125.9, 121.1, 119.7, 119.0, 108.7, 88.1, 84.8, 72.4, 69.3, 69.2, 58.5, 55.9.

4.4.17. 1-Ethyl-6-{3-[(2-hydroxyethyl)oxy]propyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (17)

Hydrogenation of compound **14** (10 g, 0.025 mol) was performed in a mixture MeOH/DCM = 3:1 (120 mL) in the presence of 10% Pd/C (2 g, 20 wt %) at 5 bar pressure for 24 h. The reaction was monitored by TLC in a solvent system DCM/MeOH/NH_4OH = 90:15:1.5. After filtration of the catalyst, the solution was concentrated to dryness under reduced pressure yielding 6.9 g (87%) of the title compound **17** as a yellow solid. The product was used in the next step without further purification.

HRMS (ES) calcd for $C_{17}H_{21}NO_5$ $[M+H]^+$ 320.1498 found 320.1486.

^{1}H NMR (300 MHz, DMSO) δ: 9.02 (s, 1H), 8.19 (s, 1H), 7.98 (d, 1H), 7.84 (dd, 1H), 4.62 (q, 2H), 3.52 (m, 2H), 3.43–3.39 (ov, 4H), 2.86 (t, 2H), 1.88 (m, 2H), 1.43 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.6, 166.3, 148.7, 140.5, 137.5, 135.1, 125.7, 124.7, 118.2, 107.5, 72.2, 69.4, 60.4, 49.1, 31.2, 30.8, 14.8.

4.4.18. 1-Cyclopropyl-6-{3-[(2-hydroxyethyl)oxy]propyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (18)

The title compound **18** as a yellowish solid was prepared in 91% yield starting from compound **15** according to the procedure used to prepare compound **17**.

HRMS (ES) calcd for $C_{18}H_{21}NO_5$ $[M+H]^+$ 332.1498 found 332.1482.

^{1}H NMR (300 MHz, DMSO) δ: 8.72 (s, 1H), 8.22 (dd, 1H), 8.16 (d, 1H), 7.88 (dd, 1H), 3.83 (m, 1H), 3.52 (t, 2H), 3.43–3.39 (ov, 4H), 2.85 (t, 2H), 1.87 (m, 2H), 1.32 (m, 2H), 1.19 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ: 178.2, 166.4, 148.4, 141.0, 139.8, 135.3, 125.3, 124.7, 118.9, 107.5, 72.5, 69.6, 60.6, 36.3, 31.5, 31.2, 7.9.

4.4.19. 6-{3-[(2-Hydroxyethyl)oxy]propyl}-4-oxo-1-propyl-1,4-dihydro-3-quinolinecarboxylic acid (19)

The title compound **19** as a yellowish solid was prepared in 85% yield starting from compound **16** according to the procedure used to prepare compound **17**.

HRMS (ES) calcd for $C_{18}H_{24}NO_5$ $[M+H]^+$ 334.1654 found 334.1667.

^{1}H NMR (500 MHz, DMSO) δ: 9.02 (s, 1H), 8.19 (d, 1H), 8.00 (d, 1H), 7.84 (dd, 1H), 4.52 (t, 2H), 3.51 (m, 2H), 3.40 (ov, 4H), 2.84 (t, 2H), 1.88 (m, 2H), 1.83 (m, 2H), 0.91 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 177.9, 166.6, 149.3, 140.8, 138.0, 135.4, 125.8, 124.9, 118.7, 107.6, 72.4, 69.6, 60.6, 55.3, 31.4, 31.1, 22.4, 10.9.

4.4.20. 6-[3-({2-[(2-Cyanoethyl)oxy]ethyl}oxy)propyl]-1-ethyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (20)

Compound **17** (27.32 g, 0.085 mol) was dissolved in 10% aq NaOH (328 mL, 0.85 mol) and the solution cooled to 5–10 °C. Acrylonitrile (28.18 mL, 0.43 mol) was added dropwise and the reaction mixture was stirred at 15–20 °C for 2 h. The reaction was monitored by TLC in a solvent system DCM/MeOH/concd NH_3 = 90:15:1.5. To the mixture water (250 mL) was added and pH was adjusted to 6.3 using 6 M aq HCl. The precipitate was filtered off and the cake washed with water yielding 44.51 g of wet title compound **20** as a yellowish solid. The product was used in next step without drying.

HRMS (ES) calcd for $C_{20}H_{24}N_2O_5$ $[M+H]^+$ 373.1763 found 373.1757.

^{1}H NMR (300 MHz, DMSO) δ: 8.57 (s, 1H), 8.09 (d, 1H), 7.67 (d, 1H), 7.59 (dd, 1H), 4.34 (q, 2H), 3.62 (t, 2H), 3.58 (m, 2H), 3.52 (m, 2H), 3.42 (t, 2H), 2.76–2.74 (ov, 4H), 1.34 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.4, 167.2, 146.7, 137.2, 137.0, 132.6, 127.5, 125.1, 119.2, 116.5, 107.0, 69.6, 69.3, 69.2, 65.2, 47.3, 31.0, 30.7, 18.0, 14.4.

4.4.21. 6-[3-({2-[(2-Cyanoethyl)oxy]ethyl}oxy)propyl]-1-cyclopropyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (21)

The title compound **21** (1.83 g, wet) as a yellowish solid was prepared from starting compound **18** (1.34 g, 0.004 mol) according to the procedure used to prepare compound **20**.

HRMS (ES) calcd for $C_{21}H_{24}N_2O_5$ $[M+H]^+$ 385.1763 found 385.1744.

^{1}H NMR (300 MHz, DMSO) δ: 8.74 (s, 1H), 8.23 (d, 1H), 8.17 (d, 1H), 7.89 (dd, 1H), 3.85 (m, 1H), 3.64 (t, 2H), 3.61–3.52 (ov, 4H), 3.45 (t, 2H), 2.86 (t, 2H), 2.77 (t, 2H), 1.87 (m, 2H), 1.33 (m, 2H), 1.21 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ: 178.6, 166.8, 148.8, 141.3, 140.2, 135.7, 125.7, 125.1, 120.5, 119.3, 107.9, 70.4, 70.2, 70.0, 66.1, 36.6, 31.9, 31.5, 18.9, 8.3.

4.4.22. 6-[3-({2-[(2-Cyanoethyl)oxy]ethyl}oxy)propyl]-4-oxo-1-propyl-1,4-dihydro-3-quinolinecarboxylic acid (22)

The title compound **22** (0.90 g, wet) as a yellowish solid was prepared from starting compound **19** (0.73 g, 0.0023 mol) according to the procedure used to prepare compound **20**.

HRMS (ES) calcd for $C_{21}H_{27}N_2O_5$ $[M+H]^+$ 387.1920 found 387.1916.

^{1}H NMR (300 MHz, DMSO) δ: 8.84 (s, 1H), 8.16 (d, 1H), 7.89 (d, 1H), 7.75 (dd, 1H), 4.44 (t, 2H), 3.63 (t, 2H), 3.56 (m, 2H), 3.51 (m, 2H), 3.42 (t, 2H), 2.81 (t, 2H), 2.78 (t, 2H), 1.89 (m, 2H), 1.80 (m, 2H), 0.91 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.1, 166.8, 148.6, 139.0, 137.9, 134.5, 126.6, 125.2, 119.7, 118.1, 107.0, 70.0, 69.8, 69.7, 65.7, 54.8, 31.4, 31.1, 22.4, 18.4, 11.0.

4.4.23. 6-[3-({2-[(2-Carboxyethyl)oxy]ethyl}oxy)propyl]-1-ethyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (23)

Into an aqueous solution of H_2SO_4 (20 wt %, 208 mL) compound **20** (44.51 g, wet precipitate from the previous step) was added in portions while keeping the temperature at 5–10 °C. The reaction mixture was stirred at 10–20 °C for 1 h and then at rt for additional 18 h. The mixture was added dropwise into a stirring water (310 mL) while cooled to 10–20 °C. The resulting mixture was stirred at 70–80 °C for 24 h. The reaction was monitored by TLC in a solvent systems DCM/MeOH/NH_4OH = 90:15:1.5 and DCM/MeOH/MeCN/concd NH_3 = 4:4:1:2. The mixture was cooled in an ice-bath, diluted with water and pH adjusted to 1.3 using 40% aq NaOH. The resulting suspension was filtered, the cake washed with water and dried in vacuum at 60 °C yielding 39.06 g of the title compound **23** as a white solid.

HRMS (ES) calcd for $C_{20}H_{25}NO_7$ $[M+H]^+$ 392.1709 found 392.1690.

^{1}H NMR (300 MHz, DMSO) δ: 9.02 (s, 1H), 8.19 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 4.60 (q, 2H), 3.62 (t, 2H), 3.52 (m, 2H), 3.49 (m, 2H), 3.41 (t, 2H), 2.83 (t, 2H), 2.45 (t, 2H), 1.87 (m, 2H), 1.43 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.4, 172.5, 166.1, 148.4, 140.3, 137.3, 134.9, 125.4, 124.5, 118.0, 107.4, 69.5, 69.3, 69.2, 66.2, 48.9, 34.7, 31.0, 30.5, 14.5.

4.4.24. 6-[3-({2-[(2-Carboxyethyl)oxy]ethyl}oxy)propyl]-1-cyclopropyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (24)

The title compound **24** (1.34 g) as a white solid was prepared from starting compound **21** (1.83 g, wet) according to the procedure used to prepare compound **23**.

HRMS (ES) calcd for $C_{21}H_{25}NO_7$ $[M+H]^+$ 404.1709 found 404.1694.

^{1}H NMR (300 MHz, DMSO) δ: 8.72 (s, 1H), 8.24 (d, 1H), 8.16 (d, 1H), 7.88 (dd, 1H), 3.85 (m, 1H), 3.61 (t, 2H), 3.54–3.47 (ov, 4H), 3.42 (t, 2H), 2.84 (t, 2H), 2.45 (t, 2H), 1.87 (m, 2H), 1.31 (m, 2H), 1.19 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ: 177.7, 172.5, 165.9, 147.9, 140.5, 139.3, 134.9, 124.9, 124.3, 118.4, 107.1, 69.6, 69.4, 69.2, 66.2, 35.8, 34.7, 31.0, 30.6, 7.5.

4.4.25. 6-[3-({2-[(2-Carboxyethyl)oxy]ethyl}oxy)propyl]-4-oxo-1-propyl-1,4-dihydro-3-quinolinecarboxylic acid (25)

The title compound **25** (0.74 g) as a white solid was prepared from starting compound **22** (0.90 g, wet) according to the procedure used to prepare compound **23**.

HRMS (ES) calcd for $C_{21}H_{28}NO_7$ $[M+H]^+$ 406.1866 found 406.1851.

^{1}H NMR (300 MHz, DMSO) δ: 8.67 (s, 1H), 8.19 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 4.53 (t, 2H), 3.61 (t, 2H), 3.51 (m, 2H), 3.48 (m, 2H), 3.41 (t, 2H), 2.83 (t, 2H), 2.42 (t, 2H), 1.88 (m, 2H), 1.81 (m, 2H), 0.91 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.9, 173.1, 166.6, 149.3, 140.3, 138.0, 135.4, 125.9, 124.9, 118.6, 107.6, 70.0, 69.8, 69.6, 66.8, 55.3, 35.4, 31.4, 31.0, 22.4, 10.9.

4.4.26. 4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (26)

Compound **23** (34.17 g, 0.09 mol) was dissolved in DCM (155 mL) and the solution cooled to 0 °C in an ice-bath under N_2 atmosphere. Into the solution EDACxHCl (33.35 g, 0.17 mol), 2′-*O*-acetyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (53.12 g, 0.07 mol) and DMAP (24.60 g, 0.20 mol) were added. The reaction mixture was stirred at 0–5 °C for 2 h and then at rt for additional 4 h. The reaction was monitored by TLC in a solvent system DCM/MeOH/concd NH_3 = 90:15:1.5. The mixture was concentrated under reduced pressure to dryness. The residue was dissolved in *i*-PrOAc (400 mL) and the solution washed with satd aq $NaHCO_3$ (2 × 200 mL). Onto the organic layer water was added and pH was adjusted to 6.3 using a mixture AcOH/H_2O = 1:1. Organic layer was separated and washed once more with fresh water at pH 6.3. Combined aqueous layers were extracted with *i*-PrOAc (4 × 50 mL). Combined organic layers were dried over K_2CO_3 and concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (800 mL) and the solution stirred at 55 °C for 18 h. The reaction was monitored by TLC in a solvent system DCM/MeOH/concd NH_3 = 90:15:1.5. MeOH was evaporated under reduced pressure. The residue was dissolved in EtOAc (250 mL), water (250 mL) was added and pH adjusted to 3.3–3.5 using AcOH. Organic layer was separated and extracted once more with water (200 mL). Combined aqueous layers were washed with DCM (2 × 100 mL). Onto aqueous layer fresh DCM (300 mL) was added and pH adjusted to 4.3 using solution NH_3/H_2O 1:1. Aqueous layer was extracted with fresh DCM (2 × 200 mL). Combined DCM extracts at pH 4.3 were washed with water at pH 8.5, dried over K_2CO_3 and evaporated under reduced pressure, precipitated from *i*-PrOAc/di-*i*-Pr_2O yielding 38.12 g (50%) of the title compound **26** as a white solid.

HRMS (ES) calcd for $C_{58}H_{95}N_3O_{18}$ $[M+H]^+$ 1122.6689 found 1122.6647.

^{1}H NMR (300 MHz, DMSO) δ: 9.03 (s, 1H), 8.18 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 4.91 (d, 1H), 4.73 (dd, 1H), 4.59 (q, 2H), 4.55 (d, 1H), 4.43 (d, 1H), 4.33 (m, 1H), 4.17 (dd, 1H), 3.66 (m, 1H), 3.64 (m, 2H), 3.50 (ov, 2H), 3.47 (ov, 1H), 3.45 (ov, 1H), 3.43 (ov, 2H), 3.38 (t, 2H), 3.22 (s, 3H), 3.05 (dd, 1H), 2.81 (t, 2H), 2.67 (ov, 1H), 2.67 (ov, 1H), 2.59 (m, 2H), 2.40 (m, 1H), 2.35 (dd, 1H), 2.31 (d, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.11 (t, 1H), 1.88 (ov, 1H), 1.85 (ov, 2H), 1.85 (ov, 1H), 1.78 (m, 1H), 1.66 (dd, 1H), 1.59 (m, 1H), 1.51 (d, 1H), 1.42 (t, 3H), 1.37 (m, 1H), 1.27 (dd, 1H), 1.12 (s, 3H), 1.1 (s, 3H), 1.09 (ov, 1H), 1.08 (d, 3H), 1.07 (dd, 3H), 1.03 (d, 3H), 1.01 (s, 3H), 0.96 (d, 3H), 0.94 (d, 3H), 0.84 (d, 3H), 0.79 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.4, 177.0, 170.9, 166.1, 148.5, 140.2, 137.4, 134.9, 125.5, 124.6, 118.0, 107.5, 102.0, 94.3, 82.7, 78.0, 77.3, 76.3, 74.9, 73.5, 72.4, 72.0, 70.5, 69.4, 69.7, 69.2, 68.6, 66.8, 66.1, 64.8, 62.2, 61.4, 48.8, 44.5, 41.6, 40.2, 35.7, 34.9, 34.2, 31.1, 31.0, 30.6, 27.3, 26.0, 22.0, 21.6, 20.9, 20.5, 17.6, 14.5, 10.9, 8.9, 6.7, 4.9.

4.4.27. 4″-*O*-(3-{2-[3-(3-Carboxy-1-cyclopropyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (27)

The title compound **27** as a white solid was prepared in 53% yield starting from compound **24** according to the procedure used to prepare compound **26**.

HRMS (ES) calcd for $C_{59}H_{95}N_3O_{18}$ $[M+H]^+$ 1134.6689 found 1134.6666.

^{1}H NMR (300 MHz, DMSO) δ: 8.73 (s, 1H), 8.22 (d, 1H), 8.16 (d, 1H), 7.87 (dd, 1H), 4.91 (d, 1H), 4.74 (dd, 1H), 4.56 (d, 1H), 4.43 (d, 1H), 4.34 (m, 1H), 4.17 (dd, 1H), 3.84 (m, 1H), 3.69 (m, 1H), 3.66 (m, 2H), 3.47 (d, 1H), 3.43 (m, 1H), 3.38 (t, 2H), 3.33–3.37 (ov, 4H), 3.23 (s, 3H), 3.04 (t, 1H), 2.83 (t, 2H), 2.67 (m, 1H), 2.58 (t, 2H), 2.40 (m, 1H), 2.38 (d, 1H), 2.33 (d, 1H), 2.21 (s, 6H), 2.19 (s, 3H), 2.11 (t, 1H), 1.89 (m, 1H), 1.83–1.86 (ov, 3H), 1.78 (m, 1H), 1.67 (dd, 1H), 1.59 (m, 1H), 1.51 (s, 1H), 1.36 (m, 1H), 1.32 (m, 2H), 1.25 (m, 1H), 1.18 (m, 2H), 1.13 (s, 3H), 1.09 (d, 3H), 1.08 (d, 3H), 1.07–1.09 (ov, 1H), 1.03 (d, 3H), 1.01 (s, 6H), 0.95 (d, 3H), 0.94 (d, 3H), 0.85 (dd, 3H), 0.79 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.7, 177.0, 170.9, 165.9, 148.0, 140.4, 139.4, 134.8, 124.9, 124.3, 118.4, 107.1, 102.0, 94.3, 82.7, 78.0, 77.4, 76.3, 74.9, 73.5, 72.4, 70.5, 69.7, 69.4, 69.2, 68.6, 66.8, 66.1, 64.8, 62.2, 61.4, 48.8, 44.6, 41.7, 41.6, 40.3, 35.8, 35.7, 35.0, 34.3, 31.1, 30.7, 30.2, 27.4, 26.0, 22.0, 21.6, 20.5, 20.2, 17.7, 17.6, 14.6, 10.9, 8.9, 7.5, 6.7.

4.4.28. 4″-*O*-(3-{2-[3-(3-Carboxy-4-oxo-1-propyl-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (28)

The title compound **28** as a white solid was prepared in 51% yield starting from compound **25** according to the procedure used to prepare compound **26**.

HRMS (ES) calcd for $C_{59}H_{97}N_3O_{18}$ $[M+H]^+$ 1136.6845 found 1136.6842.

^{1}H NMR (300 MHz, DMSO) δ: 9.02 (s, 1H), 8.18 (d, 1H), 7.98 (d, 1H), 7.82 (dd, 1H), 4.95 (d, 1H), 4.75 (dd, 1H), 4.56 (q, 2H), 4.53 (d, 1H), 4.43 (d, 1H), 4.34 (m, 1H), 4.17 (d, 1H), 3.69 (m, 1H), 3.66 (t, 2H), 3.53–3.45 (ov, 4H), 3.47 (ov, 1H), 3.45 (ov, 1H), 3.38 (ov, 2H), 3.22 (s, 3H), 3.05 (dd, 1H), 2.81 (t, 2H), 2.67 (m, 2H), 2.58 (t, 2H), 2.41 (m, 1H), 2.34 (dbr, 1H), 2.28 (d, 1H), 2.22 (s, 6H), 2.19 (s, 3H),

2.12 (tbr, 1H), 1.88 (ov, 1H), 1.86–1.80 (ov, 2H), 1.83 (ov, 1H), 1.77 (m, 1H), 1.67 (dd, 1H), 1.61 (m, 1H), 1.52 (d, 1H), 1.38 (m, 1H), 1.27 (ddbr, 1H), 1.12 (s, 3H), 1.09 (d, 6H), 1.05 (ov, 1H), 1.02 (d, 3H), 1.01 (s, 6H), 0.95 (ov, 6H), 0.92 (t, 3H), 0.85 (d, 3H), 0.80 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.6, 177.2, 171.1, 166.3, 149.0, 140.5, 137.8, 135.1, 125.6, 124.7, 118.4, 107.4, 102.2, 94.5, 82.9, 78.2, 77.6, 76.5, 75.1, 73.7, 72.6, 70.62, 70.6, 69.9, 69.6, 69.5, 68.8, 66.3, 65.0, 32.4, 61.7, 55.0, 49.0, 44.7, 41.8, 41.5, 40.4, 35.9, 35.2, 34.4, 31.2, 30.8, 30.3, 27.5, 26.2, 22.2, 21.8, 21.4, 21.1, 20.7, 17.9, 17.8, 14.8, 11.1, 10.7, 9.1, 6.9.

4.4.29. 4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-clarithromycin (29)

Compound **23** (300 mg, 0.77 mmol) was dissolved in dry DMF (4 mL) and the solution cooled to 0 °C under N_2 atmosphere. To this solution EDACxHCl (293 mg, 1.53 mmol) was added and the mixture was stirred for 5 min. Then, 2′-*O*-acetylclarithromycin (468 mg, 0.59 mmol) solution in dry DCM (4 mL) was added followed by addition of DMAP (281 mg, 2.3 mmol). The mixture was stirred at rt for 24 h and monitored on TLC in a solvent system CH_2Cl_2/MeOH/concd NH_3 = 90:15 1.5. Saturated $NaHCO_3$ (20 mL) and EtOAc (20 mL) were added and the layers separated. Aqueous layer was extracted with EtOAc (2×20 mL). Combined organic layers were dried over K_2CO_3 and concentrated to dryness. The residue was dissolved in MeOH (100 mL) and the solution stirred at 55 °C for 24 h. MeOH was evaporated and the residue purified by column chromatography on silica-gel in a solvent system DCM/MeOH/concd NH_3 = 90:9:1.5. The residue was precipitated from EtOAc/*n*-hexane yielding 205 mg (*Y* = 31%) of the title compound **29** as a white solid.

HRMS (ES) calcd for $C_{58}H_{92}N_2O_{19}$ $[M+H]^+$ 1121.6373 found 1121.6404.

^{1}H NMR (300 MHz, DMSO) δ: 9.04 (s, 1H), 8.19 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 5.06 (dd, 1H), 4.86 (d, 1H), 4.60 (q, 2H), 4.56 (d, 1H), 4.43 (d, 1H), 4.27 (dq, 1H), 3.68 (m, 2H), 3.66 (t, 2H), 3.62 (br s, 1H), 3.60 (d, 1H), 3.55 (d, 1H), 3.50 (t, 2H), 3.46 (t, 2H), 3.39 (t, 2H), 3.21 (s, 3H), 3.04 (dd, 1H), 2.95 (dq, 1H), 2.91 (s, 3H), 2.85 (dq, 1H), 2.82 (t, 2H), 2.64–2.59 (m, 2H), 2.54 (m, 1H), 2.41 (m, 1H), 2.36 (d, 1H), 2.21 (s, 6H), 1.87–1.84 (m, 2H), 1.86 (ov, 1H), 1.83 (m, 1H), 1.76 (dd, 1H), 1.67 (dd, 1H), 1.59 (br d, 1H), 1.45 (dd, 1H), 1.42 (t, 3H), 1.39 (m, 1H), 1.23 (ov, 2H), 1.17 (ov, 1H), 1.13 (s, 3H), 1.09 (d, 3H), 1.06 (d, 3H), 1.04 (ov, 12H), 0.76 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 219.7, 177.4, 175.0, 170.9, 166.1, 148.5, 140.2, 137.4, 134.9, 125.5, 124.6, 118.1, 107.5, 101.9, 95.4, 79.9, 79.2, 77.8, 77.7, 75.9, 74.1, 72.1, 70.6, 69.6, 69.4, 69.2, 68.8, 66.8, 66.1, 64.5, 62.4, 50.1, 48.9, 48.8, 44.2, 43.7, 40.2, 38.6, 38.2, 37.9, 34.9, 34.2, 31.0, 30.6, 30.1, 21.5, 20.6, 20.3, 19.7, 18.1, 17.6, 16.9, 15.6, 14.6, 11.9, 10.4, 8.9.

4.4.30. 9-Ethyloxymino-4″-*O*-(3-{2-[3-(3-carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-erythromycin A (30)

The title compound **30** as a white solid was prepared in 27% yield starting from compounds **23** and 2′-*O*-acetyl-9-ethyloxyimino-erythromycin A according to the procedure used to prepare compound **29**.

HRMS (ES) calcd for $C_{59}H_{95}N_3O_{19}$ $[M+H]^+$ 1150.6638 found 1150.6667.

^{1}H NMR (300 MHz, DMSO) δ: 8.98 (s, 1H), 8.18 (d, 1H), 7.95 (d, 1H), 7.79 (dd, 1H), 5.12 (dd, 1H), 4.96 (q, 2H), 4.83 (d, 1H), 4.57 (q, 2H), 4.55 (d, 1H), 4.45 (d, 1H), 4.28 (m, 1H), 3.88 (d, 1H), 3.69–3.63 (ov, 3H), 3.50–3.44 (ov, 6H), 3.38 (t, 2H), 3.21 (s, 3H), 3.05 (ov, 1H), 3.02 (dd, 1H), 3.00 (m, 1H), 2.81 (ov, 3H), 2.67–2.57 (ov, 2H), 2.39 (m, 1H), 2.33 (d, 1H), 2.19 (s, 6H), 1.92 (m, 1H), 1.89–1.83 (ov, 3H), 1.66 (dd, 1H), 1.58 (br d, 1H), 1.47–1.36 (ov, 5H), 1.28 (s, 3H), 1.16 (t, 3H), 1.11 (d, 3H), 1.09 (ov, 1H), 1.08–1.06 (ov, 9H), 1.03 (d, 3H), 1.00–0.97 (ov, 9H), 0.75 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.2, 174.8, 170.8, 169.6, 166.2, 148.3, 140.0, 137.3, 134.6, 125.8, 124.6, 118.8, 107.0, 101.7, 95.0, 82.6, 78.0, 77.9, 76.3, 75.8, 73.7, 72.2, 70.5, 69.8, 69.7, 69.6, 69.2, 68.1, 66.7, 66.1, 64.6, 62.2, 48.8, 48.7, 44.2, 44.1, 40.2, 38.8, 38.7, 37.0, 34.9, 34.3, 31.0, 30.6, 30.0, 21.5, 21.0, 20.9, 20.3, 18.5, 17.7, 16.9, 15.7, 14.5, 14.3, 10.5, 8.9.

4.4.31. 4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-6-*O*-methyl-8a-aza-8a-homoerythromycin A (31)

The title compound **31** as a white solid was prepared in 41% yield starting from compounds **23** and 2′-*O*-acetyl-6-*O*-methyl-8a-aza-8a-homoerythromycin A according to the procedure used to prepare compound **29**.

HRMS (ES) calcd for $C_{58}H_{94}N_3O_{19}$ $[M+H]^+$ 1136.6482 found 1136.6501.

^{1}H NMR (300 MHz, DMSO) δ: 9.03 (s, 1H), 8.18 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 7.54 (d, 1H), 4.94 (d, 1H), 4.88 (dd, 1H), 4.60 (q, 2H), 4.56 (d, 1H), 4.39 (d, 1H), 4.30 (m, 1H), 4.06 (s, 1H), 3.95 (m, 1H), 3.86 (d, 1H), 3.69–3.62 (ov, 3H), 3.54 (d, 1H), 3.52–3.46 (ov, 4H), 3.40–3.37 (ov, 3H), 3.22 (s, 3H), 3.03 (dd, 1H), 2.97 (s, 3H), 2.83 (t, 2H), 2.64 (dq, 1H), 2.61–2.52 (ov, 2H), 2.40–2.35 (ov, 2H), 2.29 (d, 1H), 2.21 (s, 6H), 1.94 (m, 1H), 1.87–1.84 (ov, 3H), 1.68 (dd, 1H), 1.63 (dd, 1H), 1.59 (br d, 1H), 1.42 (t, 3H), 1.37 (m, 1H), 1.20 (s, 3H), 1.11–1.07 (ov, 6H), 1.05 (ov, 1H), 1.03–1.01 (ov, 6H), 1.01 (s, 3H), 0.98 (d, 3H), 0.97–0.94 (ov, 6H), 0.80 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 177.9, 177.8, 173.5, 171.3, 166.6, 148.9, 140.7, 137.8, 135.3, 125.9, 125.0, 118.5, 107.9, 102.7, 94.6, 79.0, 78.5, 78.4, 76.3, 76.0, 74.5, 72.8, 71.2, 70.9, 70.1, 69.8, 69.7, 67.3, 66.5, 65.1, 62.7, 51.7, 49.3, 49.2, 45.3, 42.4, 41.9, 40.7, 40.6, 35.4, 34.4, 31.4, 31.3, 31.0, 30.7, 24.6, 21.9, 21.6, 20.9, 20.4, 18.3, 17.3, 15.0, 14.9, 14.3, 11.6, 9.8, 9.3.

4.4.32. 11-*N*,12-*O*-Carbonyl-4″-*O*-(3-{2-[3-(3-carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-11-desoxy-6-*O*-methyl-11-methylamino-erythromycin A (32)

The title compound **32** as a white solid was prepared in 27% yield starting from compounds **23** and 2′-*O*-acetyl-11-*N*,12-*O*-carbonyl-11-desoxy-6-*O*-methyl-11-methylamino-erythromycin A according to the procedure used to prepare compound **29**.

HRMS (ES) calcd for $C_{60}H_{93}N_3O_{19}$ $[M+H]^+$ 1160.6482 found 1160.6508.

^{1}H NMR (300 MHz, DMSO) δ: 9.04 (s, 1H), 8.19 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 4.85 (d, 1H), 4.78 (dd, 1H), 4.59 (q, 2H), 4.57 (d, 1H), 4.44 (d, 1H), 4.26 (dq, 1H), 3.66 (ov, 3H), 3.63 (ov, 1H), 3.53–3.46 (ov, 6H), 3.39 (t, 2H), 3.21 (s, 3H), 3.07–3.01 (ov, 2H), 2.87 (ov, 1H), 2.85 (s, 3H), 2.82 (t, 2H), 2.64–2.59 (ov, 2H), 2.45 (m, 1H), 2.42 (m, 1H), 2.35 (d, 1H), 2.23 (s, 6H), 2.09 (s, 3H), 1.85 (m, 2H), 1.81 (ov, 1H), 1.71–1.68 (ov, 3H), 1.62 (br d, 1H), 1.54 (ov, 2H), 1.42 (t, 3H), 1.38 (s, 3H), 1.22 (s, 3H), 1.16 (d, 3H), 1.10 (ov, 7H), 1.05 (ov, 3H), 1.04 (ov, 3H), 1.02 (s, 3H), 0.88 (d, 3H), 0.78 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 215.2, 177.4, 176.2, 170.9, 166.1, 156.5, 148.5, 140.2, 137.4, 134.9, 125.5, 124.5, 118.1, 107.5, 101.9, 95.2, 82.4, 78.7, 78.1, 77.7, 76.3, 75.1, 72.1, 70.5, 69.7, 69.4, 69.3, 66.9, 66.1, 64.5, 62.4, 61.2, 49.4, 48.9, 48.8, 44.9, 44.5, 40.2, 38.6, 38.5, 38.0, 34.9, 34.1, 31.9, 31.0, 30.6, 30.1, 21.5, 21.4, 20.3, 19.6, 18.0, 17.8, 15.4, 14.6, 13.4, 13.3, 10.1, 8.8.

4.4.33. 4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-roxythromycin (33)

The title compound **33** as a white solid was prepared in 30% yield starting from compounds **23** and 2′-*O*-acetyl-roxythromycin according to the procedure used to prepare compound **29**.

HRMS (ES) calcd for $C_{61}H_{99}N_3O_{21}$ $[M+H]^+$ 1210.6849 found 1210.6946.

^{1}H NMR (500 MHz, DMSO) δ: 9.04 (s, 1H), 8.19 (d, 1H), 7.99 (d, 1H), 7.83 (dd, 1H), 5.13 (dd, 1H), 5.05 (m, 2H), 4.82 (d, 1H), 4.60 (q, 2H), 4.56 (d, 1H), 4.44 (d, 1H), 4.29 (dq, 1H), 3.92 (nrdd, 1H), 3.66 (ov, 6H), 3.50 (ov, 3H), 3.41 (ov, 6H), 3.38 (d, 1H), 3.24 (s, 3H), 3.21 (s, 3H), 3.03 (d, 1H), 2.86 (ov, 4H), 2.81 (ov, 3H), 2.70 (dq, 1H), 2.55 (ov, 2H), 2.41 (m, 1H), 2.33 (d, 1H), 2.21 (s, 6H), 1.86 (ov, 4H), 1.68 (dd, 1H), 1.60 (m, 1H), 1.50 (ov, 2H), 1.42 (t, 3H), 1.38 (m, 1H), 1.28 (s, 3H), 1.17 (ov, 4H), 1.09 (ov, 9H), 1.02 (d, 3H), 1.00 (0v, 9H), 0.76 (t, 3H).

^{13}C NMR (125 MHz, DMSO) δ: 177.9, 176.0, 171.5, 171.4, 171.3, 166.6, 149.0, 140.7, 137.8, 135.4, 125.9, 125.0, 119.5, 107.9, 102.3, 97.1, 95.5, 83.0, 79.1, 78.4, 76.3, 75.4, 74.2, 72.6, 71.6, 70.9, 70.8, 70.1, 69.8, 69.6, 67.3, 67.2, 66.5, 65.2, 62.7, 58.4, 49.4, 49.3, 44.6, 40.6, 39.8, 39.4, 37.6, 35.4, 34.8, 31.4, 31.0, 30.3, 26.8, 26.7, 21.4, 21.3, 20.9, 19.1, 18.2, 17.5, 16.3, 15.1, 15.0, 11.0, 9.4.

4.4.34. 11-Amino-11-*N*,12-*O*-carbonyl-4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propiony)-11-desoxy-6-*O*-methyl-erythromycin A (34)

The title compound **34** as a white solid was prepared in 70% yield starting from compounds **23** and 2′-*O*-acetyl-11-amino-11-*N*,12-*O*-carbonyl-11-desoxy-6-*O*-methyl-erythromycin A according to the procedure used to prepare compound **26**.

HRMS (ES) calcd for $C_{59}H_{91}N_3O_{19}$ $[M+H]^+$ 1146.6325 found 1146.6326.

^{1}H NMR (300 MHz, DMSO) δ: 9.03 (s, 1H), 8.19 (d, 1H), 7.98 (d, 1H), 7.83 (dd, 1H), 7.71 (s, 1H), 4.87 (dd, 1H), 4.84 (d, 1H), 4.60 (q, 2H), 4.55 (d, 1H), 4.43 (d, 1H), 4.27 (m, 1H), 3.62–3.67 (ov, 4H), 3.47–3.52 (ov, 5H), 3.46 (d, 1H), 3.39 (t, 2H), 3.21 (s, 3H), 3.04 (dd, 1H), 2.95 (dq, 1H), 2.83 (ov, 1H), 2.81 (t, 2H), 2.8 (s, 3H), 2.61 (ov, 1H), 2.53 (ov, 1H), 2.35–2.41 (ov, 2H), 2.33 (d, 1H), 2.20 (s, 6H), 1.85 (m, 2H), 1.82 (ov, 1H), 1.76 (ov, 1H), 1.69 (dd, 1H), 1.68 (m, 1H), 1.60 (m, 1H), 1.54 (m, 1H), 1.50 (ov, 1H), 1.42 (t, 3H), 1.37 (s, 3H), 1.22 (s, 3H), 1.16 (d, 3H), 1.07–1.09 (ov, 6H), 1.06 (ov, 1H), 1.01–1.04 (ov, 9H), 0.97 (d, 3H), 0.79 (t, 3H).

^{13}C NMR (75 MHz, DMSO) δ: 216.2, 177.4, 176.4, 170.8, 166.1, 157.2, 148.5, 140.2, 137.3, 134.9, 125.5, 124.6, 118, 107.6, 101.9, 95.1, 83.1, 79.1, 77.9, 77.8, 76.4, 74.9, 72.1, 70.5, 69.6, 69.4, 69.2, 66.9, 66.1, 64.6, 62.4, 56.7, 49.5, 48.9, 48.8, 44.6, 44.4, 40.2, 38.4, 39.1, 36.9, 34.9, 34.1, 30.9, 30.6, 30.1, 19.5, 21.5, 21.4, 20.3, 17.9, 17.6, 15.2, 14.5, 13.4, 13.1, 10.3, 8.7.

4.4.35. 4″-*O*-(3-{2-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-ethoxy}-propionyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (26) diisopropyl ether solvate single crystals

Compound **26** (24.4 g) was dissolved by heating in isopropylacetate (41.5 mL). The resulting solution was cooled to room temperature and added into diisopropylether (487 mL) during 45 min under vigorous stirring. The obtained precipitate was filtered off and the mother liquor was maintained in closed vessel at room temperature for 48 h. Generated single crystals were sent for single-crystal X-ray diffraction studies without isolation.

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References and notes

1. Schönfeld, W.; Mutak, S. Azithromycin and Novel Azalides. In *Macrolide Antibiotics*; Schönfeld, W., Kirst, H. A., Eds.; Brinkauser: Basel, 2002; pp 73–97.
2. Chu, D. T. W. *Med. Chem. Res. Rev.* **1999**, *19*, 497.
3. Bryskier, A. *Expert Opin. Investig. Drugs* **1999**, *8*, 1171.
4. Fiese, E. F.; Steffen, S. H. *J. Antimicrob. Chemother.* **1990**, *25*, 39.
5. Kurath, P.; Jones, P. H.; Egan, R. S.; Perun, T. J. *Experientia* **1971**, *27*, 362.
6. Morimoto, S.; Misawa, Y.; Adachi, T.; Nagate, T.; Watanabe, Y.; Omura, S. *J. Antibiot.* **1990**, *43*, 286.
7. Beekmann, S. E.; Heilmann, K. P.; Richter, S. S.; Garcia-de-Lomas, J.; Doern, G. V. *Int. J. Antimicrob. Agents* **2005**, *25*, 148.
8. Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H. W.; Scheld, W. M.; Bartlett, J. G.; Edwards, J., Jr. *J. Clin. Infect. Dis.* **2008**, *46*, 155.
9. Payne, D. J. *Science* **2008**, *321*, 1644.
10. Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. *J. Clin. Infect. Dis.* **2009**, *48*, 1.
11. Tennakoon, M. A.; Henninger, T. C.; Abbanat, D.; Foleno, B. D.; Hilliard, J. J.; Bush, K.; Macielag, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6231.
12. Andreotti, D.; Bientenesi, I.; Bionid, S.; Donati, D.; Erbetti, I.; Lociuor, S.; Marchioro, C.; Pozzan, A.; Ratti, E.; Terreni, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5265.
13. Heggelund, A.; Undheim, K. *Bioorg. Med. Chem.* **2007**, *15*, 3266.
14. Đokić, S.; Kobrehel, G.; Lazarevski, G.; Lopotar, N.; Tamburašev, Z.; Kamenar, B.; Nagl, A.; Vicković, I. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1881.
15. (a) Đokić, S.; Kobrehel, G.; Lopotar, N.; Kamenar, B.; Nagl, A.; Mrvoš, D. *J. Chem. Res. (S)* **1988**, 152; (b) Tamburašev, Z.; Đokić, S. *Croat. Chem. Acta* **1998**, *40*, 93.
16. Kobrehel, G.; Lazarevski, G.; Đokić, S.; Kolacny-Babić, L. *J. Antibiot.* **1992**, *45*, 527.
17. Kobrehel, G.; Lazarevski, G.; Kelnerić, Ž.; Đokić, S. *J. Antibiot.* **1993**, *46*, 1239.
18. Kujundžić, N.; Kobrehel, G.; Banić, Z.; Kelnerić, Ž.; Koić-Prodić, B. *Eur. J. Med. Chem.* **1995**, *30*, 455.
19. Hutinec, A.; Đerek, M.; Lazarevski, G.; Šunjić, V.; Čipčić Paljetak, H.; Alihodžić, S.; Eraković Haber, V.; Dumić, M.; Mutak, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3244.
20. Fajdetić, A.; Čipčić Paljetak, H.; Lazarevski, G.; Hutinec, A.; Alihodžić, S.; Đerek, M.; Štimac, V.; Andreotti, D.; Šunjić, V.; Berge, J. M.; Mutak, S.; Dumić, M.; Lociuro, S.; Holmes, D. J.; Maršić, N.; Eraković Haber, V.; Spaventi R. *Bioorg. Med. Chem. Lett.*, in press.
21. Ceccheti, V.; Clementi, S.; Cruciani, G.; Fravolini, A.; Pagella, P. G.; Savino, A.; Tabarrini, O. *J. Med. Chem.* **1995**, *38*, 973.
22. Hooper, D. C.; Rubinstein, E. *Quinolone Antimicrobial Agents*; ASM Press: Washington, DC, USA, 2003.
23. Ellis, J.; Gellert, E.; Robson, J. *Aust. J. Chem.* **1973**, *26*, 907.
24. Jones, P. H.; Forest, J.; Perun, T. J. US 3736313, 1973.
25. Jones, P. H.; Perun, T. J.; Rowely, E. K.; Baker, E. J. *J. Med. Chem.* **1972**, *15*, 631.
26. Baker, W. R.; Clark, J. D.; Stephens, L.; Kim, K. H. *J. Org. Chem.* **1988**, *53*, 2340.
27. Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46–49; (b) Rossi, R.; Carpita, A.; Bellina, F. *Org. Prep. Proced. Int.* **1995**, *27*, 129.
28. Wiegand, J. M.; Schäfer, C.; Palaoro, M.; Skranc, W.; Maurer, O. WO 2007096034, 2007.
29. (a). *Org. Synth.* **1943**, *Coll. Vol. 2*, 25; (b). *Org. Synth.* **1941**, *Coll. Vol. 1*, 436.
30. http://www.zirchrom.com/organic.htm, Dissociation Constants of Organic Acids and Bases.
31. Saleh, M. S.; Idriss, K. S.; Abu-Bakr, M. S.; Hashem, E. Y. *Analyst* **1992**, *117*, 1003.
32. Williams, A.; Ibrahim, I. T. *J. Am. Chem. Soc.* **1981**, *103*, 7090.
33. Gamet, J.-P.; Jacquier, R.; Verducci, J. *Tetrahedron* **1984**, *40*, 1995.
34. Performance Standards for Antimicrobial Susceptibility Testing: 15th Informational Supplement M100-S15. Clinical Laboratory Standards Institute CLSI, Wayne, PA, 2005.
35. Oxford Diffraction, Oxford Diffraction Ltd, Xcalibur CCD System, CrysAlis Software System, Version 1.170, 2003.
36. Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837.
37. Altamore, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343.
38. Sheldrick, M. SHELXL-97, Program for the Refinement of Crystal Structures. University of Göttingen, Germany, 1997.
39. DeLano, W. L. The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, USA, 2002.