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Synthesis and properties of macrolones characterized by two ether bonds in the linker

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ABSTRACT

In this paper synthesis of macrolones **1–18** starting from azithromycin is reported. Two key steps in the construction of the linker between macrolide and quinolone moiety, are formation of central ether bond by alkylation of unactivated OH group, and formation of terminal C–C bond at 6-position of the quinolone unit. Due to the difficulty in formation of these two bonds the study of alternative synthetic methodologies and optimization of the conditions for the selected routes was required. Formation of C-4″-*O*-ether bond was completed by modified Michael addition, whereas O-alkylation via diazonium cation proved to be the most effective in formation of the central allylic or propargylic ether bond. Comparison of Heck and Sonogashira reaction revealed the former as preferred route to the C–C bond formation at C(6) position of the quinolone unit. Most of the target compounds exhibited highly favorable antibacterial activity against common respiratory pathogens, without significant cytotoxicity profile when tested in vitro on eukaryotic cell lines.

1. Introduction

The search for new generation of antibiotics derived from the natural product erythromycin via azithromycin and clarithromycin resulted in specific chemical modifications of these two structures. These can be summarized as rationalization of the natural structure by removal of cladinose sugar[1,2] and incorporation of additional potential pharmacophore units. The resulting 3-hydroxy derivatives of macrolides are further modified by acylation,[3,4] oxidation to 3-keto derivatives,[3,5–7] and further modifications of these later structures.[8–11]

The goal of this project is a modification of the structures of known macrolide antibiotics in order to obtain new class of semisynthetic antibiotics with improved potency, especially against resistant and Gram-negative bacterial strains. Recently we reported the synthesis of azithromycin-congeners, 4″-*O*-acyl derivatives of 8a-aza-8a-homoerythromycin[12] which showed potent antibacterial activity against sensitive pathogens and improved activity against several species of efflux (M) and inducible ($iMLS_B$) macrolide-resistant Gram-positive pathogens. This group of derivatives that comprise (hetero)aromatic units tethered to macrolides represent an inventive departure from the concepts mentioned above since the cladinose is kept in place.

Further extensive exploration of the idea to attach different aromatic units to 4″-*O*-position of 8a-aza-8a-homoerythromycin via appropriate alkyl linker resulted in compounds with quinolone pharamacophore unit that showed overall improved antibacterial profile.[13] As we had been trying to define structure–activity relationship, various structural units were modified: macrocyclic scaffold (azithromycin, clarithromycin, erythromycin 9-oxime), length of the linker between a macrolide and quinolone units, position of the linker on the quinolone unit, type of heteroatoms, and their different positions in the linker.

It was repeatedly observed that the number and position of heteroatoms in the linker, O and N in particular, significantly contribute to the biological profile, ADME and in vitro activity.[14,15]

In extension to this project macrolones linked through a methylene group to C(6) position of the quinolone unit were explored.[16] The results described in this paper are following these structures but with one major difference: 4″-ester bond is replaced by an ether. In addition, triggered by their high potency across tested bacteria, eventual non-specific in vitro cytotoxicity potential of macrolone compounds has been investigated on defined set of standard eukaryotic cell lines.

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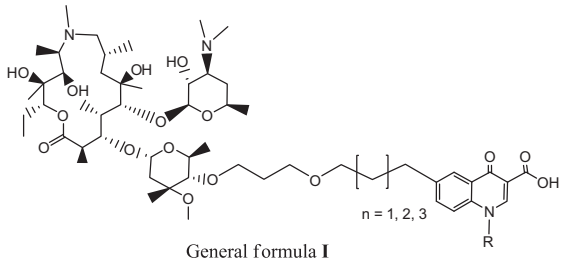
Structures reported in this paper are presented by the general formula **I**. These structures are characterized by the C–C bond at C(6) position of the quinolone-3-carboxylic acid, specific linker length, and two ether bonds: the 'central one' and the 'terminal one' connected to C(4″) position of L-cladinose on the azithromycin scaffold.



General formula **I**

The apparently simple synthetic concept of linking the heterocycle to the 4″-oxygen by a flexible ether link proved a formidable task. This is in particular due to a requirement to introduce two ether bonds in the presence of many reactive groups in the macrolide subunit. Site-selective O-alkylation of the functionality-rich macrolides requires fine tuning of various factors, in particular selection of the protecting group, most convenient alkylating agent and promotion of its reactivity by selection of the solvent, temperature, order of addition, etc. A large body of experimental results revealed that many well-known ether bond forming reactions, such as Williamson synthesis in its classic form,[17,18] PTC catalyzed process,[19–21] homogeneous[22,23] or heterogeneous Lewis-acid catalyzed process,[24] as well as modern approaches via specific activation of hydroxy group or alkylating agent, such as the Mitsunobu reaction,[25–27] Maruoka dehydrative etherification of alcohols,[28] Mukaiyama oxidation–reduction condensation to ethers,[29,30] reductive etherification of aldehydes,[31] anomalous reduction of esters,[32–37] and lactones[38] to ethers, are not applicable on the route to target macrolones **I**. Promising preliminary results were obtained with Pd-catalyzed O-alkylation by allyl-*tert*-butylcarbonate,[39,40] previously applied on the macrolide scaffold.[41,42] In the second key step the C–C bond on the quinolone unit was formed. Two approaches, Heck and Sonogashira reactions were studied in some detail. The routes reported hereafter are selected after many attempts to introduce specific bonds in the linker and connect it to the terminal subunits.

2. Results and discussion

2.1. Chemistry

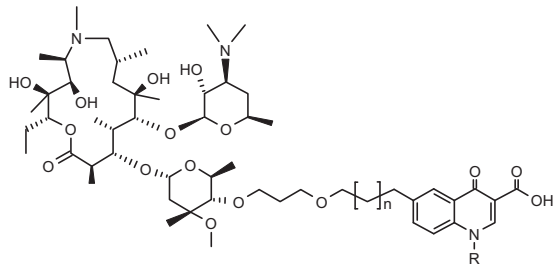
The structures of all target compounds are shown in Figure 1. Based on our previous results,[12–16] we focused our synthetic efforts on the three groups of the target macrolones, macrolide 4″-*O*, quinolone C(6) and N(1), by implementing various linker lengths and substituents of diverse polarity, Figure 1. All central oxygen atoms are three methylene groups away from 4″-*O*.

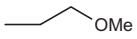
The first set of macrolones comprises compounds **1**–**9** with a fixed length of the C_6O_2 linker and selected substituents at N(1) position of quinolone ring. The second group (**10–14**) has (3 + 4) or (3 + 5) methylene units in the linker, and the third group (**15-18**) has a conformationally restricted tricyclic quinolone moiety.

2.1.1. Synthetic route to the target macrolones via Heck reaction in the key C–C bond forming step

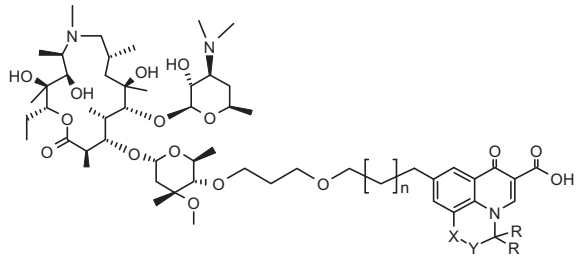
The Heck reaction was evaluated on the route to macrolones **1–7** with various N-substituents on the quinolone-3-carboxylic acid and fixed C_6O_2-linker, according to Scheme 1.

Starting from 2′-*O*-acetylazithromycin 11,12-cyclic carbonate (**19**), whose preparation has been repeatedly reported,[43,44] γ-ami-



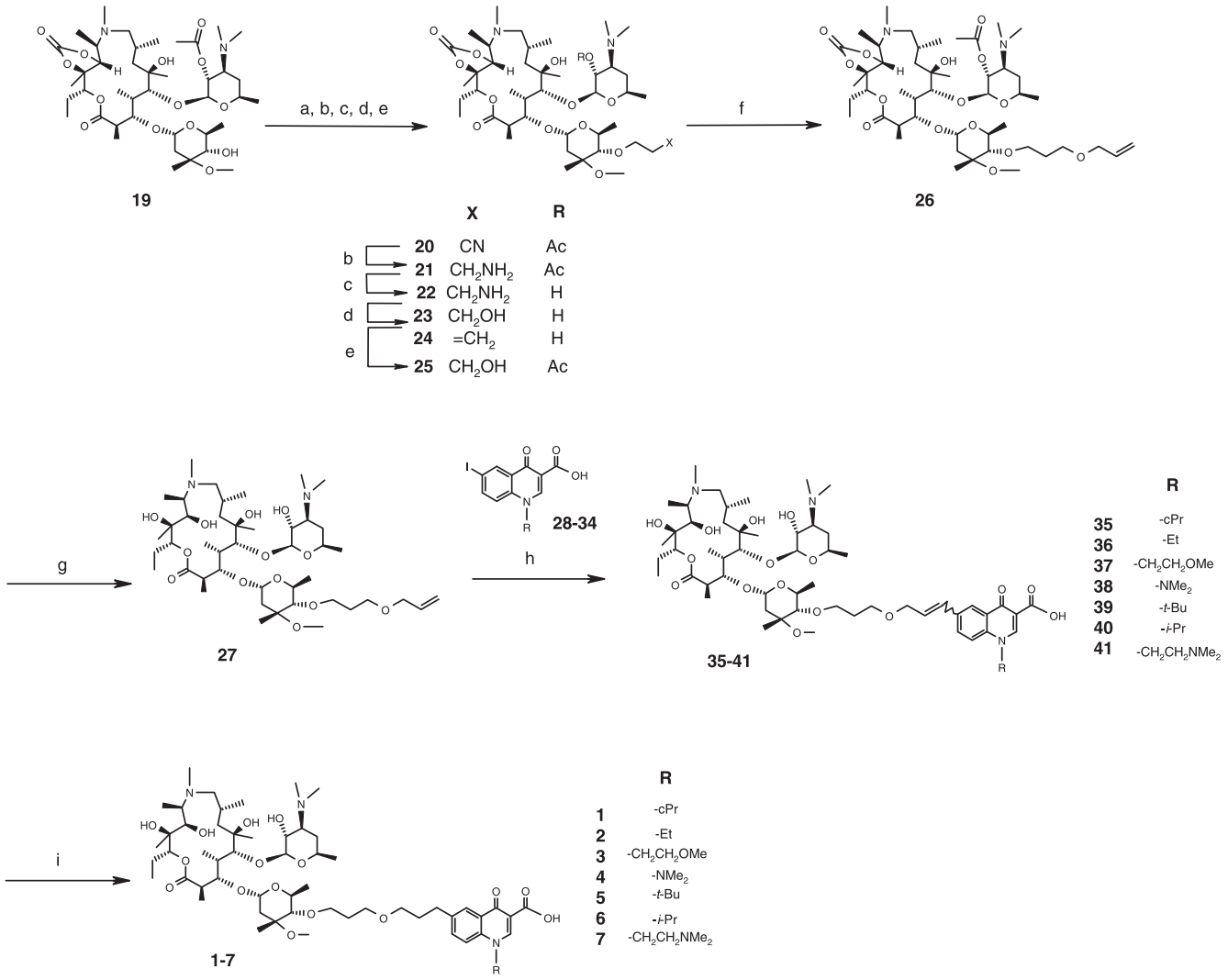
Compound	n	R
1	1	
2	1	-Et
3	1	
4	1	$-NMe_2$
5	1	-*t*-Bu
6	1	-*i*-Pr
7	1	
8	1	-*n*-Pr
9	1	-*n*-Bu

Compound	n	R
10	2	
11	2	-Et
12	2	$-NMe_2$
13	3	-Et
14	3	$-NMe_2$



Compound	n	R	X	Y
15	1	Me	CH_2	O
16	1	H	O	CH_2
17	1	Me	O	CH_2
18	2	Me	O	CH_2

Figure 1. Structures of the targeted macrolones **1**–**18**.

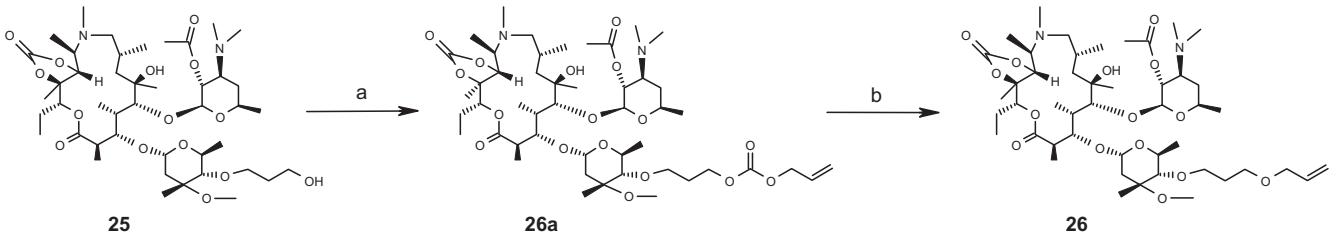


Scheme 1. Synthetic route to macrolones **1**–**7**. Reagents and conditions: (a) acrylonitrile 250 equiv, *t*-BuOH 3.2 equiv, NaH 1.1 equiv, 0 °C-rt, 24 h; (b) acetic acid, 20 wt % PtO_2, H_2 5 bar, 18 h; (c) MeOH, pH 8, 55 °C, 24 h; (d) 10% aq sol. AcOH, $NaNO_2$ 6 equiv, 0-rt, 2 h, column chromatography, separation of **23** and **24**; (e) *i*-PrOH/EtOAc 1:1, acetic anhydride 1.3 equiv, rt 8 h; (f) allyl-*t*-butyl carbonate 29 equiv, $Pd_2(dba)_3$ 0.1 equiv, dppb 0.2 equiv, 80 °C, 4 h, $Pd(PPh_3)_4$ 0.17 equiv, 80 °C, 1 h; (g) MeOH/H_2O 3:1, K_2CO_3 15 equiv, 55 °C, 2 h; (h) DMF, $Pd(OAc)_2$ 0.2 equiv, tri-*o*-tolyl phosphin 0.4 equiv, quinolones **28**–**34** 2.5 equiv, Et_3N 4 equiv, N_2 atmosphere, 65 °C, 2 h, 75 °C, 18 h; (i) MeOH, 10% Pd/C 10 wt %, H_2 3 bar, 15 h.

no derivative **22** was prepared via **21** according to the reported procedure.[45] The first key step of this route turned out to be diazotation of **22** followed by hydrolytic decomposition of diazonium salt to **23**. The first attempts resulted in a high ratio of *O*-allyl ether **24** as the by-product. This step was improved when $NaNO_2$ was substituted for *iso*-amylnitrite as diazotating agent and the reaction performed in $CHCl_3$ with 8 equiv of AcOH at 60 °C for 30 min. In the next step central ether bond in **26** was formed by Pd(II) catalyzed alkylation of **25**, according to Haight et al.[46] Allylation of the primary OH group in the linker was studied on the fully protected derivative **25**. Initial conditions 1–3 mol % $Pd(OAc)_2$/2–6 mol % Ph_3P in THF at 60–65 °C, or 1–3 mol % of $Pd(Ph_3)_4$, or 2–4 mol % $Pd_2(dba)_3$/4–8 mol % Ph_3P in THF, were optimized by performing the reaction in allyl-*tert*-butyl carbonate as a solvent at 80 °C using 0.1 equiv ($Pd_2(dba)_3$)/$CHCl_3$ complex/0.25 equiv dppb according to Scheme 2. After 4 h LC/MS revealed only 20% of the desired product **26** and 80% of the intermediate **26a**. By adding 0.17 equiv of $Pd(PPh_3)_4$ decarboxylation was completed in 1 h, LC/MS revealed complete conversion to allyl ether derivative **26**. Site-selective decarboxylation of the allyl carbonate unit in **26a** in the presence of cyclic carbonate at 11,12-position is remarkable. Deprotection was completed on brief treatment by potassium carbonate at slightly elevated temperature to obtain key intermediate **27**, Scheme 1.

Although workable for gram-scale preparation of **27** from **25**, the expensive palladium catalyst precluded scale-up of this process. In search for a scaleable method we started from the assumption that a diazonium cation, formed on transformation of **22**–**23**, may be capable of alkylating allyl alcohol. Having in hand workable protocol for diazo-alkylation of allylic alcohol, we decided to explore a shortcut approach, allyloxylation of the intermediary diazonium salts. This route was checked with **21** as a starting amine. The reactions were performed in allyl alcohol with 6 equiv of $NaNO_2$ at 0 °C to rt for 24 h, Scheme 3. Several different acids (H_3PO_4, AcOH, HCOOH, HCl) were tested and the best result was obtained when 4.5 equiv of HCOOH were used. We were delighted to see >80% purity of **27** obtained upon deprotection of **26** and isolation by column chromatography with 25% overall yield from **21**.

This result prompted the study of the second key step, C–C bond formation to 6-iodo-quinolones **28–34**. The Heck reaction[47,48] was selected and $Pd(OAc)_2$/tris-*o*-tolyl phosphine was used as a catalytic complex. The optimized protocol required 2.5 mol excess of quinolones in DMF as the solvent and two different temperatures. Compounds **35–41** were isolated in 60–90% yield, purified by



Scheme 2. Model reaction, approach to compound **26**. Reagents and conditions: (a) Allyl-*tert*-butyl carbonate 29 equiv, $Pd_2(dba)_3$ 0.1 equiv, dppb 0.25 equiv, 80 °C, 4 h; (b) $Pd(PPh_3)_4$ 0.17 equiv, 80 °C, 1 h.

extraction at various pH values and precipitated from EtOAc/*n*-hexane. Samples of the compounds **35–41** were isolated with >90% purity. Site-selective hydrogenation of allylic C=C bond in **35–41** was completed, and hydrogenolysis of allylic C–O bond and hydrogenation of the cross-conjugated C=C bond in the quinolone ring being suppressed, at 3 bar of H_2 with 10% Pd/C as a catalyst, as monitored by LC/MS. Under optimized conditions complete conversion of starting compounds and >90% selectivity was achieved to give **1–7**. Chromatographic purification of final products was performed with a mixture of DCM/MeOH/aq NH_3 (90:9:0.5) as the eluent and compounds **1–7** were isolated with >92% purity.

2.1.2. Synthetic route to target macrolones via Sonogashira reaction in the key C–C bond forming step

The Heck reaction is known for incomplete site-selectivity in the reaction of C=C bond[49] and we also observed a formation of significant amount of the *endo*-products. During the preparation of compound **4** on the multi-gram scale ~10–15% of *endo*-product was obtained. Due to the difficulties in the separation of the *endo*-isomers an alternative synthetic approach was investigated using the Sonogashira reaction in the key step.

On the route to terminal alkyne precursors **43–45** a reaction of diazonium ion with alkynyl alcohols, Scheme 4, was optimized. Removal of 11,12-carbonate and 2′-acetyl groups was favorably performed before a formation of the ether bond in **43–45**.

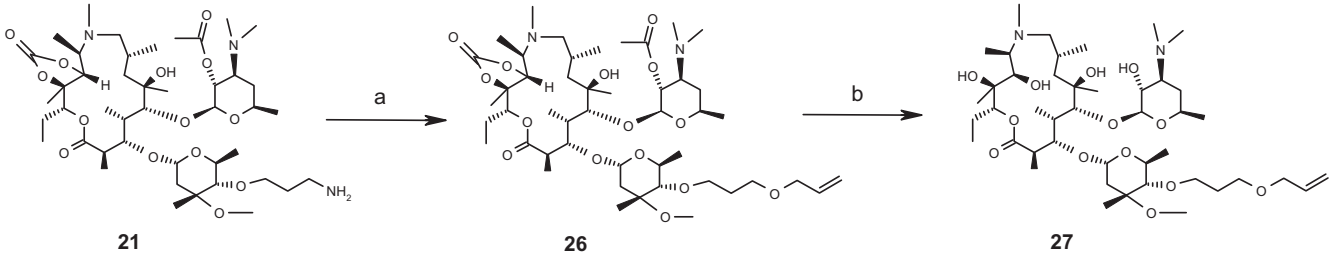
Intermediary macrolides with terminal triple bond and various number of carbons in the linker ($C_3 + C_3$, C_4, or C_5) **43–45** were purified by column chromatography before use in the next step. Macrolones **1**, **2**, **4**, and **8–14** were prepared according to Sonogashira protocol, as outlined in the Scheme 5.

Synthesis of the third targeted group of macrolones (**15–18**), that incorporate a tricyclic quinolone-3-carboxylic acid unit was also performed using the Sonogashira reaction, Scheme 6.[50,51] Solubility problem with intermediary quinolones were solved either by the alkynylation of the free acid **58** in EtOH instead of MeCN, or by using ethyl esters **59**, **60** of the parent acids.

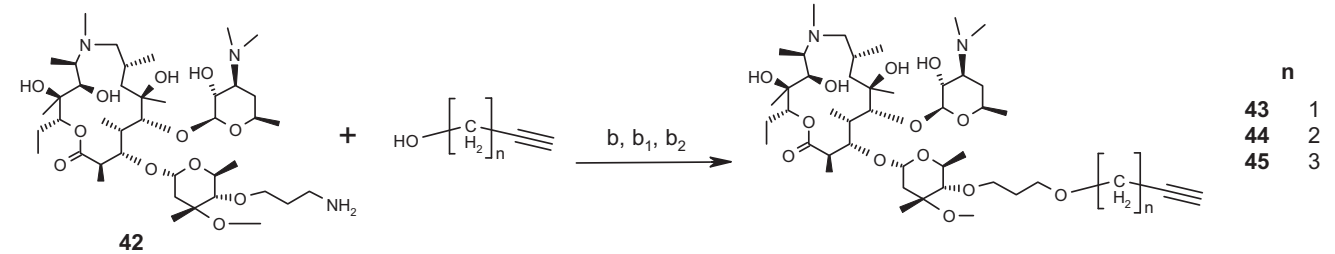
To our satisfaction when this reaction was performed with more soluble ethyl esters **59** and **60** reaction times were significantly shortened, yields improved and satisfactory purity obtained, enabling more effective chromatographic purification of the final compounds. In the next step hydrogenation of **61–64** was performed at 2 bar of H_2 over Pd/C with complete site-selectivity. In the last step esters were hydrolyzed to target carboxylic acids **16–18**.

2.2. Antibacterial activity

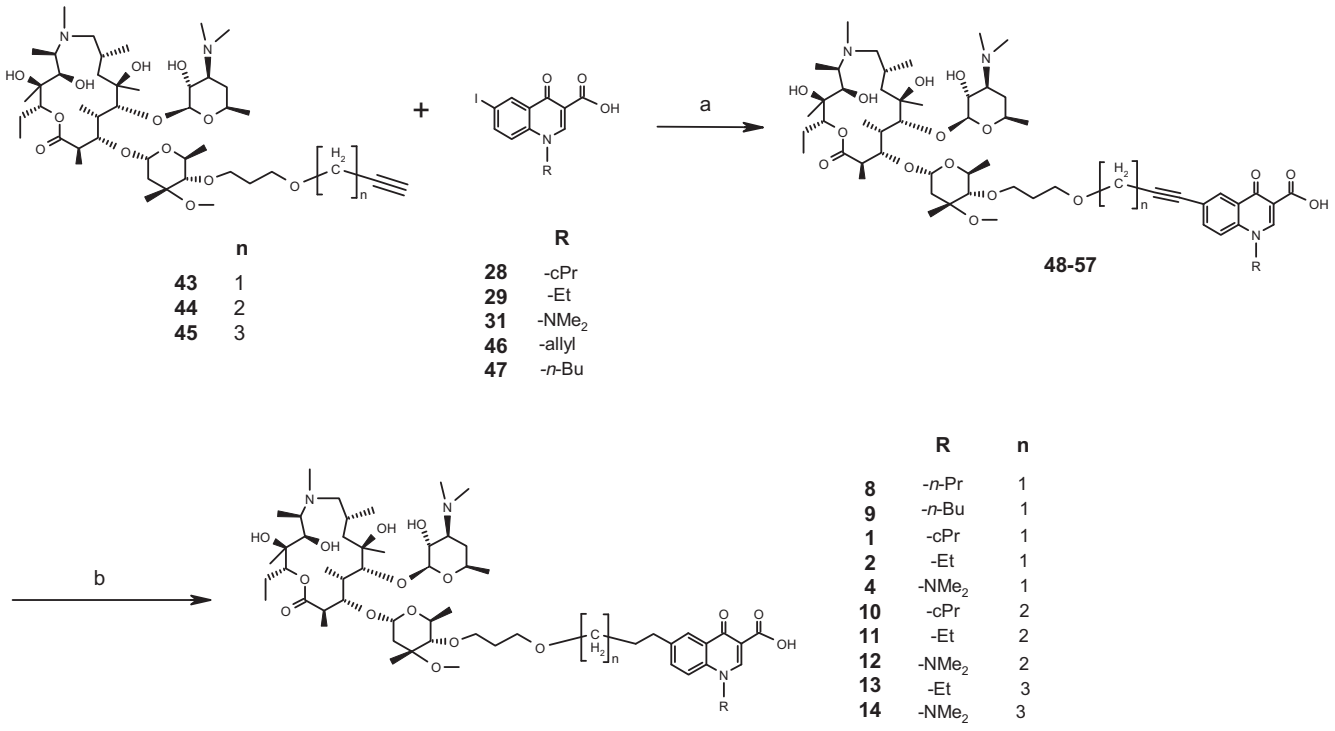
Antibacterial activity of prepared compounds was determined by a standard broth microdilution method[52] and azithromycin and telithromycin were used as comparators. The results are shown in



Scheme 3. Synthetic route to macrolide intermediate **27**. Reagents and conditions: (a) allyl alcohol 160 equiv, $NaNO_2$ 9 equiv, HCOOH 4.5 equiv, 0 °C-rt, 24 h; (b) MeOH/H_2O 2:1, K_2CO_3 15 equiv, 55 °C, 2 h.



Scheme 4. Preparation of terminal alkynyl ethers **43–45**. Reagents and conditions: (a) MeOH/H_2O 2:1, K_2CO_3 15 equiv, 55 °C, 4 h; (b) propargyl alcohol 100 equiv, HCOOH 4.5 equiv, $NaNO_2$ 6 equiv, 0–5 °C, 8 h; (b_1) 3-butyn-1-ol 100 equiv, HCOOH 4.5 equiv, $NaNO_2$ 3.5 equiv, −5 °C to +4 °C, 15 h; (b_2) 4-pentyn-1-ol 100 equiv, HCOOH 4.5 equiv, $NaNO_2$ 7 equiv, −5 °C to rt, 46 h.

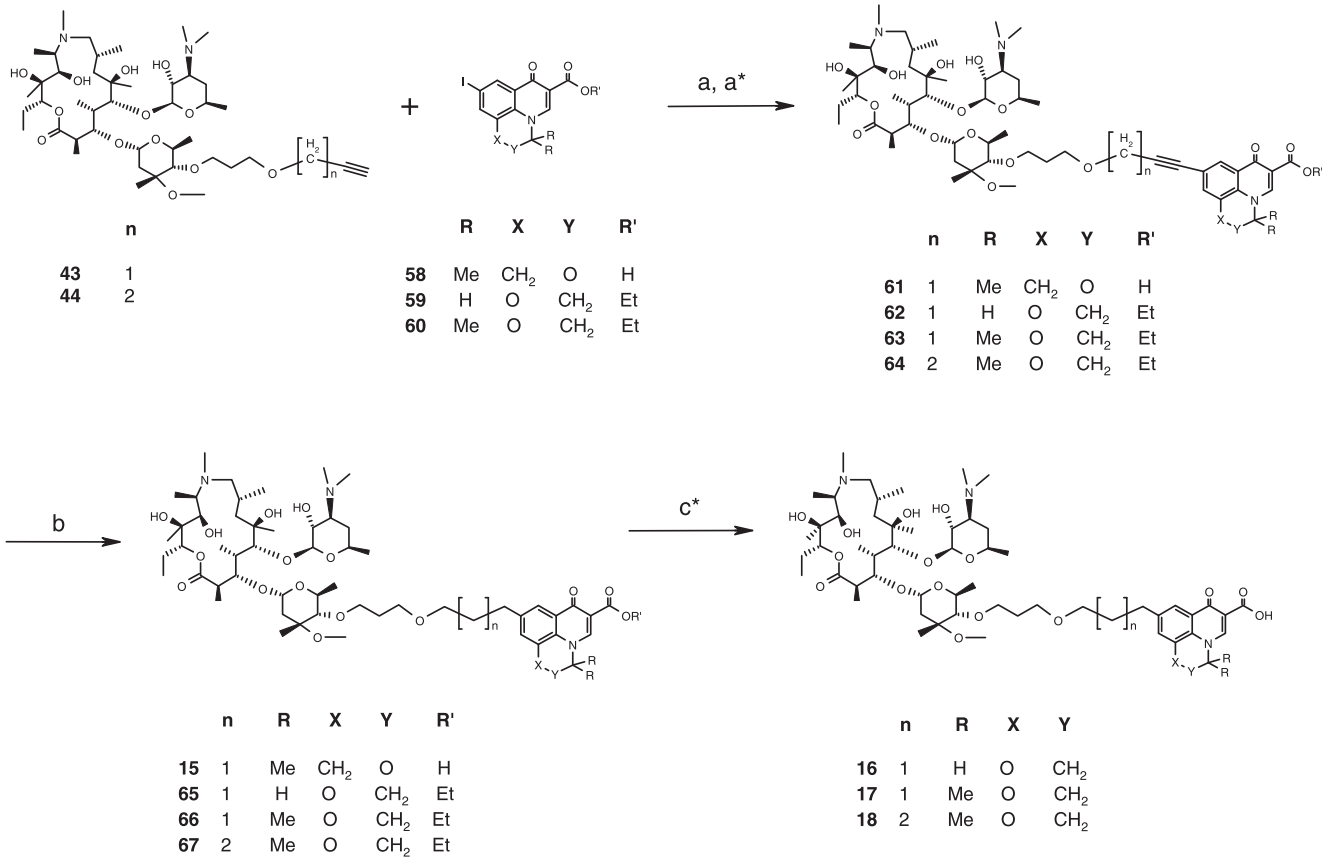


n	
43	1
44	2
45	3

R	
28	-cPr
29	-Et
31	-NMe_2
46	-allyl
47	-*n*-Bu

	R	n
8	-*n*-Pr	1
9	-*n*-Bu	1
1	-cPr	1
2	-Et	1
4	-NMe_2	1
10	-cPr	2
11	-Et	2
12	-NMe_2	2
13	-Et	3
14	-NMe_2	3

Scheme 5. Synthetic route to macrolones **1**, **2**, **4**, **8**–**14**. Reagents and conditions: (a) MeCN, CuI 0.2 equiv, Et_3N 10 equiv, **28**, **29**, **31**, **46**, or **47** 1.8 equiv, $Pd(PPh_3)_2Cl_2$ 0.05 equiv, 50 °C 16 h; (b) 10% Pd/C 20 wt %, MeOH, H_2 2.2 bar, 15 h.



n	
43	1
44	2

	R	X	Y	R'
58	Me	CH_2	O	H
59	H	O	CH_2	Et
60	Me	O	CH_2	Et

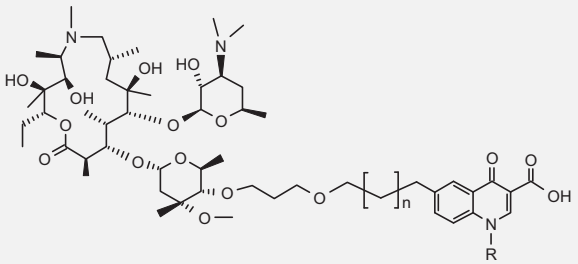
	n	R	X	Y	R'
61	1	Me	CH_2	O	H
62	1	H	O	CH_2	Et
63	1	Me	O	CH_2	Et
64	2	Me	O	CH_2	Et

	n	R	X	Y	R'
15	1	Me	CH_2	O	H
65	1	H	O	CH_2	Et
66	1	Me	O	CH_2	Et
67	2	Me	O	CH_2	Et

	n	R	X	Y
16	1	H	O	CH_2
17	1	Me	O	CH_2
18	2	Me	O	CH_2

Scheme 6. Synthetic route to macrolones **15**–**18**. Reagents and conditions: (a) EtOH, CuI 0.2 equiv, Et_3N 10 equiv, **58** 2.5 equiv, $Pd(PPh_3)_2Cl_2$ 0.05 equiv, 50 °C, 16 h; (a^*) MeCN, CuI 0.2 equiv, Et_2N 10 equiv, **59** or **60** 2 equiv, $Pd(PPh_3)_2Cl_2$ 0.05 equiv, 50 °C, 1 h; (b) MeOH, 10% Pd/C 10 wt %, H_2 2 bar, 16 h; (c^*) THF/H_2O 2:1, 0.5 M LiOH 2.5 equiv, 3 h.

Table 1
Antimicrobial activity of compounds **1**-**18**, given as minimum inhibitory concentration (MIC) in units of μg/mL.



			Organism and strain								
			S. aureus 90265/97	*S. pneumoniae* 134 GR-M	*S. pyogenes* Finland 11	*S. aureus* PK1	*S. pneumoniae* Ci137	*S. pyogenes* Finland 2	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATTC 49247
	Phenotype		iMLS	iMcLS	iMLS	M	M	M	cMLS	cMLS	
No.	n	R									
TEL	—	—	⩽0.125	0.25	⩽0.125	0.125	0.5	0.5	0.25	16	1
AZM	—	—	>64	>64	16	>64	8	8	>64	>64	1
1	1	-cPr	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
2	1	–Et	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
3	1	$-CH_2CH_2OMe$	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
4	1	$-NMe_2$	⩽0.125	0.25	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
5	1	-*t*-Bu	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
6	1	-*i*-Pr	⩽0.125	<0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	4
7	1	$-CH_2CH_2NMe_2$	⩽0.125	0.25	⩽0.125	⩽0.125	⩽0.125	0.25	⩽0.125	1	4
8	1	-*n*-Pr	0.25	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	2
9	1	-*n*-Bu	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
10	2	-cPr	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.5
11	2	–Et	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.5
12	2	$-NMe_2$	⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	0.5	4
13	3	–Et	⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	2
14	3	$-NMe_2$	⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.25	1
15	1		⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
16	1		⩽0.125	⩽0.125	⩽0.06	⩽0.125	⩽0.06	0.03	⩽0.125	0.25	2
17	1		0.06	⩽0.125	⩽0.02	0.13	⩽0.02	⩽0.02	⩽0.125	1	2
18	2		⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	0.5	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.

Table 1 and are expressed as minimum inhibitory concentrations (MICs) in units of μg/mL. The organisms tested represent relevant Gram-positive (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*) and Gram-negative (*Haemophilus influenzae*) 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 phenotype), or ribosome modification by methylation. Methyltransferase expression was either inducible (iMLS phenotype) or constitutive (cMLS phenotype).

All analogs showed MIC $\leqslant$ 0.125 μg/mL against erythromycin sensitive G-positive bacteria *S. aureus*, *S. pneumoniae,* and *S. pyogenes*. Overall potency of this compound class provides a clear advantage over currently used macrolide antibiotic azithromycin, as well as the ketolide telithromycin, which is inactive against constitutively resistant *S. pyogenes*.

2.3. Cytotoxicity profile of the tested compounds

Cytotoxicity was tested in vitro on THP-1 and HepG2 cell lines by using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] assay.[53] At IC_{50} concentration cells treated with the compounds produced 50% of the optical density (OD) values of the non-treated cells. In general, compounds having shorter linkers (<9 atoms) showed no significant cytotoxic effect (IC_{50} >10 μM), Table 2. Only compounds **13** and **14**, with the longest linker (nine atoms) between macrolide and quinolone moiety showed some weak cytotoxic effect.

3. Conclusion

In summary, synthesis of the three designed sets of macrolones was successfully completed and some of the target compounds exhibited valuable biological profiles. In the course of this work a practical and scaleable process for the synthesis of new macrolone derivatives from azithromycin was developed. A simple isolation and purification procedure was used in the large-scale preparation of some selected products. The efficiency and feasibility of the process for a production of **4** from **42** via **43** and **52**, Schemes 3 and 4, was demonstrated by the synthesis of about 15 g of pure **4**. In general, tested compounds showed excellent efficacy against common respiratory pathogens, including resistant strains, and a large majority of them demonstrated no significant cytotoxic effect on THP-1 and HepG2 cell lines at concentrations up to 50 μM, suggesting specificity of action.

Table 2
Cytotoxicity profile of the macrolones at 0.4–50 μM

	Cytotoxicity (IC_{50}) (μM)	
Compd	HepG2	THP-1
1	>50	48
2	>50	>50
3	>50	>50
4	>50	48
5	>50	40
6	>50	46
7	>50	>50
8	40	18
9	49	27
10	47	20
11	46	22
12	42	20
13	20	9
14	13	8
15	>50	40
16	>50	>50
17	>50	40
18	46	11

Viability of THP-1 and HepG2 cell lines following 24 h exposure to the tested compounds was determined by MTS, and presented as micromolar IC_{50} values.

4. Experimental section

4.1. General

All commercial reagents were analytically pure and solvents were purified by distillation. ES (Electrospray) mass spectra were recorded on Platform LCZ (Micromass, UK) and LCQ Deca (Finnigan, USA) instruments.

NMR spectra were recorded on Varian Unity Inova 600, Bruker Advance DRX 500, and Bruker Advance DPX 300 spectrometers using TMS as internal standard. $CDCl_3$ and DMSO-d_6 were used as solvents, chemical shifts are given as δ values in ppm related to TMS.

All compounds were isolated as amorphous solid.

Progress of reactions and purity of products were followed by TLC on Merck plate (Darmstadt, DE) using systems of solvents as follows:

- DCM/MeOH/NH_4OH (90:9:0.5)
- DCM/MeOH/NH_4OH (90:9:1.5)
- DCM/MeOH/NH_4OH NH_3 (90:15:1.5).

Abbreviations used: s, singlet; d, dublet; t, triplet; q, quartet; dd, double dublet; dq, double quartet; br s, broad singlet; m, multiplet; ov, overlap MTS = 3-(4,5-dimethylthiazole-2-yl)-5-(3-carboximethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazole; ECACC = European collection of cell cultures; THP-1 = human monocytic leukemia; HepG2 = human Caucasian hepatocyte carcinoma.

List of acronyms:

- $Pd_2(dba)_3$—Tris(dibenzylideneacetone)dipalladium(0)
- dppb—1,4-bis(diphenylphosphino)butane
- $Pd(Ph_3)_4$—Tetrakis(triphenylphosphine)palladium
- $Pd(OAc)_2$—palladium(II)-acetate
- $Pd(PPh_3)_2Cl_2$—dichloro-bis(triphenylphosphine)palladium.

4.2. Biological methods and materials

4.2.1. In vitro antibacterial testing

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method as described by CLSI guidelines,[52] except that for *Streptococcus* medium, lysed blood was substituted with 5% horse serum. Dilutions of tested compounds in 96-well microtitre plates 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*, Mueller-Hinton chocolate agar for *H. Influenzae* and Mueller-Hinton agar for *Staphylococci*.

4.2.2. Cell lines and cytotoxicity assay

Cell lines were purchased from the ECACC—THP-1, monocyte, ECACC-88081201[54] and HepG2, epithelial, ECACC-85011430.[55] Cells were maintained in complete RPMI 1640 medium (Institute of Immunology, Zagreb, Croatia) supplemented with 10% Fetal Bovine Serum (BioWest, S04382S1810) at 37 °C in a 5% CO_2 atmosphere. Cytotoxicity assay was performed by using the MTS CellTiter 96® Aqueous One Solution Cell Proliferation Assay (G358B, 18824201, Promega, USA). Each culture in 96-well plate contained 50,000 (for HepG2) or 75,000 (for THP-1) cells. Cultures exposed to tested compounds were incubated for 24 h at 37 °C in 5% CO_2. Thereafter,

15 μL of MTS reagent[53] was added directly to the cell lines. After an additional 2 h of incubation at 37 °C in 5% CO_2, the absorbance was recorded at 490 nm using a spectrophotometric plate reader (Ultra, TECAN, USA). The method was programmed for the TECAN robotic system in GEMINI pipetting software.[56]

4.3. Synthetic procedures

2′-*O*-Acetylazithromycin 11,12-cyclic carbonate (**19**) was prepared as previously described,[43,44] 6-iodoquinolino-3-ethylcarboxylate and tricyclic quinolone derivatives **58–60** were synthesised according to known procedures.[14,57–59]

4.3.1. 2′-*O*-Acetyl-4″-*O*-(3-cyanoethyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (20)

To a solution of the macrolide **19** (30 g, 37 mmol) in acrylonitrile (400 mL, 5.78 mol, 150 equiv) in N_2 atmosphere, *t*-BuOH (11 mL, 0.11 mol, 3 equiv) was added and the reaction mixture was cooled in an ice bath (0 °C). NaH (0.9 g, 1.5 g, 60% in mineral oil, 37 mmol, 1 equiv) was added in portions during 1 h and the reaction mixture was stirred at rt for additional 3 h. Acrylonitrile was evaporated under reduced pressure. EtOAc (250 mL) was added and the solution washed with satd aq $NaHCO_3$ (250 mL). The organic layer was dried over K_2CO_3 and evaporated under reduced pressure yielding 35 g of crude product. Diethyl ether (60 mL) was added and the mixture stirred for 30 min in an ice bath. The product precipitated and was filtered off yielding the title compound **20** as a white powder (19 g, *Y* = 50%).

4.3.2. 2′-*O*-Acetyl-4″-*O*-(3-aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (21)

A high pressure reactor was filled with a solution of **20** (17 g, 19.5 mmol) in AcOH (150 mL). PtO_2 (1.5 g) was added and the reaction mixture stirred under H_2 at 5 bar overnight. The catalyst was filtered off through Celite and the solvent evaporated under reduced pressure.

The oily residue was dissolved in DCM (200 mL) and extracted with water (200 mL). Aqueous layer was extracted with fresh DCM (200 mL) at pH 6. The organic layer was dried over K_2CO_3 and evaporated under reduced pressure yielding 15 g of foamy solid. Diethyl ether (60 mL) was added and stirred for 30 min in an ice bath. Product precipitated, filtered off yielding the title compound **21** as a white powder (10 g, *Y* = 57%).

4.3.3. 4″-*O*-(3-Aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A-11,12-cyclic carbonate (22)

Compound **21** (5 g, 5.7 mmol) was dissolved in MeOH (50 mL) and the solution stirred at 55 °C for 24 h. pH of the reaction mixture was adjusted to 8 using NH_4OH/H_2O = 1:1. DCM (50 mL) and water (50 mL) were added and pH adjusted to 6 using 0.25 M HCl. The layers were separated and the organic layer removed under reduced pressure yielding 3 g of crude product that was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:15:1.5) yielding the title compound **22** as a white foamy solid (2.5 g, *Y* = 47%).

4.3.4. 4″-*O*-(3-Hydroxypropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (23) and 4″-*O*-allyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (24)

Compound **22** (2 g, 2,4 mmol) was dissolved in 10% aq AcOH and cooled to 0 °C in an ice bath. $NaNO_2$ (1 g, 14.6 mmol, 6 equiv) was added during 1 h and the reaction stirred at rt for additional 2 h. The reaction mixture was diluted with water (20 mL), pH was adjusted to 10.5 using 20% aq NaOH and extracted with DCM (40 mL). The organic layer was dried over K_2CO_3 and removed under reduced pressure yielding 1.95 g of crude yellow solid that was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:5:0.5) yielding the title compound **23** as a white foamy solid (1.2 g, *Y* = 58%) and the title compound **24** as a white foamy solid (0.25 g, *Y* = 12%).

4.3.5. 2′-*O*-Acetyl-4″-*O*-(3-hydroxypropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (25)

Compound **23** (120 mg, 0.14 mmol) was dissolved in *i*-PrOH/EtOAc = 1:1 (3 mL) and cooled to 0 °C in an ice bath. Acetic anhydride (0.015 mL, 0.16 mmol, 1.1 equiv) was added and the reaction mixture stirred at rt for 4 h. Another portion of acetic anhydride (0.2 equiv) was added and the stirring continued for additional 4 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with aqueous $NaHCO_3$ (2 × 15 mL). The organic layer was removed under reduced pressure yielding the title compound **25** as a white foamy solid (130 mg, *Y* = 99%).

4.3.6. 2′-*O*-Acetyl-4″-*O*-(3-allyloxy-propyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic carbonate (26)

Procedure A. Into a mixture of allyl-*tert*-butyl carbonate (7.5 g, 0.05 mol, 29 equiv) and compound **25** (1.5 g, 1.7 mmol) $Pd_2(dba)_3$ (0.18 g, 0.17 mmol, 0.1 equiv) and dppb (0.18 g, 0.42 mmol, 0.25 equiv) were added. The reaction mixture was stirred at 80 °C for 4 h. $Pd(PPh_3)_4$ (0.33 g, 0.3 mmol, 0.17 equiv) was added and the stirring continued for another 1 h. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, eluent EtOAc/*n*-hexane/Et_2N = 1:1:0.2) resulting in pure title compound **26** as a white foamy solid (1.0 g, *Y* = 51%).

Procedure B. To a solution of compound **21** (800 mg, 0.92 mmol) in allyl alcohol (10 mL, 0.15 mol, 160 equiv) was added HCOOH (0.3 mL, 7.9 mmol, 8.5 equiv) under N_2 atmosphere. $NaNO_2$ (400 mg, 5.8 mmol, 6 equiv) was added in portions during 15 min and the reaction mixture stirred at rt for 5 h. Then another portion of $NaNO_2$ (3 equiv) was added and the stirring continued overnight. The reaction mixture was filtered and the solvent evaporated under reduced pressure. EtOAc (20 mL) was added and the solution washed with satd aq $NaHCO_3$ (2 × 30 mL). Evaporation of the organic layer gave the title compound **26** as a yellow foamy solid (860 mg, *Y* = 26%) that was used in the next step without further purification.

4.3.7. 4″-*O*-(3-Allyloxy-propyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (27)

Compound **26** (0.8 g, 0.87 mmol) was dissolved in MeOH (30 mL). A solution of K_2CO_3 (1.8 g, 13 mmol, 15 equiv) in water (15 mL) was added and the reaction stirred at 55 °C for 2 h. MeOH was evaporated, EtOAc (30 mL) was added and the organic layer washed with satd aq $NaHCO_3$ (2 × 20 mL). Organic layer was dried over K_2CO_3 and evaporated under reduced pressure yielding 0.7 g of oily residue that was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:9:0.5) yielding the title compound **27** as a white foamy solid (0.5 g, *Y* = 64%).

4.4. General procedure for the preparation of compounds 35–41

To a stirring solution of compound **27** (250–400 mg, 1 equiv) in DMF (5 mL) $Pd(OAc)_2$ (0.2 equiv) and tri-*o*-tolylphosphin (0.4 equiv) were added under N_2 atmosphere. After 60 min a quinolone (2.5 equiv) and Et_3N (4 equiv) were added. The reaction mixture was stirred at 65 °C for 2 h and then at 75 °C for another 18 h. The

catalysts were filtered off and the filtrate partitioned between EtOAc (40 mL) and aq $NaHCO_3$ (2 × 50 mL). The organic layer was extracted with water (2 × 20 mL) at pH 3 and combined aqueous layers extracted with EtOAc (40 mL) at pH 8.5. The organic layer (40 mL) was dried over K_2CO_3 and concentrated under reduced pressure. Precipitation from EtOAc/*n*-hexane yielded final products 35–**41**.

4.5. General procedure for the preparation of compounds 1–7 by a hydrogenation of derivatives 35–41

A solution of a macrolone (**35**–**41**) in MeOH was hydrogenated in a high-pressure reactor in the presence of 10% Pd/C (10 wt %) at rt and 3 bar for 15 h. The catalyst was filtered off and the solvent removed under reduced pressure. The residue was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:9:0.5) and then precipitated from EtOAc/*n*-hexane to obtain compounds **1–7**.

4.5.1. 4″-*O*-(3-Aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (42)

The same method was followed as for the synthesis of compound **27** but starting from compound **21** (10 g, 11.4 mmol) to obtain the title compound **42** (6 g, *Y* = 65%) as a white powder.

4.5.2. 4″-*O*-(3-Prop-2-ynyloxy-propyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (43)

To a solution of compound **42** (3.5 g, 4.2 mmol) in propargyl alcohol (25 mL, 0.43 mol, 100 equiv) HCOOH (0.72 mL, 19.1 mmol, 4.5 equiv) was added under N_2 atmosphere. $NaNO_2$ (1.7 g, 24.6 mmol, 6 equiv) was added in portions during 45 min and the reaction mixture stirred at −5 °C to 0 °C for 8 h. The mixture was diluted with EtOAc (50 mL) and washed with satd aq $NaHCO_3$ (2 × 70 mL). The organic layer was then extracted with water (50 mL) at pH 3.5. Aqueous layer was extracted with fresh DCM (2 × 25 mL) at pH 6.3. Combined organic layers were washed with water (20 mL) at pH 10.5, dried over K_2CO_3 and concentrated under reduced pressure yielding the title compound **43** as a yellow oil (1.7 g, *Y* = 24%).

4.5.3. 4″-*O*-(3-But-3-ynyloxy-propyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (44)

The same method was followed as for the synthesis of compound **43** but starting from compound **42** (3.6 g, 4.5 mmol) in 3-butyn-1-ol (35 mL, 0.45 mol, 100 equiv) to obtain the title compound **44** as a white foamy solid (1.0 g, *Y* = 23%).

4.5.4. 4″-*O*-(3-Pent-3-ynyloxy-propyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (45)

The same method was followed as for the synthesis of compound **43** but starting from compound **42** (3.6 g, 4.5 mmol) in 4-pentyn-1-ol (47 mL, 0.51 mol, 100 equiv) to obtain the title compound **45** as a white foamy solid (1.15 g, *Y* = 21%).

4.6. General procedure for the preparation of compounds 48–57

To a solution of a macrolide (**43, 44**, or **45**) in MeCN CuI (0.2 equiv), Et_3N (10 equiv), quinolone (2 equiv) and $Pd(PPh_3)_2Cl_2$ (0.05 equiv) were added. The reaction mixture was stirred at 50 °C for 16 h in N_2 atmosphere. The solvent was removed under reduced pressure and EtOAc added. A solid that precipitated was filtered off and discarded. The filtrate was washed with satd aq $NaHCO_3$ and then extracted with water at pH 3 (adjusted with 2 M HCl). Aqueous layer was washed twice with DCM at pH 4.5. Aqueous layer was extracted with fresh DCM at pH 5.5–6. The organic layer was washed with water at pH 10 and concentrated under reduced pressure. Foamy solid was precipitated from EtOAc/*n*-hexane and then purified by column chromatography (DCM/MeOH/NH_4OH = 90:15:1.5). Homogenous fractions of the product were combined, concentrated to dryness and precipitated from EtOAc/*n*-hexane resulting in pure compounds **48–57**.

4.7. General procedure for the preparation of compounds 1, 2, 4 and 8–14 by a hydrogenation of compounds 48–57

The same method was followed as earlier described in general procedure for the preparation of compounds **1–7**, but starting from compounds **48–57**. Compounds **1, 2, 4**, and **8–14** were obtained as a white powder.

4.7.1. 4″-*O*-{3-[3-(6-Carboxy-3,3-dimethyl-6-oxo-1*H*,6*H*-2-oxa-3a-aza-phenalen-8-yl)-prop-2-ynyloxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (61)

To a suspension of 8-iodo-3,3-dimethyl-6-oxo-1*H*,6*H*-2-oxa-3a-aza-phenalene-5-carboxylic acid **58** (364 mg, 0.95 mmol, 2 equiv) in EtOH (5 mL), CuI (17.7 mg, 0.09 mmol, 0.2 equiv) and Et_3N (0.66 mL, 4.7 mmol, 10 equiv) were added. The reaction mixture was stirred at rt for 20 min in N_2 atmosphere and then heated to 50 °C. A solution of compound **43** (400 mg, 0.47 mmol) in EtOH (10 mL) and $Pd(PPh_3)_2Cl_2$ (16.5 mg, 0.023 mmol, 0.05 equiv) were added and the stirring continued at 50 °C for 16 h in N_2 atmosphere. EtOH was removed under reduced pressure, EtOAc added and the precipitate that formed filtered off and discarded. The filtrate was washed twice with satd aq $NaHCO_3$ and then concentrated to dryness. DCM (20 mL) and water (30 mL) were added and pH adjusted to 3 using 2 M HCl. Aqueous layer was washed with DCM (20 mL) at pH 4.5 and then extracted with fresh DCM (30 mL) at pH 5.7. The organic layer was washed with water at pH 10.5 and concentrated to dryness. The foamy residue was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:15:1.5) yielding the title compound **61** as a white foamy solid (100 mg, *Y* = 17%).

4.7.2. 4″-*O*-{3-[3-(6-Carboxy-3,3-dimethyl-6-oxo-1*H*,6*H*-2-oxa-3a-aza-phenalen-8-yl)-propoxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (15)

The same method was followed as described in general procedure for the preparation of compounds **1–7**, but starting from compound **61** (100 mg, 0.09 mmol) to obtain the title compound **15** as a white powder (60 mg, *Y* = 54).

4.7.3. 4″-*O*-{3-[3-(6-Carboxy-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-prop-2-ynyloxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (62)

The same method was followed as described in general procedure for the preparation of compounds **48–57**, but starting from compound **43** (470 mg, 0.56 mmol) and ethyl 9-iodo-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylate (**59**) (430 mg, 1.11 mmol, 2 equiv) to obtain the title compound **62** as a yellow solid (650 mg, *Y* = 67%).

4.7.4. 4″-*O*-{3-[3-(6-Carboxy-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-prop-2-ynyloxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (65)

The same method was followed as described in general procedure for the preparation of compounds **1–7**, but starting from compound **62** (420 mg, 0.38 mmol) to obtain the title compound **65** as

a brown foamy solid (430 mg, *Y* = 100%) that was used in the next step without further purification.

4.7.5. 4″-*O*-{3-[3-(6-Carboxy-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-propoxy]-propyl}–9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (16)

To a solution of compound **65** (430 mg, 0.39 mmol) in THF (6 mL), water (3 mL), and LiOH (23.4 mg, 0.975 mmol, 2.5 equiv) were added. The reaction mixture was stirred at rt for 3 h. The mixture was diluted with EtOAc (30 mL) and washed with satd aq $NaHCO_3$ (2 × 30 mL). To the organic layer water (30 mL) was added and pH adjusted to 3. Aqueous layer was extracted with DCM at pH 6. The organic layer was washed with water (15 mL) at pH 10 and evaporated under reduced pressure yielding 435 mg of yellow foamy solid that was purified by column chromatography (SiO_2, eluent DCM/MeOH/NH_4OH = 90:15:1.5) yielding the title compound **16** as a white powder (0.172 g, *Y* = 40%).

4.7.6. 4″-*O*-{3-[3-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-prop-2-ynyloxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (63)

The same method was followed as described in general procedure for the preparation of compounds **48**–**57**, but starting from compound **43** (310 mg, 0.36 mmol) and ethyl 9-iodo-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylate (**60**) (300 mg, 0.73 mmol, 2 equiv) to obtain the title compound **63** as a beige solid (250 mg, *Y* = 60%).

4.7.7. 4″-*O*-{3-[3-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-propoxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (66)

The same method was followed as described in general procedure for the preparation of compounds **1**–**7**, but starting from compound **63** (250 mg, 0.22 mmol) to obtain the title compound **66** as a brown foamy solid (260 mg, *Y* = 100%).

4.7.8. 4″-O-{3-[3-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-propoxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (17)

The same method was followed as for the synthesis of compound **16** but starting from compound **66** (260 mg, 0.22 mmol) to obtain the title compound **17** as a white powder (119 mg, *Y* = 46%).

4.7.9. 4″-*O*-{3-[4-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-but-3-ynyloxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (64)

The same method was followed as described in general procedure for the preparation of compounds **48**–**57**, but starting from compound **44** (420 mg, 0.49 mmol) and ethyl 9-iodo-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylate (**60**) (300 mg, 0.73 mmol, 1.5 equiv) to obtain the title compound **64** as a yellowish powder (230 mg, *Y* = 28%).

4.7.10. 4″-*O*-{3-[4-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-butoxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A ethyl ester (67)

The same method was followed as described in general procedure for the preparation of compounds **1**–**7**, but starting from compound **64** (230 mg, 0.22 mmol) to obtain the title compound **67** as a yellow foamy solid (250 mg, *Y* = 88%).

4.7.11. 4″-*O*-{3-[4-(6-Carboxy-3,3-dimethyl-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-9-yl)-butoxy]-propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (18)

The same method was followed as for the synthesis of compound **16** but starting from compound **67** (250 mg, 0.19 mmol) to obtain the title compound **18** as a white powder (95 mg, *Y* = 43%).

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Supplementary data

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