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Design, Synthesis, and in Vitro Activity of Novel 2′-*O*-Substituted 15-Membered Azalides

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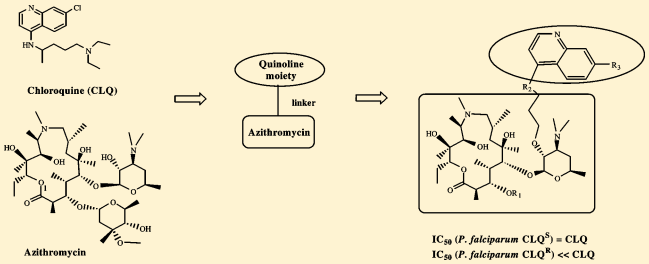
Supporting Information

ABSTRACT: Malaria remains one of the most widespread human infectious diseases, and its eradication will largely depend on antimalarial drug discovery. Here, we present a novel approach to the development of the azalide class of antimalarials by describing the design, synthesis, and characterization of novel 2′-*O*-substituted-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A derivatives consisting of different quinoline moieties covalently liked to a 15-membered azalide scaffold at position 2′. By multistep straightforward synthesis, 19 new, stable, and soluble compounds were created and biologically profiled. Most active compounds from the 4-amino-7-chloroquinoline series showed high selectivity for *P. falciparum* parasites, and in vitro antimalarial activity improved 1000-fold over azithromycin. Antimalarial potency was equivalent to chloroquine against the sensitive strain (3D7A) and up to 48-fold enhanced over chloroquine against the chloroquine-resistant strain (W2). Concurrently, the antibacterial activity of the compounds was eliminated, thus facilitating the development of malaria-specific macrolide agents.



INTRODUCTION

Malaria is by far the most serious and widespread parasitic disease in humans. Despite enormous global control efforts that have resulted in a reduction of the estimated mortality, malaria still caused 781000 deaths in 2009.[1] The protozoal parasites that cause the infection belong to the genus *Plasmodium* with species *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*, and *Plasmodium falciparum* known to infect humans. *P. falciparum*, the species responsible for the most serious form of the disease, has acquired resistance to the widest range of antimalarial drug monotherapies; therefore, the World Health Organisation is arguing against monotherapeutic approaches to malaria treatment.[2] Consequently, combination therapies including a fast-eliminating drug with a slow-eliminating partner agent have been developed over the past decades, which are now part of standard clinical practice and have contributed to a reduction in the emergence and spread of drug-resistant parasites.[3]

An additional strategy to overcome drug-resistant malaria parasites would be the design and introduction of hybrid molecules. Such chemical entities are defined as chemicals having two or more structural domains that bring distinct biological properties into one molecule.[4,5] This would eventually enable more efficient parasite eradication and consequently restrain the emergence of resistance. As new malaria drug candidates are expected to be more active than their parent compounds while remaining a low-cost treatment,[6] applying a hybrid molecule strategy could ensure an approach that is both expedient and affordable.

Macrolide antibiotics, pioneered by azithromycin (AZI), have demonstrated in vitro[7] and in vivo activity in malaria prophylaxis[8,9] and treatment, both as monotherapy[10] and in combinations.[11−13] AZI is of particular interest due to its proven safety record and favorable pharmacokinetic parameters such as its long half-life enabling once-daily dosing.[14] However, AZI as a monotherapy was proven inadequate in treating malaria,[10] while its therapeutic value in different combination therapies is still under evaluation in human clinical studies.[11,12,15−19]

In the design of novel hybrid molecules, we were guided by the idea of creating macrolide compounds that would have enhanced antimalarial efficacy while retaining AZI pharmacokinetic properties. Our previous work has shown that modifying a 15-membered azalide scaffold at position 9a-*N* with various aromate substituents could yield these desired properties.[20,21] We envisaged our new molecules comprising a (chloro)-quinoline moiety covalently linked to a macrolide scaffold at position 2′ (Figure 1). Novel design of the 15-membered azalide would bring favorable pharmacokinetic properties of AZI together with superior quinoline activity against *Plasmodium* while enabling the elimination of antibacterial activity due

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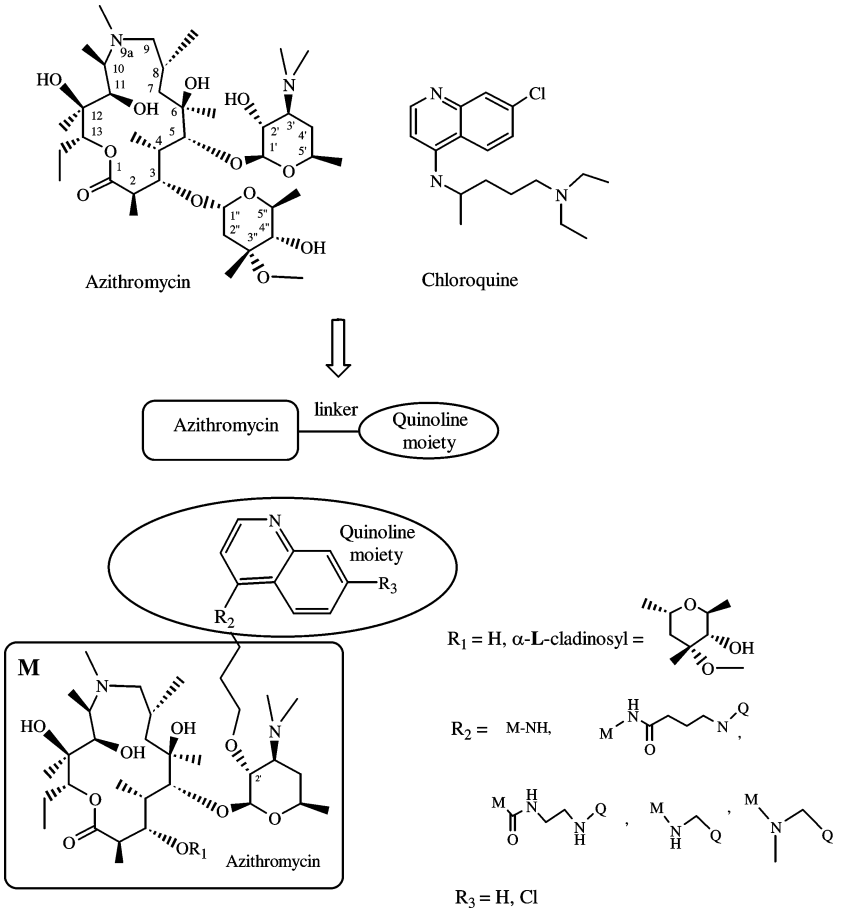
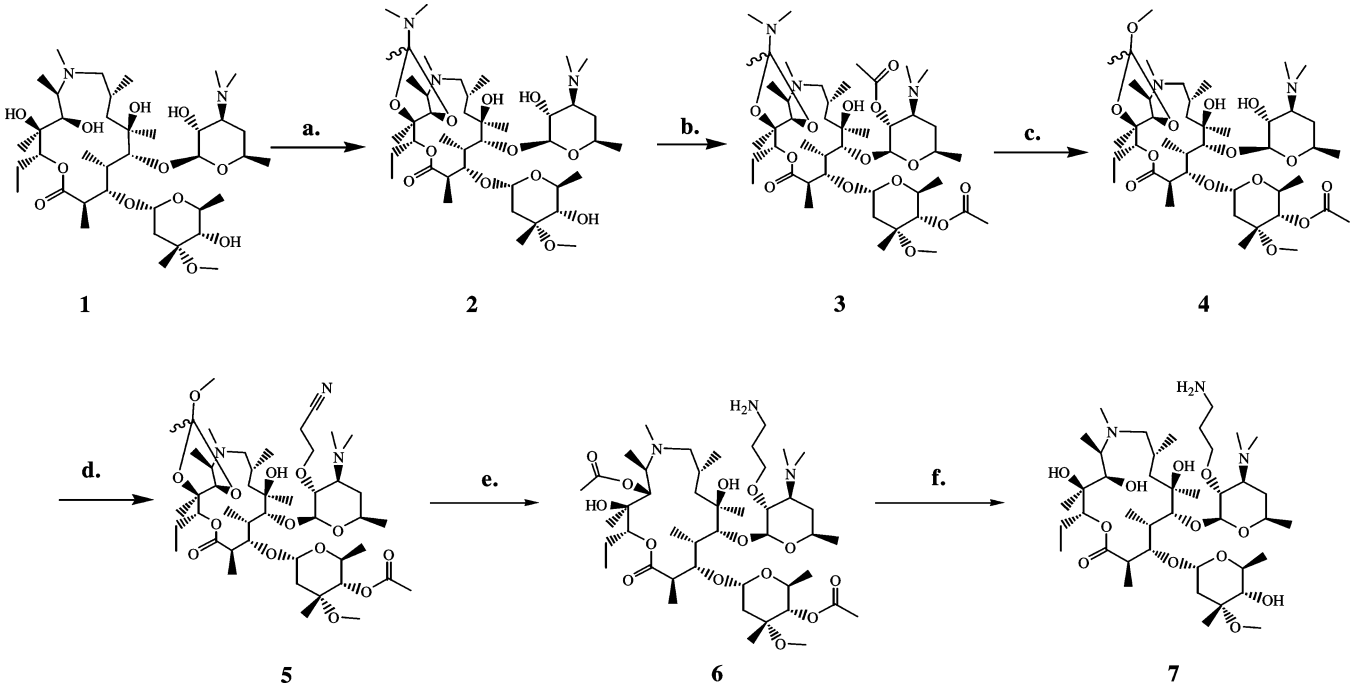


Figure 1. Schematic representation of novel azalide-quinoline concept.

Scheme 1. Synthesis of 2′-*O*-(3-Aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (7)[a]



[a]Reagents and conditions: (a) DMA/DMA, chloroform, reflux. (b) Ac_2O, DMAP, TEA, DCM, rt. (c) MeOH, rt, 45 °C. (d) Acrylonitrile, NaH, *t*-BuOH. (e) Hydrogen, PtO_2, AcOH; LiOH, MeOH/water.

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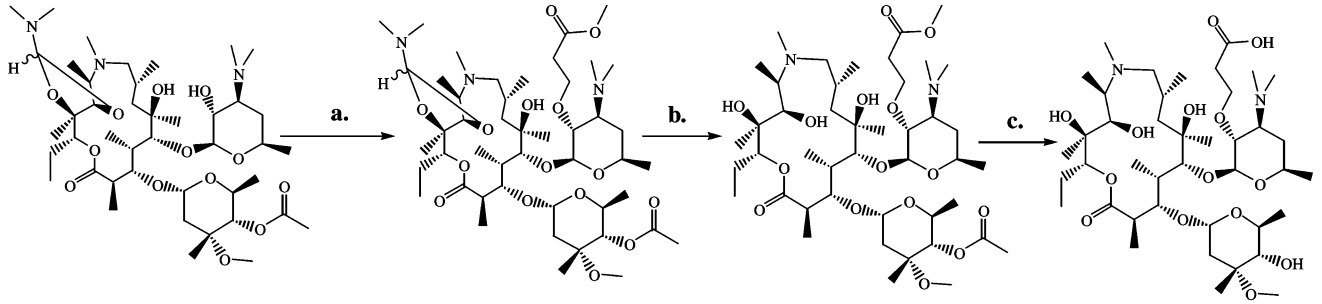
Scheme 2. Synthesis of 2′-*O*-(3-Carboxyethyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (10)[a]



4 8 9 10

[a]Reagents and conditions: (a) Methyl acrylate, NaH, *t*-BuOH. (b) HCOOH, MeOH, rt. (c) LiOH, THF/water.

to the position on the macrolide scaffold used for derivatization. The exclusion of antibacterial action is a desirable property for a new antimalarial because it would allow the eradication of the parasites without affecting the commensal bacteria or the spread of antibacterial macrolide resistance.

AZI's antibacterial activity arises from the inhibition of protein synthesis on bacterial ribosomes. The 2′-OH position on desosamine sugar contributes greatly to macrolides' antibacterial activity and to their ribosome binding by forming important interactions with the ribosome exit tunnel in the 50S subunit.[22] Consequently, there is limited evidence for the pursuit of chemical transformations at the 2′-position of the macrolide scaffold and, if available, report mainly the introduction of protecting groups.[23−25] Chemical modification of this position is challenging, and we have developed novel synthetic approaches for obtaining 2′-alkylated 15-membered azalide derivatives. Furthermore, by this alteration and by the introduction of quinoline moieties at this position, 19 novel compounds with unique biological profiles were created, thus making an important step forward in the discovery of novel antimalarial agents. Herein, we report the original synthesis and biological characterization of novel 2′-*O*-substituted-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A derivatives where a quinoline moiety was linked via an aminoalkyl linker at the 2′ position (Figure 1).

RESULTS

Chemistry. As a part of the synthetic program focused on new hybrid macrolide/chloroquinoline analogues, we investigated the construction of compounds with the goal of identifying novel azalide class of antimalarials. The complexity of the azalide scaffold in combination with our goal to explore structural variations posed a formidable synthetic challenge. Our basic synthetic approach envisioned construction of appropriately functionalized and protected azalide compounds that would permit the exploration of C-2′ position modifications and limited variation in the basic structure of the desosamine amino sugar (Scheme 1). The crucial intermediate 2′-*O*-(3-aminopropyl) derivative **7** allowed a smooth modification of the primary amino group by amidations and aminations at the C-2′ position.[26]

Starting from available precursor, 9-deoxo-9a-methyl-9a-aza-9a homoerythromycin A (**1**), the method provided an easy access to a variety of novel analogues having various groups tethered to the C-2′ position of the azalide skeleton.

Taking into account the azalide's delicate and complex structure and several functional groups with similar reactivity, it was very important to elaborate a protecting group strategy that would allow regioselective alkylation at position 2′. The 2′-OH group is the most reactive group of both erythronolides and azalides; thus, it should be initially protected and later selectively deprotected. Vicinal diols at C-11 and C-12 are often protected as cyclic carbonates, which allowed further transformations on carbohydrate hydroxyl groups.[24,27] However, this strategy was not useful in this case because cyclic carbonates are unstable in basic conditions used in the key step of 2′-OH etherification. Cyclic acetals, on the other hand, are stable in neutral and basic media and seemed as an attractive alternative for 11-OH, 12-OH protection. *N*,*N*-Dimethylacetamide dimethyl acetal (DMA/DMA) is a commercially available reagent used in mild transacetalysation without the need for acid catalysis.[28,29] Cyclic amide acetal **2** was readily prepared by treatment of **1** with DMA/DMA in chloroform under reflux. Reaction of **2** with acetic anhydride in the presence of dimethylaminopyridine (DMAP) resulted in the protection of both C-4″ and C-2′ hydroxyl groups and the formation of **3**. Treatment of **3** with methanol allowed selective deacetylation of the 2′-OH group due to autocatalytic participation of neighboring 3′-dimethylamino group.[24,25] Furthermore, under these reaction conditions, acetal protection was almost quantitatively converted into 11,12-*O*-methoxyethylidne derivative **4**. The hydrolytic instability of this acetal was actually expected in view of its orthoester structure.[29] Michael condensation of **4** with acrylonitrile in the presence of sodium hydride in *t*-BuOH and subsequent reduction of the resulting cyanoethyl aduct **5** afforded primary amine **6**. It is worth noting here that cyclic acetal protection was cleaved in this step to afford diacetate **6**. Final hydrolysis of diacetate **6** afforded 2′-*O*-(3-aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**7**) in an overall 20% yield. Using an analogous synthetic procedure, free acid azalide derivative **10** was obtained in a 15% overall yield from **4** (Scheme 2).

Having the macrolide intermediates needed for the preparation of hybride molecules, we turned our attention to quinoline partners. Therefore, the commercially available 4,7-dichloro-quinoline (**11**) was regioselectively turned into 7-chloro-4-(substituted)aminoquinolines **12**−**14** (Scheme 3).

An appropriate combination of the 4-amino-7-chloroquinoline derivative and a macrolide subunit allowed synthesis of the final hybrid compounds (Schemes 4 and 5). Amidation of amino acids **12** and **13** with primary amine **7** proceeded in the

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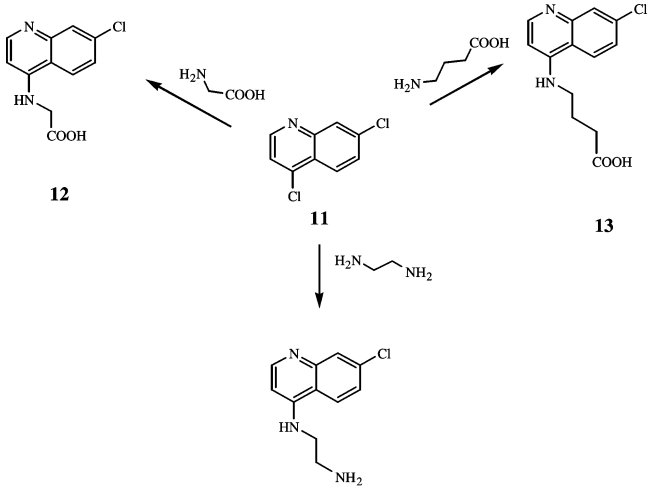
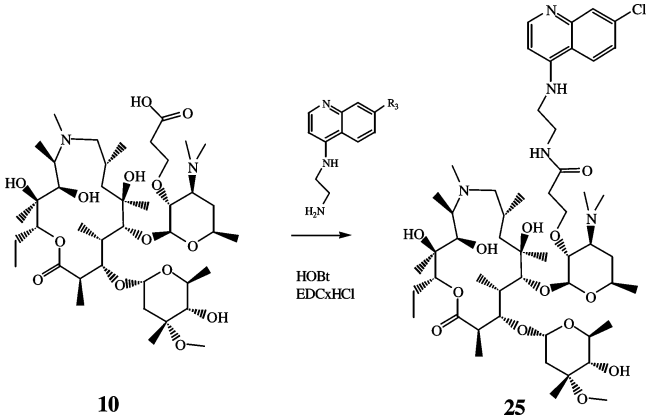
Scheme 3. Synthetic Approach to 4-Amino-7-chloroquinoline Intermediates



Scheme 5. Synthesis of Novel 15-Membered Azalides: 4-Aminoquinoline Derivative 25 Prepared by Amidation of 10



presence of 1-hydroxybenzotriazole (HOBt) and PS-carbodiimide (PS-CDI) or HOBt and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCxHCl) (Scheme 4). By analogy, amide **25** was obtained under similar conditions by coupling azalide acid **10** and amine **14**. Nucleophilic substitution of the chlorine atom at position 4 in **11** with amine **7** led to the formation of compound **22**.

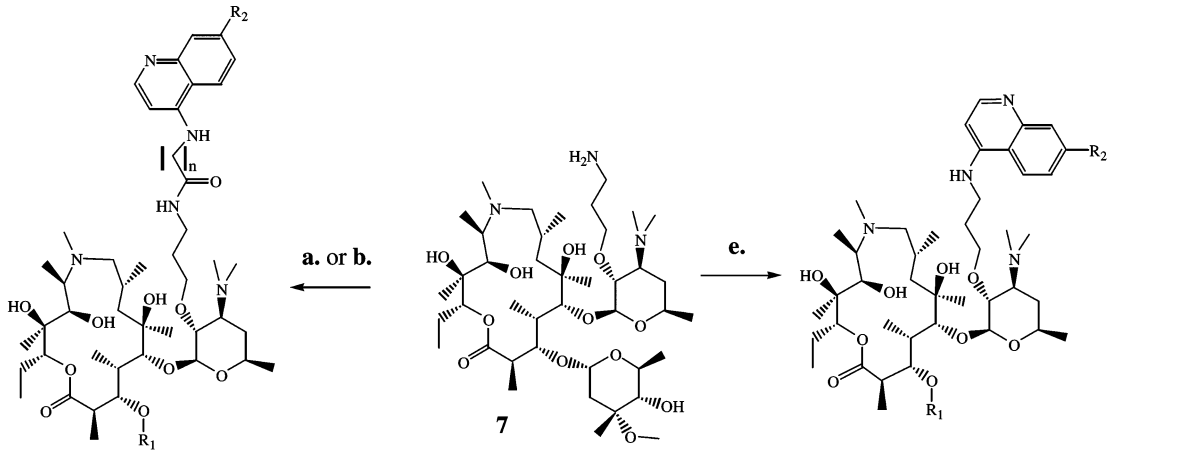
A characteristic common to both erythronolides and azalides is decomposition in aqueous acidic media comprising hydrolysis of a cladinose sugar glycosidic bond.[30−32] Several decladinosyl derivatives were prepared (**17**, **18**, **21**, and **24**) to investigate the influence of cladinose sugar removal on biological activity (Scheme 4).

We also investigated whether the presence of a chlorine atom in the quinoline ring was necessary for the activity of the hybrid molecules. Catalytic hydrogenation afforded a set of compounds **16**, **18**, **20**, and **23** in good yields.

Furthermore, the aminoquinoline moiety was substituted with a quinolinyl methanamine moiety (**26**−**29**), as well as the linking position of the quinoline ring, to investigate its influence on antiparasitic activity. Compounds **26a**, **26b**, and **26c** were prepared by reductive amination of **7** with quinoline carbaldehydes (Scheme 6). Additionally, *N*-methyl derivatives (**27** and **28**) were prepared to verify the influence of these structural features on antimalarial activity.

For the purpose of investigating the chemical stability of the 2′-ether bond, a set of solubility and solution stability studies

Scheme 4. Synthesis of Novel 15-Membered Azalides: 4-Aminoquinoline Derivatives Prepared by Amidation of Amine 7[a]



15 R_1 = α-L-cladinosyl, R_2 = Cl, n = 1
16 R_1 = α-L-cladinosyl, R_2 = H, n = 1
17 R_1 = H, R_2 = Cl, n = 1
18 R_1 = R_2 = H, n=1
19 R_1 = α-L-cladinosyl, R_2 = Cl, n = 3
20 R_1 = α-L-cladinosyl, R_2 = H, n = 3
21 R_1 = H, R_2 = Cl, n = 3

22 R_1 = α-L-cladinosyl, R_2 = Cl
23 R_1 = α-L-cladinosyl, R_2 = H
24 R_1 = H, R_2 = Cl

[a]Reagents and conditions: (a) 12, HOBt, PS-CDI, DCM, rt. (b) 13, HOBt, EDCxHCl, TEA, DCM, rt. (c) H_2/Pd/C. (d) H^+/H_2O. (e) 11, DIPEA/DMSO.

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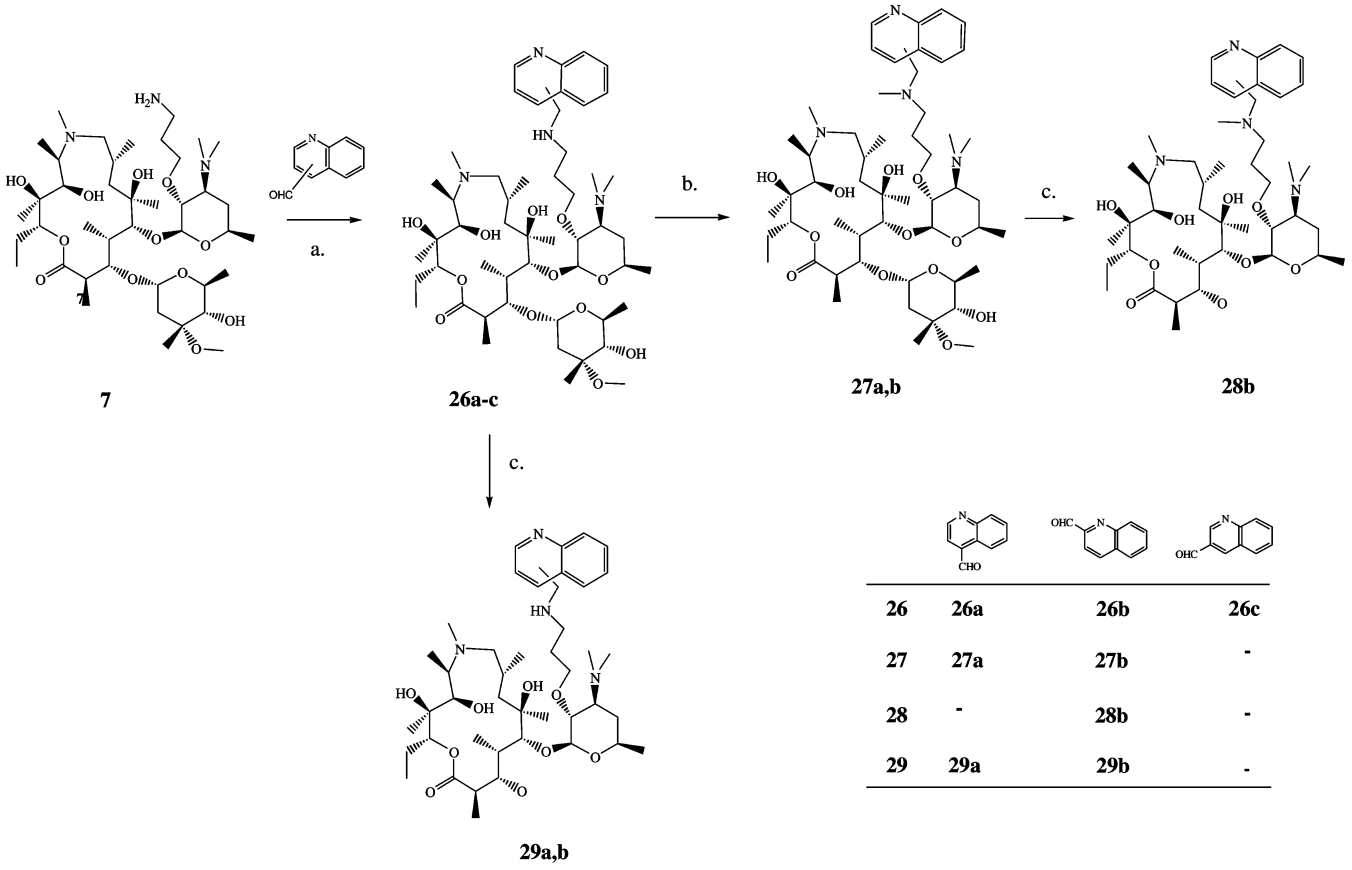
Scheme 6. Synthesis of Quinolinyl Methanamine Derivatives 26−29[a]



26	**26a**	**26b**	**26c**
27	**27a**	**27b**	-
28	-	**28b**	-
29	**29a**	**29b**	-

[a]Reagents and conditions: (a) TEA, NaBH4, rt. (b) HCHO, HCOOH, $CHCl_3$, rt. (c) 3 M HCl, rt.

were performed using compound **22**. Results revealed a high stability at pH 5−6 and degradation at pH 1.5−3,[33] attributable to a very well-known decomposition of 15-membered azalides: hydrolysis of the cladinose sugar glycosidic bond.[30−32] The newly introduced 2′-ether linkage was found to be stable in variable media, pH, and temperatures. Water solubility of the 2′-ether derivatives was determined to be >10 mg/mL (pH 4), which increased up to 100-fold for some derivatives when prepared in acetic salt form.

Biology. The in vitro antimalarial activity of the compounds was compared with that of AZI and chloroquine (CHL). Using the methodology described in the in vitro screening protocol, compounds listed in Tables 1 and 2 were profiled for their antimalarial activity against two different *P. falciparum* strains (the CHL-resistant W2 and CHL-sensitive 3D7A strains) and their inhibition of human hepatocellular carcinoma cell line HepG2 (used for determining potential cytotoxicity). The results of the biological evaluation were expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite or cell growth. Also, the antibacterial activity was verified by determining the minimal inhibitory concentration (MIC) of the compounds against phenotypically macrolide-sensitive strains of three Gram-positive bacterial species: *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*.

As shown in Tables 1 and 2, all tested compounds exhibited in vitro antimalarial activity with a substantial improvement over AZI (3−843-fold increase). Interestingly, the tested compounds followed the trend of AZI in exhibiting lower IC_{50} values against the CHL-resistant strain (W2) than against the CHL-sensitive strain (3D7A). A remarkable characteristic of these novel molecules was its activity against the W2 strain, which was improved, as compared to CHL, for almost all of the tested compounds.

Among the 19 compounds assayed against the 3D7A and W2 strains, six compounds (**15**, **19**, **22**, **23**, **24**, and **25**) exhibited activity with an IC_{50} in the low nanomolar range, which is equivalent to CHL and several orders of magnitude superior to AZI. Compounds **15**, **19**, and **22**−**25** showed greatly enhanced activity against the W2 (chloroquinoline-resistant) strain as compared to CHL and consistently low IC_{50} values against both strains tested.

Overall, the best antimalarial activity was observed for the compounds that retained both sugars and were substituted with 4-amino-7-chloroquinoline moiety (**15**, **19**, and **22**). Activity decreased when the chlorine atom on the quinoline core was substituted with hydrogen (**15** vs **16**, **17** vs **18**, **19** vs **20**, and **22** vs **23**) and when cladinose was removed from the azalide backbone (**15** vs **17**, **19** vs **21**, and **22** vs **24**). Modification of the linker by insertion of the amide moiety also resulted in decreased antiparasitic activity (**22** vs **15** and **22** vs **19**). When the 4-aminoquinoline moiety was replaced with quinolinyl-methanamine substituents (Table 2, compounds **26**−**29**), activity against the 3D7A strain weakened substantially. However, for compounds **26a**−**c** and **27a**,**b** (derivatives with both sugars present), activity against the CHL-resistant W2 strain remained in the low nanomolar range.

The inhibition of the human hepatocellular carcinoma HepG2 cell line was determined to assess the cytotoxicity of

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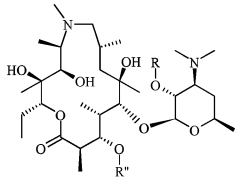
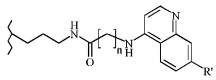
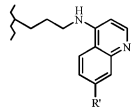
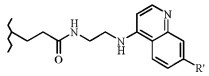
Table 1. In Vitro Activity of 4-Aminoquinoline Derivatives of 2′-*O*-Substituted 9-Deoxo-9a-methyl-9a-aza-9a-homoerythromycin A: Antimalarial Activity against Two *P. falciparum* Strains (W2 and 3D7A), Inhibition of Human Hepatocellular Carcinoma HepG2 Cell Line (Cytotoxicity), and Antibacterial Activity against Three Macrolide Sensitive Bacterial Strains (*S. pneumoniae*, *S. pyogenes*, and *S. aureus*)



Compd	R	n	R'	R''	*P. falciparum* IC_{50} (nM) 3D7A	*P. falciparum* IC_{50} (nM) W2	IC_{50} (μM) HepG2	MIC (μg/mL) *S. pneumoniae*	MIC (μg/mL) *S. pyogenes*	MIC (μg/mL) *S. aureus*
AZI					11513	2680	203	≤0,125	≤0,125	0,5
CHL					16	431	116	>64	>64	>64
15		1	Cl	cladinosyl	14	9	28	>64	>64	>64
16		1	H	cladinosyl	119	68	>100	>64	>64	>64
17		1	Cl	H	138	84	>100	>64	>64	>64
18		1	H	H	1657	815	ND*	ND	ND	ND
19		3	Cl	cladinosyl	29	19	50	>64	>64	>64
20		3	H	cladinosyl	220	135	>100	>64	>64	>64
21		3	Cl	H	402	224	75	>64	>64	>64
22		-	Cl	cladinosyl	21	10	45	64	>64	>64
23		-	H	cladinosyl	64	35	80	32	>64	16
24		-	Cl	H	37	25	83	>64	>64	>64
25		-	Cl	cladinosyl	18	13	18	64	>64	64

*ND, not determined.

the compounds as well as their selectivity for the *P. falciparum* parasites. Selectivity is defined as the ratio between HepG2 and *P. falciparum* IC_{50} values whereby a compound with a value above 100 is considered to be selective for the parasite. The results demonstrated that the factor of selectivity for all of the compounds was greatly above 100 for the W2 strain (from 231 up to 4356), as well as for the 3D7A strain for 4-aminoquinoline derivatives and (2- and 4-quinolinyl)-methamine (from 132 to 2240), while only the (3-quinolinyl)-methamine derivatives had selectivity lower than 100 (49 for **27b**, 88 for **26b**, and for **28b** and **29b** selectivity could not be precisely calculated).

Because of the fact that the 2′ position of macrolide antibiotics is known for its importance in enabling ribosome binding and antibacterial effect, the potency of the novel compounds on bacterial pathogens was verified. The bacteria tested represent the species most affected by the action of macrolide antibiotics, and the strains used were all fully sensitive to AZI. The data demonstrated a complete loss of antibacterial activity for the compounds tested, with some negligible activity of compounds **22** and **23** remaining against *S. pneumoniae* and *S. aureus*.

DISCUSSION

Novel antimalarial agents are urgently needed since the implementation of an effective antimalarial drug policy is often hindered by the emergence of resistant strains resulting in treatment failures. Macrolides, and AZI in particular, have shown the potential to be used in malaria treatment and prophylaxis. The current bottleneck in AZI implementation as an effective antimalarial agent is the lack of strong clinical evidence for overall equivalence or superiority over existing therapies, probably due to its low efficacy when used as a monotherapy.[19] Our work on novel and improved azalides for the treatment of malaria has already lead to the identification of several classes of 15-membered macrolides with improved in vitro and in vivo activity over AZI.[20] Encouraged by these findings, we extended our investigations with a focus on the most active examples. Key chemical features of the most promising compound included an AZI-based scaffold with a

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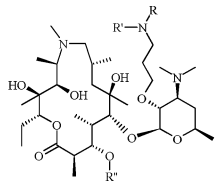
Table 2. In Vitro Activity of Quinolinyl-Methanamine Derivatives of 2′-*O*-Substituted 9-Deoxo-9a-methyl-9a-aza-9a-homoerythromycin A Derivatives: Antimalarial Activity against Two *P. falciparum* Strains (W2 and 3D7A), Inhibition of Human Hepatocellular Carcinoma HepG2 Cell Line (Cytotoxicity), and Antibacterial Activity against Three Macrolide Sensitive Bacterial Strains (*S. pneumoniae*, *S. pyogenes*, and *S. aureus*)



				P. falciparum IC_{50} (nM)		IC_{50} (μM)	MIC (μg/mL)		
Compd	R	R'	R''	3D7A	W2	HepG2	*S. pneumoniae*	*S. pyogenes*	*S. aureus*
26a		H	cladinosyl	141	78	45	>64	>64	>64
26b		H	cladinosyl	228	46	20	>64	>64	>64
26c		H	cladinosyl	227	55	30	>64	>64	>64
27a		CH_3	cladinosyl	248	49	45	>64	>64	>64
27b		CH_3	cladinosyl	466	47	23	>64	>64	>64
28b		CH_3	H	1286	311	>100	>64	>64	>64
29a		H	H	540	432	>100	>64	>64	>64
29b		H	H	1367	286	>100	>64	>64	>64

chloroquinoline substituent attached at the 9a-*N* position, which resulted in the increased in vitro and in vivo potency and favorable pharmacokinetic and druglike properties.[20,21] A step forward was achieved with the class of azalides presented here in that different quinoline substituents were added to the 15-membered azalide scaffold at the 2′-*O* position. This combined the favorable synergistic properties of these two pharmacophores observed in our previous compounds by utilizing a chemically novel and alternative method, which allowed even further improvements in their properties. Within the presented set of molecules, the most active antimalarial azalides so far were obtained (**15** and **22**) with very favorable in vitro profiles (i.e., cytotoxicity and selectivity).

In our drug design, we aimed for the elimination of antibacterial activity. An antibacterially inactive drug in the contemporary treatment of malaria would definitively have a therapeutic and ethical advantage in that such a drug would not affect the emergence and spread of bacterial resistance nor would it further narrow the spectrum of accessible antibiotics to already vulnerable and underprivileged target populations in malaria endemic countries.

The addition of quinoline substituents on the 2′-*O* position of 15-membered azalide scaffold completely abolished antibacterial activity of the new derivatives. This effect was irrespective of the removal of the cladinose.

Furthermore, because the target of macrolide antibiotics is 70S prokaryotic ribosome, which is also present in the parasite apicoplast organelle, we considered the testing of antibacterial activity as an indirect measurement of the macrolide mode of action. The elimination of antibacterial potency indicated that 2′-OH position is highly important for target binding and that the addition of quinoline substituents at this position leads to the disruption of the compound-ribosome steric complementarity.

Moreover, the antimalarial activity of analogues **15**, **19**, **22**, and **25** was substantially increased over AZI and CHL, with stronger inhibition of the CHL-resistant parasite. The obtained results point to the existence of a mechanism delivering the chloroquinoline moiety to the target site (the food vacuole) possibly by contributing to its uptake and accumulation into this organelle. The mechanism could have a physicochemical rationale since these compounds have higher lipophilicity (according to the calculated log *P* values, data not shown) than their parent compound and could thus penetrate membranes more efficiently. In addition, the mechanism could involve biochemical reasons like changing the substrate specificity for the transport proteins involved in CHL efflux.

The results reported here demonstrate a proof of concept for the linkage of AZI and a 4-amino-7-chloroquinoline moiety in a single molecule that retains, and evidently enhances, the antimalarial activity of the parent compounds. This novel generation of 15-membered azalides deserves additional investigation, and some of the later stage assays are already under way to assess the developability and mode of action of the most promising leads from this series.

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CONCLUSION

With malaria still devastating endemic countries, novel antimalarials are urgently needed. Here, we describe a novel concept in antimalarial drug design: a set of hybrid molecules obtained by covalently linking quinoline moieties to an azalide macrolactone scaffold. Following an original multistep synthetic route, 19 novel 2′-*O*-substituted-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A derivatives were obtained and analyzed. Screening of the in vitro antimalarial and cytotoxic activity of the aforementioned compounds enabled the identification of compounds with high selectivity for the *P. falciparum* parasite, antimalarial activity comparable to CHL against a sensitive strain (3D7A), and greatly enhanced activity against a CHL-resistant strain (W2). At the same time, the antibacterial activity of the compounds was eliminated, facilitating the development of malaria-specific macrolide agents. Taken all together, 2′-*O*-substituted-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A derivatives with a covalently linked 4-amino-7-chloroquinoline moiety are recognized as promising lead molecules for preclinical development. The exact mode of action of the presented compounds is not yet fully elucidated, and detailed target analyses, as well as in vivo profiling, are currently in progress.

EXPERIMENTAL SECTION

Biology. *P. falciparum Inhibition in Vitro.* The activity of the test compounds against P. falciparum in vitro was determined using a modification of the semiautomated microdilution technique of Desjardins.[34] Strains used were the standard sensitive 3D7A and CHL/pyrimethamine-resistant W2 strains. Compounds were dissolved in DMSO and subsequently diluted in culture medium (RPMI 1640) supplemented with 10% v/v human plasma. Serial 1:2 drug dilutions were prepared in triplicate in microtiter plates. Culture medium supplemented with 10% v/v human plasma and type A Rhesus positive human erythrocytes infected with *P. falciparum* strains (3D7A or W2) was added to yield a hematocrit of 3% and a parasitemia of 0.25−0.55%. [^{3}H]Hypoxanthine was added to give a final concentration of 12.5−16 μCi/mL of culture. The plates were incubated at 37 °C in a modular incubator flushed with a mixture of 5% oxygen, 3% carbon dioxide, and 92% nitrogen. After incubation for 48 h, particulate matter was harvested on fiberglass strips, and hypoxanthine incorporation was determined by scintillation spectrophotometry. From the concentration−response curves analyzed by nonlinear regression, the 50% inhibitory concentrations (IC_{50}) for each test compound were calculated.

Cytotoxicity Assay. The detailed protocol for cytotoxicity measurement is described elsewhere.[35] In brief, Hep G2 cells were maintained in complete RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 atmosphere. The cytotoxicity assay was performed using the MTS CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, United States). Each culture in the 96-well plates contained 50000 cells, which were exposed to serial dilutions (1:2) of tested compounds (initially dissolved in DMSO and subsequently diluted in supplemented RPMI 1640 medium). Plates were incubated for 24 h at 37 °C in 5% CO_2. After the addition of MTS reagent and 2 h of incubation at 37 °C in 5% CO_2, the absorbance at 490 nm was recorded, and IC_{50} was determined based on the obtained response curves.

Antibacterial Screen. The compounds were tested against bacteria by a standard broth microdilution method[36] with the exception that for the testing of *Streptococcus* strains, lysed horse blood in the broth was substituted with 5% horse serum. Bacteria were grown on appropriate agar plates (Becton Dickinson, United States): Columbia agar with 5% sheep blood for streptococci and Mueller−Hinton agar for *S. aureus*. AZI and CHL were used as controls. MICs were expressed in μg/mL. The panel consisted of two macrolide-sensitive streptococci clinical isolates (*S. pneumoniae* SP030 and *S. pyogenes* 3565) and one reference strain obtained from American Type Culture Collection (*S. aureus* ATCC13709). These organisms are the major bacterial pathogens targeted by macrolide antibiotics. The method for automated preparation of microtiter plates with compound double dilutions was programmed for TECAN robotic system in GEMINI pipetting software.[35]

Chemistry. *General Methods.* All commercial reagents (Merck, Sigma-Aldrich) were used as provided unless otherwise indicated, and all solvents were of the highest purity unless otherwise noted. NMR spectra were recorded on a Bruker Avance DRX500 or Bruker Avance DPX300 spectrometer in $CDCl_3$ or DMSO, and chemical shifts were reported in ppm using TMS as an internal standard. 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. In synthetic procedures, column chromatography was carried out over Merck Kieselgel 60 (230−400 mesh) or on an solid-phase extraction (SPE) cartridge with average size silica of 50 μm. Thin-layer chromatography was performed on 0.24 mm silica gel plates Merck TLC 60F254. The indicated eluent was used, and the solvent ratios refer to the volume. In general, organic solutions were dried with anhydrous Na_2SO_4 or K_2CO_3, and evaporation and concentrations were carried out under reduced pressure below 40 °C unless otherwise noted.

The purity of each compound was determined on an Agilent 1100 Series LC/MSD trap. The analysis was carried out with MS and UV detection at 240 nm on a YMC Pack Pro C_{18} column, 150 mm × 4.6 mm i.d., 3 μm particle size. The gradient elution at a flow rate of 1.0 mL/min started with 10% acetonitrile/90% 40 mM ammonium acetate buffer solution and ended after 15 min with 90% acetonitrile/10% 40 mM ammonium acetate buffer solution. The ESI source was operated in the positive ion mode. Nitrogen was used as a nebulizing and drying gas at 350 °C. The m/z range scanned in the MS measurement was 200−1200. Complete system control, data acquisition, and processing were done using the ChemStation for LC/MSD version 4.2 from Agilent. The acceptable range of purity for each compound was ≥95%.

2′-O-(3-Aminopropyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (7). Compound **6** (486 mg, 0.54 mmol) was dissolved in THF (4,5 mL), and LiOH (6 mL of 0.5 M) was added, heated at 40 °C for 2 h, and stirred at rt for an additional 72 h. H_2O (10 mL) was added to the reaction mixture, followed by extraction with EtOAc. Organic layers were collected and dried over Na_2SO_4. Solvent was evaporated yielding 380 mg (87%) of the white solid. MS (ES+): 806 $[MH]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm: 0.79 (t, J = 7.4 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 7.6 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 1.01 (s, 3H), 1.07 (d, J = 6.1 Hz, 3H), 1.09 (d, J = 7.5 Hz, 3H), 1.10−1.11 (m, 1H), 1.13 (s, 3H), 1.15 (d, J = 6.3 Hz, 3H), 1.17 (s, 3H), 1.31 (dd, J = 14.8, 7.9 Hz, 1H), 1.34−1.40 (m, 1H), 1.51 (d, J = 14.8 Hz, 1H), 1.51 (d, J = 15.0 Hz, 1H), 1.54−1.61 (m, 2H), 1.61−1.66 (m, 1H), 1.73−1.81 (m, 1 H), 1.85−1.89 (m, 1H), 1.90−1.94 (m, 1H), 2.13 (dd, J = 11.9, 10.5 Hz, 1 H), 2.20 (s, 3H), 2.22 (s, 6H), 2.25 (d, J = 15.0 Hz, 1H), 2.35 (dd, J = 11.0, 3.5 Hz, 1H), 2.46 (ddd, J = 12.2, 10.4, 4.1 Hz, 1H), 2.64−2.70 (m, 3H), 2.72−2.77 (m, 1H), 2.85 (dd, J = 10.1, 7.5 Hz, 1H), 2.91 (d, J = 9.6 Hz, 1H), 3.22 (s, 3H), 3.42−3.44 (m, 1H), 3.47−3.50 (m, 1H), 3.54 (d, J = 7.2 Hz, 1H), 3.62−3.68 (m, 1H), 3.87−3.92 (m, 1H), 4.06 (dq, J = 9.5, 6.2 Hz, 1H), 4.20 (dd, J = 4.0, 1.9 Hz, 1H), 4.31 (s, 1H), 4.32−4.35 (m, 1H), 4.40 (d, J = 7.3 Hz, 1H), 4.75 (dd, J = 10.2, 2.7 Hz, 1H), 4.85 (d, J = 5.1 Hz, 1H). ^{13}C NMR (600 MHz, DMSO-d_6) δ/ppm: 177.02, 101.84,

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94.26, 82.18, 79.72, 77.22, 76.98, 76.22, 74.78. 73.50, 72.75, 72.42, 70.55, 68.55, 66.77, 64.58, 63.78, 61.34, 48.75, 44.69, 41.66, 41.43, 40.16, 38.62, 35.64, 34.52, 30.57, 30.49, 27.35, 25.91, 22.04, 21.34, 20.91, 20.84, 18.45, 17.58, 14.72, 10.86, 8.18, 6.68.

*2′-O-(3-Carboxyethyl)-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**10**).* A 0.5 M concentration of LiOH was added (25 mL) to a solution of **9** (3.2 g) in THF (50 mL).[26] The reaction was stirred at room temperature for 48 h, and the solvent was evaporated to yield 2.78 g of crude product subsequently purified on an SPE column (50 g). A total of 130.7 mg of the title product was isolated. MS (ES+): 821.4 $[MH]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm: 0.79 (t, J = 7.5 Hz, 3H), 0.85 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 7.5 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 1.02 (s, 3H), 1.09 (m, 3H), 1.11 (m, 3H), 1.14 (s, 3H), 1.15 (d, J = 6.0 Hz, 3H), 1.18 (s, 3H), 1.19 (m, 1H), 1.32 (m, 1H), 1.39 (m, 1H), 1.50 (m, 1H), 1.51 (d, J = 15 Hz, 1H), 1.76 (m, 1H), 1.78 (m, 1H), 1.88 (m, 1H), 1.93 (m, 1H), 2.15 (m, 1H), 2.18 (m, 1H), 2.22 (bs, 3H), 2.26 (d, J = 15 Hz, 1H), 2.32 (m, 1H), 2.35 (m, 1H), 2.40 (s, 6H), 2.66 (m, 1H), 2.67 (m, 1H), 2.68 (m, 1H), 2.91 (d, J = 9 Hz, 1H), 3.02 (dd, J_1 = 10.5, 7.5 Hz, 1H), 3.21 (s, 3H), 3.43 (bs, 1H), 3.53 (d, J = 7.0 Hz, 1H), 3.68 (m, 1H), 3.71 (m, 1H), 3.81 (m, 1H), 4.04 (m, 1H), 4.16 (m, 1H), 4.23 (bs, 1H), 4.35 (bs, 1H), 4.44 (d, J = 7.5 Hz, 1H), 4.75 (dd, J_1 = 10.5, 2.5 Hz, 1H), 4.85 (d, J = 4.5 Hz, 1H), 7.60 (bs, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) δ/ppm: 7.13, 8.55, 11.32, 15.15, 18.05, 18.91, 21.30, 21.38, 21.67, 22.48, 26.34, 27.71, 31.21, 34.97, 36.12, 36.89, 40.56, 41.87, 41.98, 45.13, 49.24, 61.88, 64.68, 65.12, 67.02, 68.48, 68.92, 72.90, 73.21, 73.97, 75.25, 76.68, 77.48, 77.68, 78.51, 83.05, 94.75, 102.08, 173.68, 177.47.

*2′-O-[3-({2-[(7-Chloro-4-quinolinyl)amino]ethanoyl}amino)-propyl]-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**15**).* PS-Carbodiimide resin (PS-CDI; loading, 1.2 mmol/g) (325 mg, 0.403 mmol) was added to a dry reaction vessel. Compound **12** (77 mg, 0.326 mmol) and 1-hydroxybenzotriazole hydrate (29.3 mg, 0.217 mmol), dissolved in a mixture of DCM (5 mL) and DMF (2.5 mL), were added to the dry resin. The mixture was stirred at room temperature for 5 min upon which **7** (250 mg, 0.310 mmol), dissolved in DCM (5 mL), was added. The reaction mixture was heated by microwave irradiation at 70 °C for 6 min. HOBt was scavenged using PS-trisamine (loading, 4.11 mmol/g) (420 mg, 1.73 mmol) for 3 h at room temperature. The product was filtered off, and the resin was washed with DCM (2 × 10 mL). After evaporation of filtrate, 278 mg of white foam was obtained. Crude material was dissolved in 3 mL of EtOAc and precipitated with the addition of *n*-hexane. Isolated precipitate was further recrystallized from acetone/petroleum ether (bp 35−60 °C), and 68 mg of the title product was isolated. Further crystallization from filtrate resulted in isolation of additional amounts (85 mg) of the title product (46%). MS (ES^+): 1024 $[MH]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm: 8.39 (d, J = 5.5 Hz, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.11 (t, J = 5.6 Hz, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.68 (t, J = 6.0 Hz, 1H), 7.49 (dd, J = 8.9, 2.1 Hz, 1H), 6.25 (d, J = 5.2 H, 1H), 4.84 (d, J = 4.9 Hz, 1H), 4.75 (dd, J = 10 Hz, 1H), 4.37 (d, J = 7.3 Hz, 1H), 4.29 (m, 2H), 4.24 (d, J = 7.6 Hz, 1H), 4.21 (dd, J = 4.0, 2.0 Hz, 1H), 4.06 (m, 1H), 3.86 (dd, J = 6.9, 5.5 Hz, 2H), 3.79 (m, 1H), 3.60 (m, 1H), 3.52 (d, J = 7.3 Hz, 1H), 3.46 (m, 2H), 3.23 (m, 1H), 3.20 (s, 3H), 3.12 (ddd, J = 12.8, 6.4 Hz, 1H), 2.91 (dd, J = 9.0, 7.8 Hz, 1H), 2.81 (dd, J = 9.9, 7.5 Hz, 1H), 2.66 (m, 2H), 2.45 (m, 1H), 2.33 (dd, J = 11.9, 1.3 Hz, 1H), 2.25 (d, J = 11.6 Hz, 1H), 2.22 (s, 6H), 2.20 (s, 3H), 2.12 (dd, J = 11.9 Hz, 1H), 1.91 (m, 1H), 1.88 (br. s., 1H), 1.77 (m, 1H), 1.57 (m, 3H), 1.49 (m, 2H), 1.38 (m, 1H), 1.31 (dd, J = 13.6, 7.8 Hz, 1H), 1.16 (s, 3H), 1.15 (d, J = 6.1 Hz, 3H), 1.12 (s, 3H), 1.09 (d, J = 7.3 Hz, 4H), 1.06 (d, J = 5.8 Hz, 3H), 1.01 (s, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 7.6 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H), 0.80 (t, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm: 177.45, 168.63, 152.13, 150.56, 149.31, 133.84, 127.86, 124.67, 124.51, 117.89, 102.41 (C-1′), 99.43, 94.78, 82.66, 80.22, 77.75, 77.52, 76.76, 75.23, 74.02, 73.2, 72.95, 70.36, 68.96, 67.18, 65.06, 64.24, 61.77, 49.21, 46.21, 45.18, 42.21, 41.90, 41.16, 37.00, 36.09, 35.03, 31.81, 30.23, 27.85, 26.39, 22.47, 21.77, 21.38, 21.38, 18.90, 18.06, 15.17, 11.34, 8.80, 7.20. HRMS (ESI) m/z calcd for $C_{52}H_{87}N_5O_{13}Cl$ (M + H^+), 1024.5989; found, 1024.5996.

*2′-O-[3-({2-[(4-Quinolinyl)amino]ethanoyl}amino)propyl]-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**16**).* To an ethanol solution of **15** (200 mg, 0.195 mmol in 30 mL), Pd/C (10%) was added (60 mg, 0.056 mmol), and the reaction mixture was stirred under hydrogen atmosphere (4 barr). After the 4 h catalyst was filtered off, EtOAc (20 mL) and water (20 mL) were added to the filtrate, and the pH was adjusted to 4 (1 M HCl). The water layer was then extracted with DCM (2 × 30 mL), and the layers were separated. By the addition of 1 M NaOH, the pH of the water layer was adjusted to pH 6.5 and extracted with DCM (2 × 30 mL), and the layers were separated. The organic layers at pH 6.5 were combined, and water was added. By addition of NH_4OH, the pH was adjusted at pH 9.5, and the layers were separated. The crude product obtained after evaporation of the solvent was recrystallized from diisopropyl-ether yielding 120 mg (62%) of the title product. MS (ES^+): 990.6 $[MH]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm: 8.38 (d, J = 5.5 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 8.08 (s, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.63 (ddd, J = 7.6, 1.2 Hz, 1H), 7.49 (t, J = 6.0 Hz, 1H), 7.45 (ddd, J = 7.5, 2.0 Hz, 1H), 6.23 (d, J = 5.2 Hz, 1H), 4.84 (d, J = 4.9 Hz, 1), 4.75 (dd, J = 10.2, 2.6 Hz, 1H), 4.37 (d, J = 7.3 Hz, 1H), 4.29 (s, 1H), 4.28 (d, J = 4.3 Hz, 1H), 4.25 (d, J = 7.3 Hz, 1H), 4.21 (dd, J = 4.0, 1.8 Hz, 1H), 4.07 (m, 1H), 3.85 (dd, J = 6.0 Hz, 1H), 3.80 (dt, J = 9.2, 5.6 Hz, 1H), 3.59 (m, 1H), 3.53 (d, J = 7.3 Hz, 1H), 3.46 (m, 2H), 3.21 (s, 3H), 3.24 (m, 1H), 3.13 (m, 2H), 2.91 (dd, J = 9.5, 7.6 Hz, 1H), 2.81 (dd, J = 10.1, 7.3 Hz, 1H), 2.67 (m, 2H), 2.45 (m, 1H), 2.33 (dd, J = 12.0, 2.0 Hz, 1H), 2.25 (d, J = 15.0 Hz, 2H), 2.22 (s, 6H), 2.20 (s, 3H), 2.12 (dd, J = 11.6 Hz, 1H), 1.91 (m, 1H), 1.88 (m, 1H), 1.77 (m, 1H), 1.58 (m, 3H), 1.50 (d, J = 14.6 Hz, 1H), 1.49 (d, J = 15.0 Hz, 1H), 1.38 (m, 1H), 1.32 (dd, J = 14.8, 7.8 Hz, 1H), 1.16 (s, 3H), 1.15 (d, J = 6.4 Hz, 3H), 1.13 (m, 1H), 1.12 (s, 3H), 1.10 (d, J = 7.6 Hz, 3H), 1.06 (d, J = 6.1 Hz, 3H), 1.01 (s, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 7.2 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.79 (t, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm: 176.86, 168.26, 150.31, 149.73, 147.98, 128.80, 128.58, 123.81, 121.47, 118.68, 101.83, 98.39, 94.24, 82.10, 79.66, 77.21, 76.98, 76.21, 74.66, 73.45, 72.62, 72.41, 69.82, 68.37, 66.61, 64.50, 63.66, 61.20, 48.63, 45.74, 44.63, 41.65, 41.32, 40.57, 36.43, 35.52, 34.47, 31.23, 29.68, 27.27, 25.83, 21.89, 21.19, 20.82, 20.78, 18.33, 17.49, 14.59, 10.76, 8.25, 6.64. HRMS (ESI) m/z calcd for $C_{52}H_{88}N_5O_{13}$ (M + H^+), 990.6379; found, 990.6364.

*2′-O-[3-({2-[(7-Chloro-4-quinolinyl)amino]ethanoyl}amino)-propyl]-3-O-decladinosyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**17**).* Compound **15** (0.08 g, 0.078 mmol) in HCl (10 mL, 3 M) was stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc (20 mL), the pH was adjusted to pH 9.5 (by addition of 6 M NaOH), and the layers were separated. Organic extracts were washed with water (2 × 20 mL). The combined organic layers were evaporated and purified by column chromatography (eluent DCM:MeOH:NH_4OH = 90:9:1.5) yielding 50 mg (74%) of the title product. MS (ES^+): 866.59 $[MH]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm: 8.40 (d, J = 5.2 Hz, 1H), 8.24 (d, J = 8.9 Hz, 1H), 8.11 (t, J = 5.5 Hz, 1H), 7.81 (d, J = 2.1 Hz, 1H), 7.68 (t, J = 5.8 Hz, 1H), 7.49 (dd, J = 8.9, 2.1 Hz, 1H), 6.27 (d, J = 5.2 Hz, 1H), 5.14 (d, J = 6.7 Hz, 1H), 4.95 (dd, J = 11.1, 1.4 Hz, 1H), 4.74 (d, J = 7.3 Hz, 1H), 4.05 (d, J = 7.6 Hz, 1H), 3.86 (dd, J = 5.2, 3.4 Hz, 2H), 3.83 (m, 1H), 3.53 (s, 1H), 3.43 (m, 2H), 3.35 (m, 1H), 3.29 (m, 1H), 3.22 (m, 1H), 3.11 (m, 1H), 2.84 (dd, J = 9.6, 7.8 Hz, 1H), 2.69 (dd, J = 13.0, 6.6 Hz, 1H), 2.54 (m, 1H), 2.43 (ddd, J = 14.0, 9.6, 5.0 Hz, 1H), 2.27 (m, 1H), 2.22 (s, 3H), 2.21 (s, 6H), 2.06 (m, 2H), 1.78 (m, 2H), 1.57 (m, 3H), 1.39 (m, 2H), 1.31 (dd, J = 13.5, 5.0 Hz, 1H), 1.17 (m, 1H), 1.12 (m, 6H), 1.07 (s, 3H), 0.97 (s, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 7.3 Hz, 3H), 0.82 (d, J = 7.0 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H). ^{13}C NMR (500 MHz, DMSO-d_6) δ/ppm: 175.97, 168.63, 152.2, 150.52, 149.32, 133.82, 127.8, 124.68, 124.50, 117.89, 100.71, 99.47, 84.29, 79.83, 76.84, 76.55, 76.53, 74.01, 72.82, 70.12, 69.18, 67.89, 64.13, 61.81, 46.19, 43.67, 41.25, 41.02, 37.18, 21.81, 21.56, 21.01, 18.21, 16.89, 10.91, 8.58, 6.69. HRMS (ESI) m/z calcd for $C_{44}H_{73}N_5O_{10}Cl$ (M + H^+), 866.5046; found, 866.5039.

*2′-O-[3-({4-[(7-Chloro-4-quinolinyl)amino]butanoyl}amino)-propyl]-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**19**).* To a solution of **7** (0.18 g, 0.22 mmol) in DCM (10 mL), 4-[(7-

chloro-4-quinolinyl)amino]butanoic acid (0.066 g, 0.25 mmol), HOBt (0.037 g, 0.286 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (0.071 g, 0.37 mmol), and triethylamine (TEA) (0.25 mL) were added, and the reaction mixture was stirred overnight at rt. Water was added, the organic layer separated, and the pH of the water layer was adjusted to pH 6.5 and extracted with DCM. To the water layer, DCM was added, and the pH was adjusted to pH 9.0 and extracted with DCM. DCM layers at pH 6.5 and 9.0 were combined and dried over Na_2SO_4. DCM was evaporated affording 0.25 g of the title product as a light yellow powder. MS (ES+): 1052.7 $[MH]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ/ppm: 8.42 (m, 1H), 8.23 (m, 1H), 8.05 (m, 1H), 8.01 (m, 1H), 7.81 (t, J = 5.2 Hz, 1H), 7.40 (m, 2H), 6.31 (m, 2H), 5.23 (m, 1H), 5.18 (d, J = 4.9 Hz, 1H), 4.73 (m, 1H), 4.38 (d, J = 7.3 Hz, 1H), 4.32 (d, J = 2.4 Hz, 1H), 4.10, 3.42 (m, 2H), 4.06 (m, 1H), 3.67 (m, 1H), 3.65 (d, J = 7.3 Hz, 1H), 3.48 (m, 1H), 3.61 (m, 1H), 3.30 (m, 1H), 3.34 (m, 2H), 3.30 (s, 3H), 3.05 (dd, J = 9.0, 7.5 Hz, 1H), 2.95 (dd, J = 10.1, 7.6 Hz, 1H), 2.70 (m, 1H), 2.69 (m, 1H), 2.59 (m, 1H), 2.56 (m, 1H), 2.07 (m, 1H), 2.44 (m, 2H), 2.34 (s, 6H), 2.33 (s, 3H), 2.33 (m, 1H), 1.61 (m, 1H), 2.16 (m, 2H), 2.04 (m, 1H), 1.95 (m, 1H), 1.89 (m, 1H), 1.67 (m, 1H), 1.46 (m, 1H), 1.75 (m, 1H), 1.24 (m, 1H), 1.69 (m, 1H), 1.63 (m, 1H), 1.29 (m, 1H), 1.32 (d, J = 6.1 Hz, 3H), 1.30 (s, 3H), 1.26 (s, 3H), 1.20 (d, J = 5.9 Hz, 3H), 1.19 (d, J = 7.5 Hz, 3H), 1.10 (d, J = 7.0 Hz, 3H), 1.09 (s, 3H), 0.94 (d, J = 7.6 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ/ppm: 179.0, 173.5, 151.7, 149.8, 147.2, 136.1, 126.8, 125.8, 123.2, 117.5, 103.1, 98.0, 94.5, 82.7, 79.8, 78.3, 77.6, 77.3, 74.6, 73.9, 73.4, 73.2, 72.2, 70.2, 68.3, 65.6, 65.1, 62.9, 49,4, 45.5, 44.5, 44.2, 42.5, 40.6, 38.4, 36.3, 34.7, 34.6, 29.0, 28.9, 27.6, 26.8, 22.3, 23.0, 21.8, 21.5, 21.5, 18.3, 16.4, 14.8, 11.4, 8.5, 7.5. HRMS (ESI) m/z calcd for $C_{54}H_{91}N_5O_{13}Cl$ (M + H^+), 1052.6302; found, 1052.6294.

2′-O-{3-[(7-Chloro-4-quinolinyl)amino]propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**22**). To a solution of **7** (25.0 g, 0.031 mol) in DMSO (250 mL), 4,7-dichloroquinoline (30.7 g, 155 mmol) and Trisma Base (18.77 g, 155 mmol) were added. The reaction mixture was heated at 105 °C for 18 h. The reaction mixture was cooled to room temperature and evaporated to yield a slurry product. The slurry product was resolved in 500 mL of dichloromethane and 1500 mL of water. The pH of the mixture was adjusted to pH 5.0 by the addition of 1 M HCl, and layers were separated. The water layer was extracted at pH 5 with DCM (5 × 500 mL). The layers were separated. Further DCM (500 mL) was added, the pH was adjusted to pH 6.0 by the addition of 1 M NaOH, and the layers were separated. The water layer was extracted at pH 6.0−6.5 with DCM (22 × 500 mL). The organic layers extracted at pH 6.0−6.5 were dried over Na_2SO_4, and the organic solvent evaporated yielding 17.91 g of the crude product as light yellow crystals. The crude product of 17.91 g was recrystallized from acetonitrile to afford 12.89 g (43%) of the title product. MS (ES+): 967.6 $[MH]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ ppm: 8.37 (d, J = 5.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.41 (dd, J = 9.2, 2.1 Hz, 1H), 7.25 (t, J = 5.2 Hz, 1H), 6.43 (d, J = 5.5 Hz, 1H), 4.82 (d, J = 4.9 Hz, 1H), 4.72 (dd, J = 10.1, 2.4 Hz, 1H), 4.41 (d, J = 7.3 Hz, 1H), 4.31 (m, 2H), 4.19 (d, J = 7.6 Hz, 1H), 4.14 (dd, J = 4.0, 1.8 Hz, 1H), 4.05 (m, 1H), 3.88 (m, 1H), 3.63 (m, 2H), 3.49 (d, J = 7.0 Hz, 1H), 3.37 (d, J = 8.2 Hz, 1H), 3.30 (m, 2H), 3.22 (s, 3H), 2.90 (dd, J = 9.2, 7.6 Hz, 1H), 2.86 (dd, J = 10.1, 7.6 Hz, 1H), 2.55 (m, 3H), 2.29 (d, J = 11.3 Hz, 1H), 2.24 (s, 6H), 2.23 (d, J = 4.5, 1H), 2.18 (br. s., 3H), 1.91 (m, 2H), 1.81 (m, 3H), 1.75 (m, 1H), 1.59 (dd, J = 10.5, 3.2 Hz, 1H), 1.49 (dd, J = 15.0, 4.9 Hz, 1H), 1.43 (d, J = 14.6 Hz, 1H), 1.37 (m, 1H), 1.14 (d, J = 6.2 Hz, 3H), 1.13 (s, 6 Me, 6H), 1.10 (m, 1H), 1.10 (m, 1H), 1.06 (d, J = 5.8 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 1.03 (s, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 7.3 Hz, 3H), 0.79 (t, J = 7.0 Hz, 3H), 0.78 (d, J = 6.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm: 177.39, 152.09, 150.45, 149.42, 133.60, 127.87, 124.37, 124.28, 117.86, 102.33, 98.98, 94.67, 82.65, 80.10, 77.73, 77.40, 76.69, 75.23, 73.95, 73.19, 72.84, 69.95, 68.96, 67.12, 65.07, 64.31, 61.85, 49.18, 45.14, 42.01, 41.85, 41.31, 39.88, 36.04, 35.00, 32.43, 28.87, 27.77, 26.20, 22.38, 21.77, 21.38, 21.31, 18.90, 18.04, 15.10, 11.32, 8.70, 7.07. HRMS (ESI) m/z calcd for $C_{46}H_{77}N_5O_{10}Cl$ (M + H^+), 967.5774; found, 967.5759.

2′-O-{3-[(4-Quinolinyl)amino]propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**23**). To an ethanol solution of **22** (150 mg, 0.15 mmol in 25 mL), Pd/C (10%) was added (75 mg, 0.07 mmol), and the reaction mixture was stirred under hydrogen atmosphere (5 barr). After 4 h, the catalyst was filtered off, and the solvent was evaporated to yield 130 mg of crude product, which was further purified by column chromatography (eluent DCM:MeOH:N-H_4OH = 90:9:1.5) yielding 90 mg (64%) of the white powder. MS (ES^+): 933.6 $[MH]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ/ppm: 8.36 (d, J = 5.2 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 7.76 (d, J = 7.9 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.08 (t, J = 5.3 Hz, 1H), 6.40 (d, J = 5.5 Hz, 1H), 4.83 (d, J = 4.9 Hz, 1H), 4.73 (dd, J = 10.2, 2.9 Hz, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.30 (m, 2H), 4.20 (d, J = 7.3 Hz, 1H), 4.17 (m, 1H), 4.07 (m, 1H), 3.90 (dt, J = 8.9, 5.8 Hz, 1H), 3.65 (m, 2H), 3.52 (d, J = 7.3 Hz, 1H), 3.39 (m, 3H), 3.23 (s, 3H), 2.91 (m, 1H), 2.87 (dd, J = 9.5, 7.0 Hz, 1H), 2.58 (m, 3H), 2.29 (d, J = 11.0 Hz, 1H), 2.24 (d, J = 15.0 Hz, 1H), 2.23 (s, 6H), 2.19 (s, 3H), 1.99 (dd, J = 11.6, 11.0 Hz, 1H), 1.86 (m, 4H), 1.76 (m, 1H), 1.60 (m, 1H), 1.50 (dd, J = 15.0, 5.0 Hz, 1H), 1.47 (dd, J = 11.8, 1.0 Hz, 1H), 1.37 (m, 1H), 1.19 (m, 1H), 1.15 (s, 3H), 1.15 (d, J = 7.0 Hz, 3H), 1.14 (s, 3H), 1.11 (d, J = 11.9 Hz, 1H), 1.06 (d, J = 6.3 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 1.02 (s, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 7.3 Hz, 3H), 0.80 (d, J = 7.5 Hz, 3H), 0.79 (t, J = 6.5 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm: 177.42, 150.83, 150.33, 148.59, 129.33, 128.95, 124.01, 121.94, 119.25, 102.36, 98.45, 94.69, 82.65, 80.13, 77.74, 77.4, 76.71, 75.23, 73.97, 73.21, 72.89, 70.09, 68.93, 67.16, 65.06, 64.38, 61.79, 49.18, 45.14, 42.09, 41.90, 41.2, 40.38, 36.07, 35.01, 32.23, 29.04, 27.81, 26.28, 22.40, 21.79, 21.3, 21.33, 18.90, 18.09, 15.11, 11.33, 8.78, 7.11. HRMS (ESI) m/z calcd. for $C_{50}H_{85}N_4O_{12}$ (M + H^+), 933.6164; found, 933.6171.

2′-O-[3-({2-[(7-Chloro-4-quinolinyl)amino]ethyl}amino)-3-oxopropyl]9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**25**). To a solution of **10** (95 mg, 0.12 mmol) in DCM (10 mL), TEA (0.11 mL, 0.81 mmol), HOBt (18 mg, 0.13 mmol), compound **14** (27 mg, 0.12 mmol), and EDCxHCl (33 mg, 0.1725 mmol) were added. The reaction mixture was stirred at room temperature for 42 h. To the reaction mixture, 30 mL of water was added (pH 8.0) and extracted with DCM (3 × 30 mL). The organic layers were collected and dried on Na_2SO_4. The solvent was evaporated to afford 130.7 mg of yellowish solid, which was purified on Isolute SPE 10 g column eluting with DCM/[MeOH/NH_4OH] resulting in 62 mg (50%) of the title product. MS (ES+) m/z: 1024.59 $[MH]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ/ppm: 8.65 (t, J = 5.5 Hz, 1H), 8.39 (d, J = 5.4 Hz, 1H), 8.13 (d, J = 9.1 Hz, 1H), 7.78 (d, J = 2.3 Hz, 1H), 7.45 (dd, J = 9.0, 2.0 Hz, 1H), 7.44 (m, 1H), 6.54 (d, J = 5.4 Hz, 1H), 4.83 (d, J = 4.7 Hz, 1H), 4.74 (dd, J = 10.1, 2.6 Hz, 1H), 4.39 (d, J = 7.3 Hz, 1H), 4.29 (br. s., 2H), 4.24 (br. s., 1H), 4.19 (d, J = 2.3 Hz, 1H), 4.04 (m, 1H), 3.88 (m, 1H), 3.66 (m, 1H), 3.63 (m, 1H), 3.52 (d, J = 7.0 Hz, 1H), 3.43 (d, J = 7.7 Hz, 1H), 3.35 (br. s., 4H), 3.14 (s, 3H), 2.89 (br. s., 1H), 2.85 (dd, J = 10.1, 7.5 Hz, 1H), 2.66 (m, 2H), 2.43 (m, 1H), 2.33 (br. s., 1H), 2.30 (m, 2H), 2.22 (d, J = 15.2 Hz, 1H), 2.20 (s, 3H), 2.18 (s, 6H), 2.11 (dd, J = 12.0 Hz, 1H), 1.90 (m, 2H), 1.76 (m, 1H), 1.60 (m, 1H), 1.49 (m, 2H), 1.38 (m, 1H), 1.30 (dd, J = 14.0, 7.5 Hz, 1H), 1.16 (s, 3H), 1.14 (d, J = 6.1 Hz, 3H), 1.11 (s, 3H), 1.08 (d, J = 7.5 Hz, 4H), 1.06 (d, J = 5.9 Hz, 3H), 1.01 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 7.5 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6) δ/ppm: 176.8, 171.5, 151.6, 149.8, 148.8, 133.1, 127.3, 123.9, 123.4, 117., 101.7 (C-1′), 98.3, 94.1, 82.3, 78.9, 77.1, 76.9, 76.1, 74.7, 73.4, 72.6, 72.3, 68.4, 67.4, 66.6, 64.5, 63.5, 61.2, 48.5, 44.5, 42.3, 41.6, 41.3, 40.3, 37.7, 36.4, 35.5, 34.4, 30.0, 27.2, 25.8, 21.8, 21.1, 20.8, 19.9, 18.3, 17.4, 14.5, 10.7, 8.1, 6.6. HRMS (ESI) m/z calcd for $C_{52}H_{87}N_5O_{13}Cl$ (M + H^+), 1024.5989; found, 1024.5978.

2′-O-{3-[(4-Quinolinylmethyl)amino]propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**26a**). To a solution of **7** (1 g, 1.24 mmol) in MeOH (35 mL), TEA (0.585 mL, 4.2 mmol), 4-quinolinecarbaldehyde (164 mg, 1.04 mmol), and the reaction mixture were stirred at room temperature for 18 h, after which $NaBH_4$ (94 mg, 2.48 mmol) was added. The reaction mixture was stirred for an additional 3 h, and after that time, the solvent was evaporated. The

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residue was dissolved in water, and the pH was adjusted to pH 9.5 and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded 1.3 g of yellow powder product, which was further purified by column chromatography, (eluentDCM:MeOH:NH_4OH = 90:9:1.5) yielded 0.35 g (33%) of the title product. MS (ES+): 947.66 $[MH]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ/ppm: 0.78 - 0.82 (m, 6H), 0.93 (t, J = 7.2 Hz, 6H), 1.02 (m, 3H), 1.05 (d, J = 5.9 Hz, 3H), 1.06−1.07 (m, 1H), 1.08 (d, J = 7.5 Hz, 3H), 1.11 (s, 3H), 1.14 (d, J = 6.1 Hz, 3H), 1.15 (s, 3H), 1.24−1.30 (m, 1H), 1.33−1.42 (m, 1H), 1.48 (d, J = 14.8 Hz, 1H), 1.49 (d, J = 15.0 Hz, 1H), 1.54−1.58 (m, 1H), 1.63−1.73 (m, 2H), 1.74−1.81 (m, 1H), 1.83−1.86 (m, 1H), 1.87−1.92 (m, 1H), 2.05 (d, J = 11.2 Hz, 1H), 2.16 (s, 6H), 2.19 (s, 3H), 2.22 (d, J = 15.0 Hz, 1H), 2.32 (d, J = 12.0 Hz, 1H), 2.44 (ddd, J = 12.2, 10.3, 4.2 Hz, 1H), 2.63−2.68 (m, 2H), 2.68−2.74 (m, 2H), 2.81 (dd, J = 10.1, 7.5 Hz, 1H), 2.89 (dd, J = 9.4, 7.5 Hz, 1H), 3.14 (s, 3H), 3.44 (d, J = 8.2 Hz, 1H), 3.51 (d, J = 7.2 Hz, 1H), 3.53−3.58 (m, 1H), 3.58−3.64 (m, 1H), 3.81−3.86 (m, 1H), 4.02−4.07 (m, 1H), 4.14−4.16 (m, 1H), 4.17 (br. S., 1H), 4.17 (s, 2H), 4.29 (br. s., 2H), 4.39 (d, J = 7.5 Hz, 1H), 4.75 (dd, J = 10.1, 2.8 Hz, 1H), 4.83 (d, J = 4.9 Hz, 1H), 7.54 (d, J = 4.4 Hz, 1H), 7.60 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 7.74 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 8.02 (dd, J = 8.5, 1.0 Hz, 1H), 8.18 (d, J = 7.7 Hz, 1H), 8.82 (d, J = 4.4 Hz, 1H). ^{13}C NMR (600 MHz, DMSO-d_6) δ/ppm: 176.91, 149.97, 147.46, 146.26, 129.33, 128.76, 126.52, 126.01, 123.69, 119.46, 101.79, 94.18, 82.13, 79.49, 77.20, 76.95, 76.20, 74.70, 73.46, 72.63, 72.41, 70.26, 68.40, 66.6, 64.52, 63.79, 61.26, 48.98, 48.53, 46.76, 44.61, 41.63, 41.36, 40.59, 35.57, 34.48, 31.31, 29.55, 27.25, 25.81, 21.88, 21.23), 20.83, 20.76, 18.34, 17.53, 14.61, 10.80, 8.23, 6.67. HRMS (ESI) m/z calcd for $C_{51}H_{87}N_4O_{12}$ (M + H^+), 947.6321; found, 947.6321.

*2′-O-{3-[Methyl(4-quinolinylmethyl)amino]propyl}-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**27a**).* To a solution of **26a** (0.15 g, 0.158 mmol) in chloroform (5 mL), formaldehyde (0.028 mL) and formic acid (0.149 mL, 4.05 mmol) were added. The reaction mixture was stirred at 60 °C. After 18 h, the reaction mixture was diluted with DCM and water. Layers were separated, and the organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent yielded 0.16 g of crude product, which was further purified by column chromatography (eluent DCM:MeOH:NH_4OH = 90:9:1.5) yielding 0.1 g (65%) of the title product. MS (ES+): 961.67 $[MH]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ/ppm: 0.73 (d, J = 6.8 Hz, 3H), 0.80 (t, J = 7.5 Hz, 3H), 0.87 (d, J = 7.5 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 1.03 (s, 3H), 1.05 (d, J = 6.1 Hz, 3H), 1.08 (d, J = 7.3 Hz, 3H), 1.10 (m, 1H), 1.12 (s, 3H), 1.12 (d, J = 4.5 Hz, 3H), 1.14 (s, 3H), 1.17−1.24 (m, 1H), 1.38 (ddd, J = 14.3, 10.1, 7.3 Hz, 1H), 1.45 (d, J = 14.0 Hz, 1H), 1.49 (dd, J = 14.9, 5.0 Hz, 1H), 1.53−1.58 (m, 1H), 1.69 (quin, J = 7.0 Hz, 2H), 1.74−1.80 (m, 1H), 1.81−1.84 (m, 1H), 1.85−1.88 (m, 1H), 2.03 (t, J = 11.7 Hz, 1H), 2.18 (s, 3H), 2.18 (br. s., 3H), 2.19 (s, 6H), 2.23 (d, J = 14.8 Hz, 1H), 2.30 (d, J = 10.6 Hz, 1H), 2.42−2.45 (m, 1H), 2.45−2.49 (m, 2H), 2.61−2.64 (m, 1H), 2.64−2.68 (m, 1H), 2.80 (dd, J = 10.0, 7.4 Hz, 1H), 2.89 (dd, J = 9.4, 7.5 Hz, 1H), 3.16 (s, 3H), 3.44 (d, J = 8.9 Hz, 1H), 3.50 (d, J = 7.2 Hz, 1H), 3.51−3.56 (m, 1H), 3.58−3.65 (m, 1H), 3.70 (dt, J = 9.0, 6.7 Hz, 1H), 3.85−3.94 (m, 2H), 4.00−4.07 (m, 1H), 4.14 (d, J = 7.5 Hz, 1H), 4.17 (dd, J = 4.2, 1.9 Hz, 2H), 4.28 (d, J = 8.2 Hz, 1H), 4.29 (s, 1H), 4.35 (d, J = 7.3 Hz, 1H), 4.75 (dd, J = 10.1, 2.8 Hz, 1H), 4.83 (d, J = 4.9 Hz, 1H), 7.47 (d, J = 4.4 Hz, 1H), 7.58 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.74 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H), 8.01 (dd, J = 8.5, 0.8 Hz, 1H), 8.28 (dd, J = 8.5, 0.8 Hz, 1H), 8.81 (d, J = 4.4 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ/ppm: 177.06, 150.04, 147.92, 144.71, 129.44, 128.97, 127.14, 126.04, 124.51, 121.24, 101.96, 94.36, 82.17, 79.85, 77.44, 77.11, 76.45, 74.89, 73.68, 72.81, 72.62, 69.92, 68.55, 66.80, 64.71, 63.92, 61.45, 58.60, 54.39, 48.78, 44.81, 42.14, 41.89, 41.54, 40.96, 35.75, 34.71, 32.60, 27.80, 27.43, 25.99, 22.00, 21.81, 21.40, 21.04, 18.52, 17.75, 14.78, 10.98, 8.49, 6.90. HRMS (ESI) m/z calcd for $C_{52}H_{89}N_4O_{12}$ (M + H^+), 961.6477; found, 961.6491.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental procedures and spectroscopic information for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AZI, azithromycin; CHL, chloroquine; PS-CDI, polymer-supported carbodiimide; SPE column, solid-phase extraction column; TEA, triethylamine; DIPEA, diisopropylethylamine

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