Synthesis and Antimalarial Efficacy of Two-Carbon Linked, Artemisinin-Derived, Trioxane Dimers in Combination with Known Antimalarial Drugs

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Abstract

Malaria continues to be a difficult disease to eradicate, largely due to the widespread populations it affects and to the resistance that malaria parasites have developed against once very potent therapies. The natural product artemisinin has been a boon for antimalarial chemotherapy as artemisinin combination therapy (ACT) has become the first line of chemotherapy. Because the threat of resistance is always on the horizon, it is imperative to continually identify new treatments, comprised of both advanced analogs of all antimalarial drugs, especially artemisinin, as well as the exploration of novel combinations, ideally with distinct mechanisms of action. Here we report, for the first time, synthesis of a series of two-carbon linked artemisinin-derived dimers, their unique structural features, and demonstration of their antimalarial efficacy via single oral dose administration in two 60-day survival studies of *Plasmodium berghei*-infected mice. Several of the new endoperoxide chemical entities consistently demonstrated excellent antimalarial efficacy, and combinations with two non-peroxide antimalarial drugs have been studied.

Introduction

As the landscape of therapeutic treatment advances, orally available small molecule inhibitors remain a prominent weapon in the fight against disease. Perhaps nowhere is this more important than in the treatment of malaria, whose parasites infected over 200 million people and killed ~655,000 people in 2010,[1] though a recent report suggests deaths of greater than 1 million people in 2010.[2] Prevention of malaria caused by the *Plasmodium* parasites has centered on the use of bed nets (of which implementation suffers from poor compliance)[3] and attempts to develop a vaccine (which has recently demonstrated protection, albeit marginal),[4] but the large number of people who still become infected continues to keep small molecule therapeutic treatment of paramount importance. The history of malaria therapy is long and dozens of small molecules have been the treatment of

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choice at some point.[5] Well known examples include quinine, chloroquine, and atovaquone (compounds **1**-**3**, respectively, Figure 1). Each of these molecules (and others) has fallen from prominence, due either to significant side effects or to the development of parasite resistance, or both.[5] These chemical entities have been largely replaced by treatments containing the natural product artemisinin.

Artemisinin (**4a**, Figure 2) could be considered one of the most significant natural product small molecule discoveries over the past 40 years. The molecule was first isolated from its natural source, *Artemisia annua*, in 1972 by a team under the direction of Dr. Youyou Tu.[6] It contains a surprisingly stable endoperoxide linkage that is essential for the demonstrated activity in a variety of diseases, most notably malaria and, more recently, several types of cancers and cytomegalovirus.[7] Unfortunately the molecule has poor bioavailability and is rapidly cleared from the body.[8] First generation derivatives that attempt to circumvent this issue include lipid-soluble artemether (**4b**) and water-soluble artesunate (**4c**), and these molecules are now part of the first line of antimalarial treatment known as artemisinin combination therapy (ACT). Example combinations are artemether (**4b**) plus lumefantrine (**6**),[9] artesunate (**4c**) plus mefloquine (**7**) and the recently FDA-approved artesunate (**4c**) plus pyronaridine (**8**, Figure 3).[10] However, both artemether and artesunate still have significant metabolic liabilities and short half-lives, so most new candidate drug combinations pair an artemisinin-derived analog with a drug that has better physical properties and a longer duration of activity. Further structure-activity relationships (SAR) have been explored around this peroxide linkage and have ranged from functionalization of the natural product[11–15] to completely synthetic peroxide structures.[16–19] The pursuit of highly efficacious artemisinin derivatives and novel drug combinations continues.

Synthesis of artemisinin-derived dimers

Efforts to improve the pharmacokinetic properties of artemisinin are ongoing. A wide variety of functional groups have been incorporated in order to improve absorption and increase the half-life of the molecule *in vivo* while also maintaining efficacy.[19] An alternative approach has been to synthetically tether two artemisinin units together to form dimeric molecules, the rationale being that, at a minimum, for every molecule that survives the metabolism and excretion processes, two of the required endoperoxide pharmacophores will be delivered to the sight of action. However, dimer trioxanes are often more than simply twice as potent as the corresponding monomers, and therefore an as yet unknown explanation must exist. Many examples of artemisinin dimers exist with varying lengths of the linker between the two units, usually tethering at the C10 position (position numbered in Figure 2). The most successful examples include linker lengths of 5,[20] 4,[21] or 3[22, 23] carbon atoms (example structures **9**, **10**, and **11**, respectively, Figure 4), with three-carbon analogs generally showing the greatest efficacy *in vivo*. There is, to the best of our knowledge, only one example of a two-atom linked dimer (**12**, Figure 4)[24] and only one example of a single-atom linked dimer (**13**, Figure 4).[25] Here we report the synthesis of a series of novel two-carbon linked dimers, their absolute stereochemical configuration, and their antimalarial efficacies. Furthermore, several of the best dimers have been screened in combination with two adjuvants in order to establish a diverse set of potential drug combinations.

Synthesis of two-carbon linked dimers

The currently used drugs artemether (**4b**) and artesunate (**4c**) contain a C10 acetal linkage. It is generally accepted that this linkage is a significant metabolic liability. Both molecules **4b** and **4c** are rapidly catabolized to dihydroartemisinin (DHA, **5**, Figure 2) and excreted following glucuronidation. Methodology has been previously developed to replace the C10 acetal linkage with a carbon-carbon bond,[26, 27] and these molecules are called C10-carba

analogs. These analogs have shown increased stability in simulated stomach conditions as compared to the first generation acetals artemether (**4b**) and artesunate (**4c**),[28] but have yet to translate into a marketed drug. By adapting established methodology, we set out to synthesize a two-carbon linked artemisinin dimer that might have distinct benefits over dimers of greater linker lengths.

The main obstacle in synthesizing such a molecule is the large steric interaction that would likely occur between two artemisinin units joined through such a short distance. Established methodology follows a pathway using a Lewis acid activation of an appropriately functionalized artemisinin precursor (Scheme 1). In this pathway, artemisinin is functionalized with an appropriate leaving group (LG) at the C10 position (general structure **14**, Scheme 1) which is thought to form oxocarbenium ion (**15**) *in situ* after Lewis-acid activation. Treatment with a nucleophile can then proceed through either one of two pathways – ideally nucleophilic addition occurs at C10, providing desired products of general structure **16**. However, the C9 proton is also easily accessible and elimination is a major competing pathway, particularly with harder nucleophiles, leading to the undesired elimination product (**17**).

With this reactivity in mind, we envisioned a two-carbon linker unit that could be made nucleophilic in successive steps. The acetylene unit became the focus because substituted acetylides have previously achieved substitution at the C10 position by reacting an electrophilic C10 fluoride analog with nucleophilic trimethylaluminum-activated acetylenes.[29] However, this methodology was low-yielding and the C10 fluoride is difficult to manipulate. As such, a more robust method was required. Organometallic reagents have achieved substitution at the C10 position,[29] and the commercially available ethynyl Grignard would allow installation of the acetylene unit. Initial attempts to introduce the acetylene unit at the C10 position using the common electrophilic precursor dihydroartemisinin acetate (DHA-OAc, **19**, Scheme 2) provided almost exclusively **17**, likely due to the highly basic (and weakly nucleophilic) nature of the Grignard acetylide reagent. Efforts were still futile upon addition of zinc(II) chloride ($ZnCl_2$), in an attempt to form a more nucleophilic zinc-magnesium hybrid species (likely $[\{Mg_2Cl_3(Et_2O)_6\}^+\{Zn(acetylene)_3\}^-]$) *in situ*.[30, 31] A new precursor containing a nucleophile assisting leaving group[32] was synthesized in order to form a chelating intermediate that would direct the organometallic reagent to the C10 position. Using standard procedure, the artemisinin lactone was reduced using diisobutylaluminum hydride (DIBALH) at –78 °C. The resulting oxyanion can be trapped with the appropriate electrophile (in this case 2,4-dimethoxybenzoyl chloride, **18**) directly *in situ* to provide exclusively the desired intermediate **20** (Scheme 2). The 2,4-dimethoxybenzoate group is an electron rich moiety to which $ZnCl_2$ or other Lewis acidic species can strongly chelate. The previously described zinc-magnesium hybrid nucleophile would be directed to the C10 position upon coordination to the benzoate. Simultaneous addition of the Lewis acid $ZnCl_2$ and ethynylmagnesium chloride to a stirring solution of **20** at –5 °C provided the previously unreported C10 β-alkyne (**21**). The best results were achieved with a 3.2:1.0 ratio of the Grignard reagent to $ZnCl_2$, which is consistent with previous studies that utilized similar reagents.[30, 31]

With **21** prepared in only two steps and in 73% overall yield from artemisinin, the two-carbon linked dimer system was within reach. Attempts to convert **21** into a Grignard reagent (and into other organometallic reagents, for example, a dimethylaluminate[29] or a tri-*n*-butylstannane, not shown) and then simply to repeat the coupling procedure with **20** led to decomposition of starting material. It was hypothesized that the large bulk of the artemisinin prevented attachment to a second artemisinin via a straight-on attack that would be required of a linear alkyne, so efforts shifted to conversion of the alkyne into alternate functionality.

Ziffer has established that trimethylsilyl (TMS) enol ethers could react with **19** to form C10 carba analogs in good yields,[27] and we believed that if the alkyne could be converted into a TMS enol ether then similar reactivity would be achieved. Treatment of **21** with *p*-toluenesulfonic acid (*p*TsOH) and mercury(II) acetate [(HgOAc)$_2$] in water and acetone afforded the C10 methyl ketone (**22**) in excellent yield (Scheme 3). Subsequent conversion into TMS enol ether (**23**) was accomplished by treatment of **22** with a solution of freshly prepared lithium diisopropylamide (LDA) in tetrahydrofuran (THF) in the presence of chlorotrimethylsilane (TMSCl) and triethylamine (Et$_3$N) at −78 °C. Compound **23** is surprisingly stable to silica gel and can be isolated and characterized as a clean material, but it must be used immediately. The final step for the formation of the two-carbon linked dimer system was then achieved by following established protocol.[22, 26] Slow addition of tin(IV) chloride (SnCl$_4$) into a solution of **23** and **19** at −78 °C gave, for the first time, the two-carbon linked dimer ketone (**24**) in good yield. Compound **24** is prepared in five linear steps starting from artemisinin in 36% overall yield, and has been prepared on 500 mg scale.

Analog synthesis

A series of oxime derivatives were prepared from **24** in order to add diversity to this chemical series and to probe SAR. The oxime functionality was desirable because it is already contained in known drugs,[33] has shown activity against protozoa,[34, 35] and evidence suggests that it is a suitable "water-soluble prodrug" that might unveil the ketone under biological conditions.[36–39] The initial series of analogs, prepared by treating **24** with the appropriate commercially available hydroxylamine in pyridine, consisted of the parent oxime (**25**), the *O*-methyl oxime (**26**), and the *O*-allyl oxime (**27**, Scheme 4a). Analogs containing bulkier alkyl groups were also prepared and included cyclopropylmethylene oxime (**28**), isobutyloxime (**29**), and *tert*-butyloxime (**30**). While two geometric isomers of the oxime would be expected, the *Z*-isomer was favored, and in some cases was the only isomer produced (stereochemical implications will be discussed, *vide infra*). Compound **25** was further converted into the *O*-propargyloxime (**31**), *O*-dimethyl carbamoyloxime (**32**), *O*-acetate oxime (**33**), and a pair of *O*-phosphate oximes (dimethyl **34**, diethyl **35**) upon treatment with sodium hydride (NaH) and the appropriate halide in THF at 0 °C (Scheme 4b).

Stereochemistry

Synthesis of the two-carbon linked dimer and its derivatives generates several stereochemical questions. Reduction of artemisinin at low temperatures leads exclusively to products with α-stereochemistry,[40] as described in the synthesis of **19** and **20**. Furthermore, nucleophilic displacement on these analogs has been shown to produce exclusively products with β-configuration,[26, 29, 40] as demonstrated in the synthesis of **21**. The proton NMR coupling constants between the C9/C10 protons are indicative of the stereochemistry at this location, as α-products position these protons in an axial-axial orientation resulting in larger coupling constants.[41] For example, the C9/C10 coupling constant for **20** (protons are trans-diaxial) is 9.8 Hz but for **21** (protons are axial-equatorial) it is 5.7 Hz (see table S1). The C5 proton is affected by C10 stereochemistry as well. Conversion of **20** to **21** increases the chemical shift from 5.47 to 5.56 ppm, and it is hypothesized that the C5 proton is positioned to interact with the deshielded anisotropic cone of the alkyne in the β-orientation. Synthesis of **22** should not change the C10 stereochemistry, and indeed the C9/C10 coupling constant and C5 chemical shift support this hypothesis ($J_{9,10}$ = 6.8 Hz; C5 δ = 5.65 ppm). The C5 proton is further deshielded, likely due to hydrogen-bonding interactions with the carbonyl oxygen. While it could be possible to epimerize the C10 position upon reaction with LDA to produce **23**, the steric hindrance is likely too great. Proton NMR again confirms this hypothesis ($J_{9,10}$ = 6.4 Hz). Finally, reaction of **23** with **19** should produce **24** with β-

configuration at both C10 positions [referred to as C10 (adjacent to the carbonyl) and C10′ (on the second artemisinin unit) in Figure 5]. The chemical shifts and coupling constants for C10 and C10′ were 4.84 ppm ($J = 6.3$ Hz) and 4.57 ($J = 6.2$ Hz), respectively, suggesting that both positions were in the β-configuration; this hypothesis was confirmed by X-ray crystallography (see Figure S1). Interestingly, the C5 and C5′ protons had very different chemical shifts – 5.57 ppm and 5.30 ppm, respectively. This provides further evidence that the C5 proton is interacting with the carbonyl oxygen, an important observation for the oxime analogs (*vide infra*). Interestingly, the endoperoxides from both artemisinin units are exposed and locked on the outside of the caged structure, a feature that could be beneficial for antimalarial activity of these two-carbon linked dimers.

Conversion of **24** into various oximes **25-30** raised an additional stereochemical question. The conditions employed could form either the *E*- or *Z*-isomer and could also epimerize the C10 position (because it is alpha to the carbonyl). The synthesis of each oxime from **24** generally formed one major product (yields shown in Scheme 4a, the less polar product by analytical TLC), and in some cases one minor product (yields shown in Scheme 4a, the more polar spot by analytical TLC). First, it should be noted that in all cases the C9-10 and C9′-10′ coupling constants are ~6 Hz, suggesting that the β-stereochemistry has been maintained at both positions. The C5 and C5′ and the C10 and C10′ protons from each molecule were again of interest. For example, the chemical shifts of the C5 and C5′ protons for the less polar oxime [designated (*Z*)-**25**] are 5.39 ppm and 5.37 ppm, respectively, and the C10 and C10′ protons are 5.68 ppm ($J = 6.9$ Hz) and 4.53 ppm ($J = 5.9$ Hz), respectively. The observation that the C5 proton is no longer deshielded suggests that the less polar compound is the *Z*-isomer – the hydroxyl group would be on the same side of the artemisinin unit, and the steric clash would force the C10-oxime bond to rotate out, thereby avoiding the interaction of the oxime nitrogen with the C5 proton (see Figure 6a). Furthermore, the C10 proton is drastically deshielded, shifting from 4.84 ppm in the starting ketone to 5.68 ppm. This suggests that the hydroxyl group is positioned to interact with the C10 proton. Conversely, the chemical shifts of the C5 and C5′ protons for the more polar oxime (minor product (*E*)-**25**) are 5.50 ppm and 5.43 ppm, respectively, and the C10 and C10′ were 5.17 ppm ($J = 6.3$ Hz) and 4.59 ppm ($J = 6.3$ Hz), respectively. In this polar isomer, the C10 and C10′ protons are both deshielded, which could be explained by interaction with the oxime nitrogen (for C10) and possibly the hydroxyl group (for C10′). This would be indicative of the *E*-isomer (see Figure 6b). The chemical shift trends for C5, C5′, C10, and C10′ are consistent for both the major and the minor isomers across the remainder of the oxime analogs (see table S1), suggesting that each major product has the same configuration (and conversely, each minor product as well). These hypotheses were confirmed by 2D NOESY NMR experiments on (*Z*)-**26** and (*E*)-**26**, where the oxime methyl-C10 NOE was observed in (*Z*)-**26** but not in (*E*)-**26** (see Supporting Information). All analogs tested have *Z*-stereochemistry unless otherwise noted.

Biological Evaluation

In vivo studies

Nearly all of the two-carbon linked dimer analogs were evaluated with *Plasmodium berghei* ANKA infected mice using a 60-day survival study. Each trioxane (0.64 mg) and mefloquine hydrochloride (1.92 mg) were dissolved in 100 μL of 7:3 Tween:EtOH mixture, and the mixture was further diluted with 965 μL of water (total volume of 1065 μL). Twenty four hours after infection with *P. berghei* ANKA strain (2×10^7 parasitized erythrocytes), each of four 5-week old C57BL/6J male mice (Jackson Laboratory, weighing approximately 20 g each) received 200 μL of the trioxane solution via oral gavage, corresponding to a dose of 6 mg/kg of trioxane and 18 mg/kg of mefloquine hydrochloride. Blood parasitemia levels at day three and duration of animal survival as compared to

survival time of animals receiving no drug were monitored, both being widely accepted measures of a drug's antimalarial efficacy. A select set were tested in the initial experiment, and these results are summarized in Table 1.

The data in this study demonstrate the overall high efficacy of this class of compounds. Mice receiving the drug artemether (**4b**) in combination with mefloquine survived an average of 40.0 days. Nearly all of the compounds tested were definitively more efficacious, with two compounds (**26** and **31**) achieving complete cures of all four mice. The cured mice gained an average of 10.7 grams and 12.0 g for **26** and **31**, respectively, as compared to an average of 7.7 gram for uninfected control mice receiving vehicle only, suggesting that the cured mice were exceptionally healthy. Another compound of note was **27**, which was tested both in combination with mefloquine and as a single dose monotherapy (at 120 mg/kg, without mefloquine). The combination therapy was much more efficacious than artemether combined with mefloquine, extending the survival time to 56.0 days, while the single dose monotherapy was equally as efficacious as artemether plus mefloquine.

Encouraged by these initial results, a second mouse experiment was initiated to further evaluate several of the lead compounds. We also wished to evaluate an alternative adjuvant to establish a broader understanding of the efficacy of these compounds. As such, the lead compounds were screened in combination with lumefantrine and also as a single dose monotherapy at 150 mg/kg. As summarized in Table 2, all of the lead compounds outperformed the drug artemether (**4b**) which prolonged survival to an average of 38.5 days and 19.0 days in combination with mefloquine and lumefantrine, respectively. One mouse receiving dimer **26** and mefloquine died early in the experiment (day 16) while the other three lived to day 60, an average of 49.0 days. Compound **26**, however, was marginally effective in combination with lumefantrine (26.3 day average) and not effective as a single dose monotherapy, with an average survival time (8.3 days) similar to that of infected mice receiving no drug (8.8 days). Compound **27** was efficacious in all three groups, extending survival time to 49.0 days, 41.3 days, and 46.8 days in combination with mefloquine, lumefantrine, or as a monotherapy, respectively. Compound **31** was once again the most efficacious compound. One mouse receiving mefloquine combination died at an extended time point (35 days) while the other three survived the entire 60 days, an average survival of 53.8 days. All four mice receiving lumefantrine combination survived the entire 60 days, with no parasitemia detected on day 60. These mice had gained as much weight as the uninfected control mice receiving vehicle only, again suggesting that the cured mice were healthy. The single dose monotherapy using **31** prolonged survival time to an average of 46.8 days, with three of the four mice having no detectable parasitemia on day 60, similar to the monotherapy results using **27**. Interestingly, the dimer *tert*-butyl oxime (**30**) combined with mefloquine was not very efficacious, prolonging survival to only 23.8 days. As the *tert*-butyl moiety would be less susceptible to metabolic oxidation (that would lead to subsequent conversion to the free oxime and ultimately to the ketone via hydrolysis),[37–39] these data suggest that the oxime analogs may be acting as prodrugs.

Conclusion

Malaria remains an oppressive disease, especially in tropical regions. Antimalarial drugs that have long term chemical stability *in vivo* have been difficult to achieve due to the complexity of the parasitic life cycle and to the parasite's ability to develop resistance (particularly when exposed to sub-lethal doses). Artemisinin combination therapy has been a staple of chemotherapy over the past 20 years, but the poor properties of most artemisinin-derived drugs make them susceptible to rapid clearance and therefore reduced efficacy. Our group has focused on the development of artemisinin-derived C10 carba analogs that might have improved physical properties to maintain an effective concentration in the blood

stream, and this is demonstrated by their efficacy in *in vivo* experiments as compared to the currently used drug artemether (**4b**). We report here the synthesis and biological evaluation of a new series of artemisinin-derived two-carbon linked dimers, whose unique conformation places the endoperoxides of each artemisinin unit (necessary for biological activity) on the outside of a caged structure. This two-carbon linked dimer system has been further functionalized into a series of oxime derivatives in an effort to improve their behavior *in vivo*. Many of our new dimers are much more efficacious than artemether (**4b**) when administered as a single oral dose combined with either mefloquine or lumefantrine. In particular, the propargyl oxime (**31**) achieved a complete cure in two separate experiments, and in combination with both either adjuvant mefloquine or lumefantrine. The average survival time of all mice receiving this new trioxane dimer was significantly higher (56.9 days in combination with mefloquine over two experiments, 60.0 days in combination with lumefantrine over one experiment) than the survival time of all mice receiving artemether combination therapy (39.3 days in combination with mefloquine over two experiments, 19.0 days in combination with lumefantrine over one experiment). The efficient synthesis of these compounds and their high antimalarial efficacy make especially the curative propargyl oxime (**31**) a leading candidate for further drug development.

Experimental

All reactions were performed under argon in oven-dried or flame-dried glassware. Dichloromethane and tetrahydrofuran were dispensed from an LC Technology Solutions SPBT-1 bench top solvent purification system. All commercially available reagents were purchased from Sigma Aldrich and used as received. All experiments were monitored by analytical thin layer chromatography (TLC) performed on Silicycle silica gel 60 Å glass supported plates with 0.25 mm thickness. Flash chromatography was performed with EMD silica gel (40–63 μM). Automated purification was performed on a Teledyne Isco CombiFlash R_f purification system (referred to as "Isco") using Teledyne RediSep R_f Gold High Performance pre-packed silica columns. Yields are not optimized. Infrared (IR) spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 MHz FT-NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker Avance 300 MHz FT-NMR spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). The following abbreviations are used in the experimental section for the description of ^{1}H NMR spectra: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (bs), doublet of doublets (dd), doublet of triplets (dt), and doublet of quartets (dq). HPLC-MS (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to an Agilent Technologies 1200 series HPLC equipped with a Luna C18 3 micron 3×75 mm column. Elution gradient was 4 to 100% acetonitrile (modified with 0.05% TFA) in water over 5 min, unless otherwise noted. High resolution mass spectrum-electron ionization sprary (HRMS-ESI) were obtained on an Agilent Technologies 1200 series Dual Absorbance Detector HPLC system equipped with a Phenomenex Luna 75×3 mm, C18, 3Ym column at 45 °C (UV detection at 220nm, BW 8nm, and 254nm BW 8nm, flow rate: 0.8 mL/min (increasing), Injection volume: 1.0 YL, sample solvent: 100% Methanol, sample conc.: ~0.01 mg/mL, mobile phase A: Water with 0.1% acetic acid, mobile phase B: Acetonitrile with 0.1% acetic acid) coupled to a Agilent 6210 time-of-flight mass spectrometer (ion source: Duel ESI, min range: 115 m/z, max range: 1400 m/z, scan rate: 0.9 seconds, gas temp: 340°C, gas flow: 10 L/min, nebulizer: 50 PSI, ion polarity: positive, VCap: 3500 V, fragmentor: 175 V, skimmer1: 65 V, OctopoleRFPeak: 250 V, ref mass: enabled (Agilent P/N G1969-85001). Data were analyzed using Agilent Masshunter Workstation Data Acquisition (v B.02.00, Patch 1,2,3) and Agilent Masshunter Qualitative Analysis (v B.02.00, Build 2.0.197.7, Patch 3).

All dimers were >95% pure based on HPLC data unless otherwise noted.

Synthesis of C10-α-2,4-dimethoxybenzoate 20

To a 100 mL round bottom flask were added artemisinin (300 mg, 1.06 mmol, 1.0 eq) and CH_2Cl_2 (10 mL). The stirring solution was cooled to −78 °C for 20 min, at which time diisobutylaluminum hydride (DIBALH, 1.0 M in CH_2Cl_2, 1.275 mL, 1.27 mmol, 1.2 eq) was added dropwise over 15 min. The reaction mixture was stirred at −78 °C for 2 h (until complete consumption of starting material was seen by TLC). At that time, 4-dimethylaminopyridine (DMAP, 143 mg, 1.17 mmol, 1.1 eq) and pyridine (py, 0.5 mL, 6.38 mmol, 6 eq) were added, followed by 2,4-dimethoxybenzoyl chloride (1.07 mg, 5.31 mmol, 5 eq). The resulting reaction mixture was allowed to gradually warm to rt and stirred overnight. Upon completion, the reaction was quenched with sat. aq. NH_4Cl and diluted with EtOAc. The organic layer was washed again with sat. aq. NH_4Cl and brine, then separated, dried on $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified directly on silica. Gradient elution (10–35% ethyl acetate in hexanes) afforded the desired product as a colorless solid: 421 mg, 98% yield; mp 127.8 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96-7.94 (d, 1 H, J = 8.6 Hz), 6.47-6.43 (m, 2 H), 5.97-5.95 (d, 1 H, J = 9.8 Hz), 5.47 (s, 1 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 2.72-2.63 (m, 1 H), 2.40-2.31 (m, 1 H), 2.04-1.98 (m, 1 H), 1.91-1.83 (m, 1 H), 1.81-1.68 (m, 2 H), 1.67-1.60 (m, 1 H), 1.54-1.43 (m, 2 H), 1.40 (s, 3 H), 1.34-1.25 (m, 2 H), 1.15-0.88 (m, 7 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 161.9, 134.5, 104.3, 98.8, 91.8, 91.5, 80.2, 55.8, 55.5, 52.9, 51.7, 45.4, 41.1, 37.2, 36.3, 34.1, 32.0, 25.9, 24.6, 22.0, 20.2, 12.2; HRMS (FAB) m/z for $C_{24}H_{33}O_8$ (M + H)$^+$ calc. = 449.2175, found = 449.2169; FT-IR (cm^{-1}) 3010, 2939, 2876, 1732, 1652, 1608, 1575, 1558, 1540, 1506, 1457, 1418, 1375, 1330, 1267, 1248, 1211, 1164, 1131, 1107, 1086, 1030, 1016, 942, 926, 878, 861, 830, 752; $[α]_D$ ^{23}=+26.27 (c=0.645, $CHCl_3$); HPLC t_r = 3.93 min.

Synthesis of β-C10-acetylene 21

To a 100 mL round bottom flask were added **20** (500 mg, 1.11 mmol, 1.0eq) and Et_2O (30 mL). The slurry was stirred at rt for 10 min until complete dissolution was achieved, and the stirring solution was subsequently cooled to 0 °C. After 10 min, zinc(II) chloride ($ZnCl_2$, 1.0 M in Et_2O, 1.34 mL, 1.43 mmol, 1.2 eq) and ethynyl magnesium chloride (0.5 M in THF, 8.03 ml, 4.01 mmol, 3.6 eq) were added dropwise simultaneously from separate syringes. The reaction mixture was stirred at 0 °C for 3 h, and then gradually warmed to rt as the ice bath melted and stirred overnight. Upon completion, the reaction was quenched with H_2O and diluted with EtOAc. The organic layer was washed with sat. aq. NH_4Cl and brine, then extracted, dried on $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified directly on silica. Gradient elution (0 to 20% EtOAc in hexanes) afforded the desired product as a white solid: 242 mg, 74% yield, mp 121.6 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 5.56 (s, 1 H), 4.74-4.72 (dd, 1 H, J = 2.5, 5.8 Hz), 2.81-2.70 (m, 1 H), 2.53-2.52 (d, 1 H, J = 2.5 Hz), 2.41-2.30 (m, 1 H), 2.24-2.00 (m, 2 H), 1.92-1.83 (m, 1 H), 1.79-1.62 (m, 2 H), 1.59-1.48 (m, 2 H), 1.42 (s, 3 H), 1.39-1.20 (m, 3 H), 1.00-0.85 (m, 7 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.2, 89.4, 81.4, 80.8, 77.2, 67.1, 52.6, 45.3, 37.3, 36.2, 34.5, 29.8, 26.0, 24.6, 22.9, 20.3, 13.7; HRMS (FAB) m/z for $C_{17}H_{25}O_4$ (M + H)$^+$ calc. = 293.1747, found = 293.1744; FT-IR (cm^{-1}) 2942, 2871, 1506, 1450, 1208, 1136, 1122, 1088, 1055; $[α]_D$ ^{23}=+112.57 (c=0.390, $CHCl_3$); HPLC t_r = 3.84 min.

Synthesis of artemisinin monomer methyl ketone 22

To a round bottom flask were added monomer acetylene **21** (695 mg, 2.38 mmol, 1.0 eq), water (H_2O, 200 μL, 5.0 eq) and acetone (15 mL). *para*-Toluenesulfonic acid (*p*TsOH, 597 mg, 1.0 eq) and mercuric acetate [$Hg(OAc)_2$, 547 mg, 0.7 eq] were added to the stirring solution, and the resulting slurry was stirred at rt overnight. Upon completion the reaction mixture was filtered through a short pad of celite and concentrated in vacuo. The residue

was purified directly on the Isco Combiflash automated purification system. Gradient elution (0 to 40% ethyl acetate in hexanes) afforded the desired product as a colorless oil: 664 mg, 90%, yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.65 (s, 1 H), 4.75 (d, J = 6.78 Hz, 1 H), 2.93 (ddd, J = 14.03, 7.54, 6.50 Hz, 1 H), 2.38 - 2.24 (m, J = 13.23, 10.69, 3.91, 3.91, 3.91 Hz, 1 H), 2.21 (s, 3 H), 2.08 – 1.98 (m, 1 H), 1.98 - 1.87 (m, 1 H), 1.76 - 1.70 (m, 1 H), 1.70 - 1.65 (m, 2 H), 1.65 - 1.60 (m, 1 H), 1.52 - 1.43 (m, 1 H), 1.40 (s, 3 H), 1.38 - 1.31 (m, 1 H), 1.31 - 1.24 (m, 2 H), 1.24 - 1.15 (m, 1 H), 1.02 (d, J = 7.68 Hz, 3 H), 0.95 (d, J = 5.98 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 210.2, 103.2, 90.1, 80.9, 79.9, 51.8, 44.2, 37.3, 36.4, 34.1, 30.0, 28.8, 25.9, 24.7, 23.7, 20.0, 13.5; ESI-HRMS m/z (M + H)$^+$ for C$_{17}$H$_{27}$O$_5$ calc. = 311.1853, found = 311.1854; FT-IR (cm^{-1}) 2930, 2874, 1783, 1711, 1672, 1566, 1548, 1532, 1526, 1514, 1457, 1430, 1366, 1279, 1196, 1102, 1062, 1012, 957, 931, 883; [α]$_D$ ^{25} +70.37 (c = 0.18, CHCl$_3$); HPLC t$_r$ = 3.66 min.

Synthesis of artemisinin monomer trimethylsilyl enol ether 23

To a round bottom flask were added artemisin monomer methyl ketone **22** (50 mg, 0.16 mmol, 1.0 eq) and THF (5 mL), and the stirring solution was cooled to −78 °C. Triethylamine (Et$_3$N, 0.045 mL, 0.32 mmol, 2.0 eq) and trimethylsilyl trifluormethanesulfonate (TMSOTf, 0.058 mL, 0.32 mmol, 2.0 eq) were added, and the reaction mixture was stirred at −78 °C for 30 min before warming to rt. Upon completion, the reaction was quenched with sat. aq. NaHCO$_3$ and the product was extracted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered, and conc in vacuo. The residue was purified directly on silica gel. Gradient elution (0 – 5% EtOAc in hexanes) afforded the desired compound as a colorless oil: 50 mg, 81% yield; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.57 (s, 1H), 4.54 (d, J=6.32 Hz, 1H), 4.37 (s, 1H), 4.14 (s, 1H), 2.83-2.65 (m, 1H), 2.29 (dt, J = 3.92, 13.96 Hz, 1H), 2.02-1.92 (m, 1H), 1.87 (tdd, J = 3.41, 6.32, 13.64 Hz, 1H), 1.70-1.47 (m, 4H), 1.42-1.34 (m, 5H), 1.30-1.15 (m, 2H), 0.91 (d, J = 6.57 Hz, 6H), 0.18 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.3, 103.1, 90.3, 89.6, 81.1, 74.7, 52.1, 44.4, 37.4, 36.5, 34.3, 26.0, 24.8, 23.3, 20.1, 13.9, 0.1.

Synthesis of dihydroartemisinin acetate **19** has been previously reported.[20–22]

Synthesis of artemisin two-carbon linked dimer ketone 24

To a round bottom flask were added artemisinin monomer trimethylsilyl enol ether **23** (36.5 mg, 0.095 mmol, 1.0 eq), dihydroartemisinin acetate **19** (31.0 mg, 0.095 mmol, 1.0 eq) and dicholoromethane (CH$_2$Cl$_2$, 1 mL). The stirring solution was cooled to −78 °C, and after 10 min tin IV chloride (SnCl$_4$, 1.0 M in CH$_2$Cl$_2$, 0.095 mL, 0.095 mmol, 1.0 eq, further diluted to 1 mL) was added in one portion. The reaction mixture was stirred at −78 °C for 2 h. Analytical TLC showed 9,10-olefin (elimination product), artemisinin monomer methyl ketone (either residual from TMS enol ether starting material or unreacted enolate), and two new spots (one of which was desired product, the other has yet to be identified). The reaction was quenched with water and the products were extracted with CH$_2$Cl$_2$. The organic layer was washed with brine, dried, filtered, and concentrated under reduced pressure. The residue was purified directly on silica gel. Gradient elution (0 – 15% ethyl acetate in hexanes) afforded the desired product as a colorless solid: 38 mg, 69% yield; mp 135–137 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.57 (s, 1 H), 5.30 (s, 1 H), 4.84 (ddd, J = 9.51, 6.17, 3.81 Hz, 1 H), 4.57 (d, J = 6.31 Hz, 1 H), 3.18 (dd, J = 15.82, 9.09 Hz, 1 H), 2.99 - 2.85 (m, J = 13.61, 7.58, 7.58, 6.03 Hz, 1 H), 2.76 (dt, J = 13.70, 7.35, 6.36 Hz, 1 H), 2.39 (dd, J = 15.71, 3.74 Hz, 1 H), 2.35 - 2.25 (m, 2 H), 2.07 - 2.02 (m, 1 H), 2.02 – 1.95 (m, 1 H), 1.94 - 1.83 (m, 2 H), 1.82 - 1.72 (m, 2 H), 1.72 - 1.62 (m, 3 H), 1.61 - 1.55 (m, 2 H), 1.53 - 1.43 (m, 1 H), 1.41 (s, 3 H), 1.37 (s, 3 H), 1.36 - 1.32 (m, 1 H), 1.31 - 1.26 (m, 2 H), 1.23 (d, J = 5.79 Hz, 2 H), 1.21 - 1.15 (m, 1 H), 1.10 (d, J = 7.68 Hz, 3 H), 0.95 (d, J = 5.98 Hz, 3 H), 0.92 (d, J = 6.26 Hz, 4 H), 0.84 (d, J = 7.49 Hz, 3 H); ^{13}C NMR (100 MHz,

CDCl$_3$) δ 211.9, 103.5, 103.2, 90.2, 88.9, 80.9, 80.8, 80.1, 71.6, 52.3, 52.1, 44.6, 44.3, 40.0, 37.5, 36.9, 36.5, 36.4, 34.4, 34.3, 30.6, 29.9, 25.9, 25.9, 24.7, 24.6, 23.9, 20.1, 13.7, 13.4; ESI-HRMS m/z (M + Na)$^+$ for C$_{32}$H$_{48}$O$_9$Na calc. = 599.3191, found = 99.3184; FT-IR (cm^{-1}) 2937, 2872, 1712, 1651, 1549, 1532, 1525, 1514, 1457, 1194, 1098, 1053, 1018, 941, 876, 829, 753; [α]$_D$ ^{25} +93.98 (c = 0.26, CHCl$_3$); HPLC (4–100% acetonitrile in water over 8 min) t$_r$ = 7.04 min.

Synthesis of dimer oximes *Z*-25 and *E*-25

To a round bottom flask were added dimer ketone **24** (15.0 mg, 0.03 mmol, 1.0 eq), hydroxyl amine hydrochloride (9.0 mg, 0.13 mmol, 5.0 eq), and pyridine (1 mL). The reaction mixture was stirred at rt overnight. Upon completion as detected by analytical TLC (both isomers were visualized), the reaction mixture was diluted with EtOAc and organic layer was washed twice with 10% citric acid. The aqueous layer was back extracted with EtOAc and the organic layers were pooled and washed with brine. The solvent was removed under reduced pressure and the residue was purified directly on silica gel. Gradient elution (0 to 40% ethyl acetate in hexanes) afforded the desired products as a colorless amorphous solid: **Z-25** – 12.5 mg, 81% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.68 (d, *J* = 6.90 Hz, 1 H), 5.39 (s, 1 H), 5.37 (s, 1 H), 4.53 (ddd, *J* = 9.13, 5.91, 3.30 Hz, 1 H), 3.48(s, 1 H), 2.92 (sxt, *J* = 7.68 Hz, 1 H), 2.82 (dd, *J* = 13.40, 7.48 Hz, 1 H), 2.54 (dd, *J* = 14.62, 9.15 Hz, 1 H), 2.45 (dd, *J* = 14.67, 3.33 Hz, 1 H), 2.37 - 2.24 (m, 2 H), 2.04 - 2.01 (m, 1 H), 2.01 – 1.94 (m, 2 H), 1.91 - 1.81 (m, 2 H), 1.81 - 1.77 (m, 1 H), 1.77 - 1.74 (m, 1 H), 1.74 - 1.69 (m, 1 H), 1.68 - 1.65 (m, 1 H), 1.65 - 1.61 (m, 1 H), 1.57 - 1.51 (m, 1 H), 1.51 - 1.44 (m, 1 H), 1.44 - 1.39 (m, 4 H), 1.39 - 1.36 (m, 3 H), 1.36 - 1.29 (m, 2 H), 1.29 - 1.24 (m, 3 H), 1.24 - 1.19 (m, 1 H), 0.98 - 0.93 (m, 7 H), 0.91 (d, *J* = 7.48 Hz, 4 H), 0.81 (d, *J* = 7.63 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.9, 103.4, 102.3, 90.4, 88.3, 81.1, 80.7, 74.1, 74.1, 69.6, 52.7, 51.2, 44.8, 43.2, 37.6, 37.3, 36.7, 36.6, 34.6, 34.2, 30.3, 29.5, 29.1, 26.1, 25.8, 25.1, 24.8, 24.6, 20.4, 19.9, 13.6, 12.7; ESI-HRMS m/z (M + H)$^+$ for C$_{32}$H$_{50}$NO$_9$ calc. = 592.3480, found = 592.3480; FT-IR (cm^{-1}) 3412, 2968, 2942, 2874, 1660, 1453, 1376, 1204, 1123, 1091, 1054, 1011, 977, 946, 879, 754; [α]$_D$ ^{25} +45.69 (c = 0.625, CHCl$_3$); HPLC (4–100% acetonitrile in water over 8 min) t$_r$ = 7.06 min.

E-25—1.4 mg, 9% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.50 (s, 1H), 5.42 (s, 1H), 5.30 (s, 1H), 5.17 (d, *J* = 6.40 Hz, 1H), 4.67-4.52 (m, 1H), 2.87-2.74 (m, 2H), 2.62-2.55 (m, 2H), 2.37-2.25 (m, 2H), 2.05-1.79 (m, 4H), 1.76-1.57 (m, 4H), 1.55-1.18 (m, 14H), 1.09-0.78 (m, 16H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 186.6, 160.4, 103.4, 102.9, 90.3, 88.5, 81.0, 81.0, 77.2, 73.8, 73.6, 53.4, 52.6, 51.9, 51.9, 44.7, 44.3, 37.4, 37.3, 36.6, 36.5, 34.6, 34.4, 31.0, 30.4, 26.8, 26.1, 25.8, 24.8, 24.8, 24.6, 24.4, 20.3, 20.1, 13.9, 13.3.

Synthesis of dimer *O*-methyl oximes *Z*-26 and *E*-26

The desired compound was prepared in a similar manner to dimer oxime **25** as described above, substituting *O*-methyl hydroxylamine hydrochloride for hydroxylamine hydrochloride. Gradient elution on silica gel (0 to 40% ethyl acetate in hexanes) afforded the desired product as a colorless amorphous solid: **Z-26** – 11.9 mg, 75% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.56 (d, *J* = 6.94 Hz, 1 H), 5.41 (s, 1 H), 5.35 (s, 1 H), 4.55 (ddd, *J* = 8.93, 5.89, 3.23 Hz, 1 H), 3.83 (s, 3 H), 3.49 (s, 1 H), 2.89 - 2.77 (m, 2 H), 2.55 (dd, *J* = 14.57, 9.00 Hz, 1 H), 2.41 (dd, *J* = 14.48, 3.23 Hz, 1 H), 2.31 (qd, *J* = 14.04, 4.38 Hz, 2 H), 2.04 - 2.01 (m, 1 H), 2.01 – 1.97 (m, 1 H), 1.97 - 1.93 (m, 1 H), 1.91 - 1.83 (m, 1 H), 1.83 - 1.77 (m, 1 H), 1.77 - 1.72 (m, 1 H), 1.72 - 1.68 (m, 1 H), 1.68 - 1.61 (m, 4 H), 1.58 - 1.53 (m, 1 H), 1.53 - 1.48 (m, 1 H), 1.48 - 1.41 (m, 1 H), 1.40 (s, 3 H), 1.38 (s, 3 H), 1.37 - 1.30 (m, 3 H), 1.30 - 1.21 (m, 4 H), 0.95 (dd, *J* = 5.97, 3.23 Hz, 7 H), 0.92 (d, *J*=7.48 Hz, 4 H), 0.77 (d, *J*=7.63 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 158.8, 103.3, 102.3, 90.3, 88.4, 81.1, 80.7, 74.2, 70.0, 61.6, 52.7, 51.2, 44.9, 43.2, 37.5, 37.3, 36.7, 36.6, 34.6, 34.3, 30.4, 30.0, 29.1,

26.1, 25.8, 25.0 24.7, 24.7, 24.6, 20.3, 19.9, 13.6, 12.7; ESI-HRMS m/z (M + H)$^+$ for C$_{33}$H$_{52}$NO$_9$ calc. = 606.3637, found = 606.3636; HPLC (4–100% acetonitrile in water over 8 min) t$_r$ = 7.54 min.

E-26—1.7 mg, 11% yield; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.62 (s, 1H), 5.35 (s, 1H), 5.09 (d, *J* = 6.26 Hz, 1H), 4.61-4.54 (m, 1H), 3.86 (s, 3H), 2.94-2.71 (m, 2H), 2.58 (dd, *J* = 7.83, 17.22 Hz, 1H), 2.45 (dd, *J* = 10.37, 13.11 Hz, 1H), 2.32 (dt, *J* = 3.72, 13.99 Hz, 2H), 2.06-1.96 (m, 2H), 1.94-1.77 (m, 4H), 1.71-1.61 (m, 4H), 1.59-1.48 (m, 6H), 1.46-1.28 (m, 8H), 1.27-1.22 (m, 2H), 1.16-1.05 (m, 1H), 1.00 (d, *J* = 7.43 Hz, 3H), 0.97-0.93 (m, *J* = 6.10, 6.10 Hz, 6H), 0.91 (d, *J* = 7.83 Hz, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 215.6, 159.8, 103.3, 103.0, 90.2, 88.4, 81.0, 80.9, 74.2, 73.9, 67.9, 52.6, 52.1, 44.7, 44.7, 37.4, 37.3, 36.6, 34.6, 34.5, 30.9, 30.4, 27.6, 26.0, 25.9, 24.7, 24.7, 24.3, 20.3, 20.1, 14.2, 13.3.

Synthesis of 2C-dimer *O*-allyl oxime 27

The desired compound was prepared in a similar manner to dimer oxime **25** as described above, substituting *O*-allyl hydroxylamine hydrochloride hydrate for hydroxylamine hydrochloride. The crude product consisted of a single oxime diastereomer. Gradient elution on silica gel (0 to 40% ethyl acetate in hexanes) afforded the desired diastereomerically pure product as a colorless amorphous solid: 8.2 mg, 75% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 6.04 – 5.91 (m, *J* = 15.99, 10.61, 6.75, 5.43, 5.43, 5.18 Hz, 1 H), 5.63 (d, *J* = 6.85 Hz, 1 H), 5.40 (s, 1 H), 5.36 (s, 1 H), 5.26 (dd, *J* = 17.31, 1.71 Hz, 1 H), 5.13 (dd, *J* = 10.37, 1.52 Hz, 1 H), 4.62 - 4.52 (m, 2 H), 2.94 - 2.75 (m, 2 H), 2.55 (dd, *J* = 14.67, 8.51 Hz, 1 H), 2.46 (dd, *J* = 14.67, 3.91 Hz, 1 H), 2.38 - 2.24 (m, *J*=13.98, 13.98, 13.73, 4.38 Hz, 2 H), 2.05 – 1.94 (m, 3 H), 1.92 - 1.84 (m, 1 H), 1.83 - 1.76 (m, 1 H), 1.76 - 1.69 (m, 2 H), 1.69 - 1.60 (m, 3 H), 1.59 - 1.50 (m, 1 H), 1.50 - 1.41 (m, 1 H), 1.39 (d, *J* = 4.99 Hz, 6 H), 1.36 - 1.30 (m, 2 H), 1.30 - 1.19 (m, 3 H), 0.96 (d, *J* = 3.57 Hz, 3 H), 0.95 (d, *J* = 4.11 Hz, 3 H), 0.91 (d, *J* = 7.48 Hz, 3 H), 0.79 (d, *J*=7.63 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.1, 134.8, 116.5, 103.3, 102.2, 90.4, 90.4, 88.6, 88.5, 81.1, 80.7, 74.6, 73.9, 70.0, 52.6, 51.2, 44.9, 43.2, 37.6, 37.3, 36.7, 36.6, 34.6, 34.3, 30.3, 30.1, 29.2, 26.1, 25.7, 25.1, 24.7, 20.3, 19.9, 13.5, 12.7; ESI-HRMS m/z (M + H)$^+$ for C$_{35}$H$_{54}$NO$_9$ calc. = 632.3793, found = 632.3797; FT-IR (cm^{-1}) 2991, 2969, 2920, 1665, 1454, 1376, 1114, 1092, 1056, 1011, 983, 945, 880, 755; [α]$_D$ ^{25} +50.15 (c = 0.40, CHCl$_3$); HPLC (4–100% acetonitrile in water over 8 min) t$_r$ = 7.65 min.

Synthesis of dimer *O*-cyclopropylmethyl oxime *Z*-28

The desired compound was prepared in a similar manner to dimer oxime **25** as described above, substituting *O*-methylcyclopropyl hydroxylamine hydrochloride for hydroxylamine hydrochloride. Gradient elution on silica gel (0 to 40% ethyl acetate in hexanes) afforded the desired diastereomerically pure product as a colorless amorphous solid: **Z**-**28** – 9.9 mg, 74% yield; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.62 (d, *J* = 6.82 Hz, 1H), 5.40 (s, 1H), 5.36 (s, 1H), 4.61-4.56 (m, 1H), 3.91-3.81 (m, 2H), 2.94-2.77 (m, 2H), 2.55 (dd, *J* = 9.09, 14.91 Hz, 1H), 2.45 (dd, *J* = 4.04, 14.65 Hz, 1H), 2.37-2.25 (m, 2H), 2.08-1.95 (m, 4H), 1.91-1.62 (m, 9H), 1.57-1.51 (m, 1H), 1.50-1.37 (m, 6H), 1.37-1.21 (m, 6H), 1.17-1.07 (m, 1H), 0.94 (m, with three doublets, *J* = 4.55, 5.95, 7.58 Hz, 10H), 0.80 (d, *J* = 7.58 Hz, 3H), 0.49-0.44 (m, 2H), 0.28-0.23 (m, 2H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 158.6, 103.2, 102.2, 90.4, 88.6, 81.2, 80.7, 78.0, 73.9, 70.1, 52.6, 51.2, 44.8, 43.3, 37.6, 37.4, 36.8, 36.6, 34.6, 34.3, 30.3, 30.1, 29.2, 26.1, 25.7, 25.1, 24.8, 24.7, 24.7, 20.3, 19.9, 13.5, 12.6, 10.6, 2.9, 2.8; ESI-HRMS m/z (M + H)$^+$ for C$_{36}$H$_{56}$NO$_9$ calc. = 646.3950, found = 646.3962; FT-IR (cm^{-1}) 3081, 2947, 2920, 2873, 1455, 1376, 1278, 1252, 1224, 1202, 1178, 1114, 1095, 1054, 1010, 943, 881, 823, 756; [α]$_D$ ^{25} +48.24 (c = 0.465, CHCl$_3$).

Synthesis of dimer *O*-isobutyl oxime *Z*-29

The desired compound was prepared in a similar manner to dimer oxime **Z**-**25** as described above, substituting *O*-isobutyl hydroxylamine hydrochloride for hydroxylamine hydrochloride. The crude product consisted of a single oxime diastereomer. Gradient elution on silica gel (0 to 40% ethyl acetate in hexanes) afforded the desired diastereomerically pure product as a colorless amorphous solid: 10.8 mg, 80% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.63 (d, J = 6.78 Hz, 1H), 5.40 (s, 1H), 5.35 (s, 1H), 4.60-4.53 (m, 1H), 3.86 (dd, J = 6.31, 10.08 Hz, 1H), 3.77 (dd, J = 6.97, 9.98 Hz, 1H), 2.93-2.73 (m, 2H), 2.53 (dd, J = 9.00, 17.71 Hz, 1H), 2.46 (dd, J = 3.77, 15.82 Hz, 1H), 2.39-2.23 (m, 2H), 2.04-1.91 (m, 4H), 1.88-1.61 (m, 6H), 1.58-1.48 (m, 2H), 1.46-1.21 (m, 14H), 1.00-0.86 (m, 18H), 0.78 (d, J = 7.54 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 158.4, 103.2, 102.2, 90.5, 88.5, 81.2, 80.7, 80.4, 74.0, 72.2, 70.0, 52.6, 51.3, 44.9, 43.3, 37.6, 37.4, 36.8, 36.6, 35.6, 34.6, 34.3, 30.4, 30.0, 29.3, 28.0, 26.1, 25.7, 25.1, 24.8, 24.7, 20.3, 19.9, 19.3, 19.3, 13.5, 12.7; ESI-HRMS m/z (M + H)$^+$ for C$_{36}$H$_{58}$NO$_9$ calc. = 648.4106, found = 648.4100; FT-IR (cm^{-1}) 2997, 2956, 2920, 2872, 2847, 1458, 1376, 1224, 1202, 1114, 1095, 1048, 1011, 945, 880, 756; [α]$_D$ ^{25} +40.65 (c = 0.515, CHCl$_3$).

Synthesis of dimer *O*-*tert*-butyl oxime *Z*-30

The desired compound was prepared in a similar manner to dimer oxime **Z**-**25** as described above, substituting *O*-*tert*-butyl hydroxylamine hydrochloride for hydroxylamine hydrochloride. The crude product consisted of a single oxime diastereomer. Gradient elution on silica gel (0 to 40% ethyl acetate in hexanes) afforded the desired diastereomerically pure product as a colorless amorphous solid: 10.5 mg, 72% yield; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.58 (d, J = 6.82 Hz, 1H), 5.38 (s, 1H), 5.36 (s, 1H), 4.67 (q, J = 6.32 Hz, 1H), 2.89-2.75 (m, 2H), 2.60 (dd, J = 7.07, 15.66 Hz, 1H), 2.52 (dd, J = 7.07, 15.92 Hz, 1H), 2.37-2.25 (m, 2H), 2.04-1.94 (m, 3H), 1.91-1.86 (m, 1H), 1.84-1.55 (m, 8H), 1.41-1.30 (m, 9H), 1.30-1.20 (m, 12H), 0.97 (d, J = 1.52 Hz, 3H), 0.95 (d, J = 1.77 Hz, 4H), 0.90 (d, J = 7.58 Hz, 4H), 0.76 (d, J=7.58 Hz, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.9, 103.0, 102.1, 90.5, 89.0, 81.3, 80.8, 77.9, 77.2, 73.4, 69.8, 52.5, 51.3, 44.8, 43.2, 37.1, 34.7, 34.3, 30.6, 30.2, 29.2, 27.6, 26.1, 25.7, 25.1, 24.8, 24.8, 20.3, 19.9, 13.2, 12.6;; ESI-HRMS m/z (M + Na)$^+$ for C$_{36}$H$_{57}$NNaO$_9$ calc. = 670.3926, found = 670.3932; FT-IR (cm^{-1}) 2970, 2948, 2922, 2873, 2848, 1453, 1377, 1255, 1197, 1112, 1096, 1051, 1010, 964, 938, 882, 821, 756; [α]$_D$ ^{25} +31.91 (c = 0.525, CHCl$_3$).

Synthesis of dimer propargyl oxime *Z*-31

To a 1 dram vial were added dimer oxime **Z**-**25** (4.8 mg, 0.01 mmol, 1.0 eq) and tetrahydrofuran (THF, 1 mL). The stirring solution was cooled to 0 °C in an ice bath, and sodium hydride (NaH, 0.3 mg, 1.5 eq) was added as a dry powder, and the reaction mixture was stirred for 20 min. At that time, propargyl bromide (2 μL, 1.5 eq) was added and the reaction mixture was stirred overnight, gradually warming to room temperature. Upon completion the reaction mixture was quenched with water and diluted with EtOAc. The organic layer was washed with sat. aq. NH$_4$Cl and brine, extracted, dried over Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified directly on silica gel. Gradient elution (0 to 50% ethyl acetate in hexanes) afforded the desired product as a colorless amorphous solid: 4.1 mg, 60% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (d, J = 6.88 Hz, 1 H), 5.41 (s, 1 H), 5.36 (s, 1 H), 4.71 - 4.60 (m, J = 15.85, 3.47, 2.40, 2.40 Hz, 2 H), 4.58 (td, J = 5.73, 3.00 Hz, 1 H), 2.95 - 2.78 (m, 2 H), 2.58 (dd, J = 14.53, 8.59 Hz, 1 H), 2.51 - 2.44 (m, J = 14.53, 3.66 Hz, 1 H), 2.39 (t, J = 2.40 Hz, 1 H), 2.38 - 2.25 (m, J = 14.04, 14.04, 13.77, 4.39 Hz, 2 H), 2.07 – 1.94 (m, 2 H), 1.93 - 1.85 (m, 1 H), 1.85 - 1.78 (m, 1 H), 1.78 - 1.70 (m, 1 H), 1.70 - 1.65 (m, 1 H), 1.65 - 1.59 (m, 4 H), 1.59 - 1.49 (m, 2 H), 1.48 - 1.42 (m, 1 H), 1.40 (d, J = 6.25 Hz, 7 H), 1.38 - 1.30 (m, 4 H),

1.30 - 1.20 (m, 4 H), 0.97 (dd, J = 6.03, 3.06 Hz, 7 H), 0.93 (d, J = 7.52 Hz, 4 H), 0.82 (d, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.4, 103.3, 102.3, 90.4, 88.5, 81.1, 80.7, 80.5, 74.0, 73.7, 70.0, 61.3, 52.6, 51.2, 44.9, 43.2, 37.6, 37.3, 36.7, 36.6, 34.6, 34.2, 30.3, 30.1, 29.2, 26.1, 25.7, 25.1, 24.7, 20.3, 19.9, 13.6, 12.7; ESI-HRMS m/z (M + H)$^+$ for C$_{35}$H$_{52}$NO$_9$ calc. = 630.3637, found = 630.3613; FT-IR (cm^{-1}) 2955, 2872, 1631, 1455, 1375, 1051, 1008, 756; [α]$_D$ ^{25} +55.72 (c = 0.105, CHCl$_3$).

Synthesis of dimer oxime dimethylcarbamate *Z*-32

The desired compound was prepared in a similar manner to propargyl oxime **Z**-**31** as described above, substituting dimethylcarbamyl chloride for propargyl bromide. Gradient elution on silica gel (10 to 100% ethyl acetate in hexanes) afforded the desired diastereomerically pure product as a colorless amorphous solid: 4.1 mg, 76% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.73 (d, J = 6.88 Hz, 1 H), 5.47 (s, 1 H), 5.38 (s, 1 H), 4.60 - 4.52 (m, 1 H), 2.98 (s, 6 H), 2.94 - 2.85 (m, 2 H), 2.85 - 2.78 (m, 1 H), 2.51 (dd, J = 13.96, 2.27 Hz, 1 H), 2.38 - 2.24 (m, 2 H), 2.08 – 1.95 (m, 3 H), 1.91 - 1.80 (m, 2 H), 1.80 - 1.72 (m, 2 H), 1.72 - 1.62 (m, 7 H), 1.56 - 1.46 (m, 2 H), 1.46 - 1.41 (m, 1 H), 1.41 - 1.37 (m, 8 H), 1.37 - 1.33 (m, 2 H), 1.33 - 1.18 (m, 5 H), 1.00 – 0.93 (m, 10 H), 0.86 (d, J = 7.64 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.5, 155.3, 103.4, 102.4, 90.7, 88.3, 81.1, 80.7, 73.9, 70.4, 52.7, 51.1, 44.8, 43.2, 37.5, 36.6, 36.6, 36.0, 35.9, 34.5, 34.1, 30.5, 30.3, 30.1, 25.7, 24.9, 24.6, 24.6, 20.3, 19.9, 13.7, 12.6; ESI-HRMS m/z (M + Na)$^+$ for C$_{35}$H$_{54}$N$_2$O$_{10}$ calc. 685.3671, found = 685.3680.

Synthesis of dimer oxime acetate *Z*-33

The desired compound was prepared in a similar manner to propargyl oxime **Z**-**31** as described above, substituting acetyl chloride for propargyl bromide. Gradient elution on silica gel (10 to 80% ethyl acetate in hexanes) afforded the desired product as a colorless amorphous solid: 8.5 mg, 99% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.67 (d, J = 6.88 Hz, 1 H), 5.43 (s, 1 H), 5.37 (s, 1 H), 4.59 - 4.52 (m, 1 H), 2.92 - 2.81 (m, 2 H), 2.76 (dd, J = 14.02, 9.66 Hz, 1 H), 2.54 (dd, J = 14.02, 2.72 Hz, 1 H), 2.36 - 2.26 (m, 2 H), 2.16 (s, 3 H), 2.06 – 1.95 (m, 3 H), 1.87 (ddd, J = 10.22, 6.74, 3.38 Hz, 1 H), 1.84 - 1.81 (m, 1 H), 1.81 - 1.761 (m, 1 H), 1.76 - 1.70 (m, J = 13.46, 3.60, 3.60, 3.47 Hz, 1 H), 1.68 - 1.62 (m, 2 H), 1.53 (dt, J = 13.63, 4.46 Hz, 1 H), 1.49 - 1.43 (m, 1 H), 1.43 - 1.35 (m, 7 H), 1.35 - 1.29 (m, 3 H), 1.29 - 1.18 (m, 3 H), 1.00 – 0.95 (m, 6 H), 0.94 (d, J = 7.14 Hz, 6 H), 0.81 (d, J = 7.64 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.5, 168.04, 103.4, 102.4, 90.4, 88.3, 81.0, 80.7, 73.9, 70.4, 52.7, 51.1, 44.8, 43.2, 37.5, 36.6, 36.6, 34.5, 34.1, 30.4, 30.3, 30.0, 25.7, 25.0, 24.6, 24.6, 20.3, 19.9, 19.8, 13.7, 12.6; ESI-HRMS m/z (M + H)$^+$ for C$_{34}$H$_{52}$NO$_{10}$ calc. 634.3586, found = 634.3564.

Synthesis of dimer oxime dimethyl phosphate *Z*-34

To a 1 dram vial were added dimer oxime **Z**-**25** (7.0 mg, 0.01 mmol, 1.0 eq) and dichloromethane (CH$_2$Cl$_2$, 1 mL), followed by triethylamine (Et$_3$N, 3 μL, 0.02 mmol, 2.0 eq) and dimethyl chlorophosphate (3 μL, 0.02 mmol, 2.0 eq). The reaction mixture was stirred overnight at rt. Upon completion the reaction mixture is further diluted with DCM and washed with sat. aq. NH$_4$Cl and brine. The organic layer was extracted, dried over Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified directly on silica gel. Gradient elution (10 to 100% ethyl acetate in hexanes) afforded the desired product as a colorless amorphous solid: 2.8 mg, 37% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.72 (d, J = 6.88 Hz, 1 H), 5.40 (s, 1 H), 5.38 (s, 1 H), 4.52 (ddd, J = 9.39, 6.02, 3.41 Hz, 1 H), 3.96 - 3.84 (m, 6 H), 2.97 - 2.89 (m, J = 15.28, 7.83, 7.58 Hz, 1 H), 2.89 - 2.83 (m, 1 H), 2.68 (dd, J = 14.08, 9.47 Hz, 1 H), 2.59 (dd, J = 14.15, 3.35 Hz, 1 H), 2.39 - 2.24 (m, 2 H), 2.06 – 1.97 (m, 3 H), 1.92 - 1.83 (m, 2 H), 1.83 - 1.79 (m, 1 H),

1.79 - 1.71 (m, 2 H), 1.71 - 1.62 (m, 10 H), 1.56 (ddd, J = 14.04, 4.82, 4.71 Hz, 1 H), 1.51 - 1.42 (m, 1 H), 1.40 (s, 4 H), 1.36 (s, 4 H), 1.35 - 1.30 (m, 2 H), 1.30 - 1.21 (m, 5 H), 0.97 (dd, J = 5.97, 3.88 Hz, 8 H), 0.93 (d, J = 7.52 Hz, 4 H), 0.85 (d, J = 7.58 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.5, 168.4, 103.2, 102.3, 90.4, 88.3, 81.0, 80.6, 73.44, 70.0, 64.6, 64.6, 54.8, 54.2, 52.7, 51.1, 44.7, 43.2, 37.5, 37.3, 36.7, 36.6, 34.6, 34.2, 30.2, 30.0, 29.7, 26.1, 25.7, 25.0, 24.8, 24.7, 20.3, 19.8, 13.5, 12.6; ESI-HRMS m/z (M + H)$^+$ for C$_{34}$H$_{55}$NO$_{12}$P calc. 700.3456, found = 700.3487.

Synthesis of dimer oxime diethyl phosphate *Z*-35

The desired compound was prepared in a similar manner to 2C-dimer-oxime-diethyl phosphate **Z-34** as described above, substituting diethyl chlorophosphate for dimethyl chlorophosphate. Gradient elution on silica gel (10 to 100% ethyl acetate in hexanes) afforded the desired product as a colorless amorphous solid: 5.4 mg, 63% yield; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.70 (d, J = 6.92 Hz, 1 H), 5.39 (s, 1 H), 5.37 (s, 1 H), 4.52 (ddd, J = 9.15, 5.83, 3.44 Hz, 1 H), 4.35 - 4.17 (m, 4 H), 2.99 - 2.80 (m, 2 H), 2.75 - 2.64 (m, J = 14.13, 9.04 Hz, 1 H), 2.57 (dt, J = 14.18, 3.44 Hz, 1 H), 2.41 - 2.24 (m, 2 H), 2.06 – 1.95 (m, 3 H), 1.93 - 1.83 (m, 2 H), 1.83 - 1.76 (m, 2 H), 1.75 - 1.63 (m, 5 H), 1.61 - 1.50 (m, 1 H), 1.50 - 1.41 (m, 1 H), 1.41 - 1.33 (m, 14 H), 1.33 - 1.20 (m, 5 H), 1.01 - .95 (m, 7 H), 0.93 (d, J = 7.49 Hz, 4 H), 0.85 (d, J = 7.58 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.6, 168.4, 103.3, 102.3, 90.4, 88.3, 81.1, 80.6, 73.44, 70.0, 65.1, 65.0, 64.6, 64.6, 52.7, 51.1, 44.7, 43.2, 37.5, 37.3, 36.7, 36.6, 34.6, 34.2, 30.2, 30.0, 29.7, 26.1, 25.7, 25.0, 24.8, 24.7, 20.3, 19.8, 16.2, 13.5, 12.6; ESI-HRMS m/z (M + Na)$^+$ for C$_{36}$H$_{58}$NNaO$_{12}$P calc. 750.3589, found = 750.3618.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations Used

ACT	artemisinin combination therapy
SAR	structure-activity relationships
DHA	dihydroartemisinin
LG	leaving group
DHA-OAc	dihydroartemisinin acetate
DIBALH	diisobutylaluminum hydride
TMS	trimethylsilyl
*p***TsOH**	*para*-toluenesulfonic acid
LDA	lithium diisopropylamide

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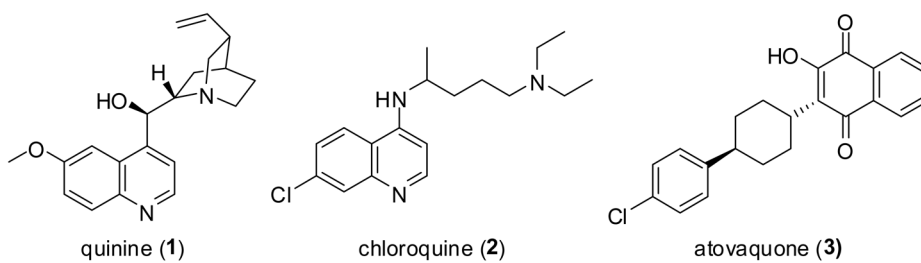
Figure 1.
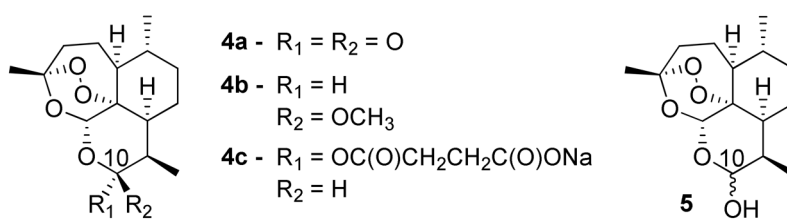Antimalarial agents to which resistance has developed

4a - $R_1 = R_2 = O$

4b - $R_1 = H$
$R_2 = OCH_3$

4c - $R_1 = OC(O)CH_2CH_2C(O)ONa$
$R_2 = H$

Figure 2.
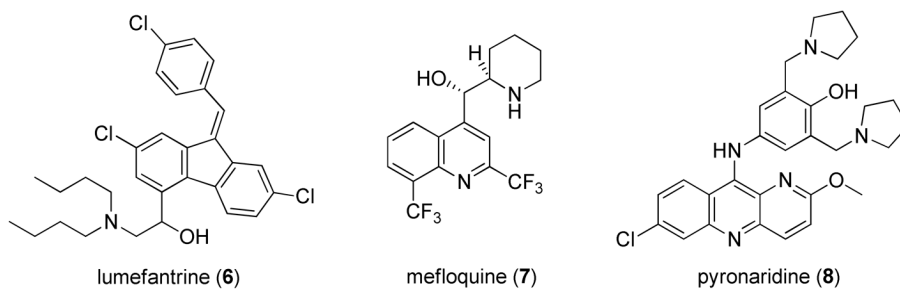artemisinin, currently used first generation derivatives, and dihydroartmesinin
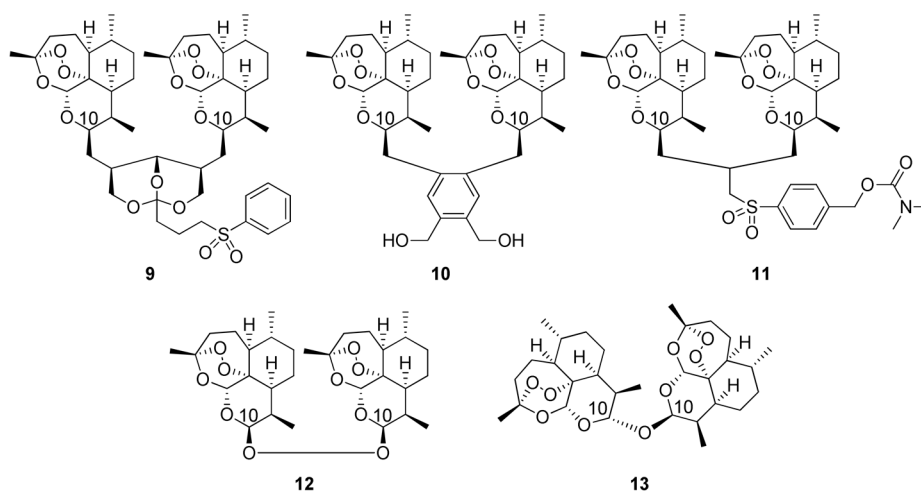
Figure 3.
currently used therapeutic drugs in ACT

Figure 4.
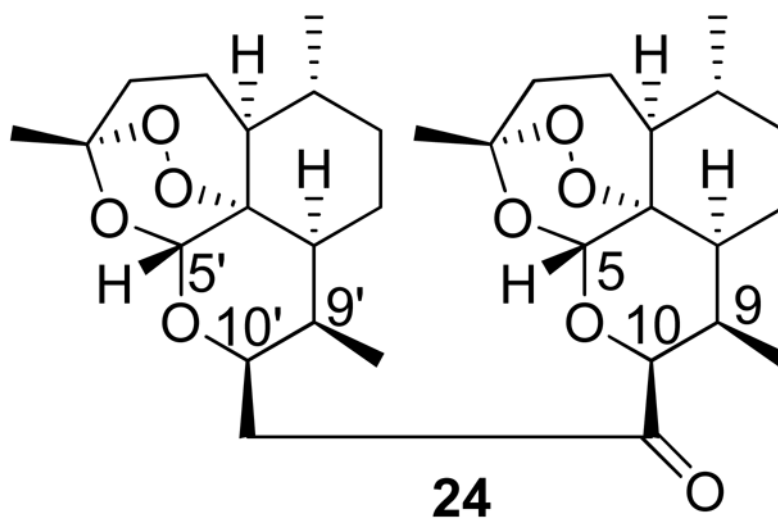Representative artemisinin dimers of varying linker length

Figure 5.
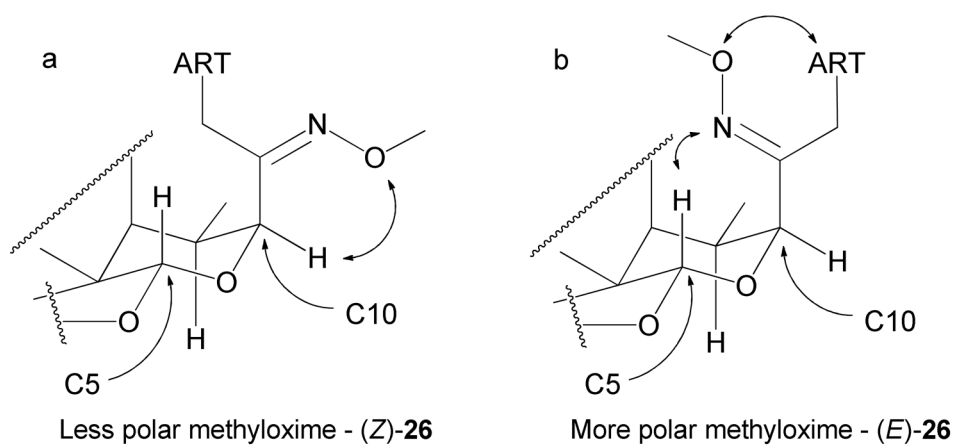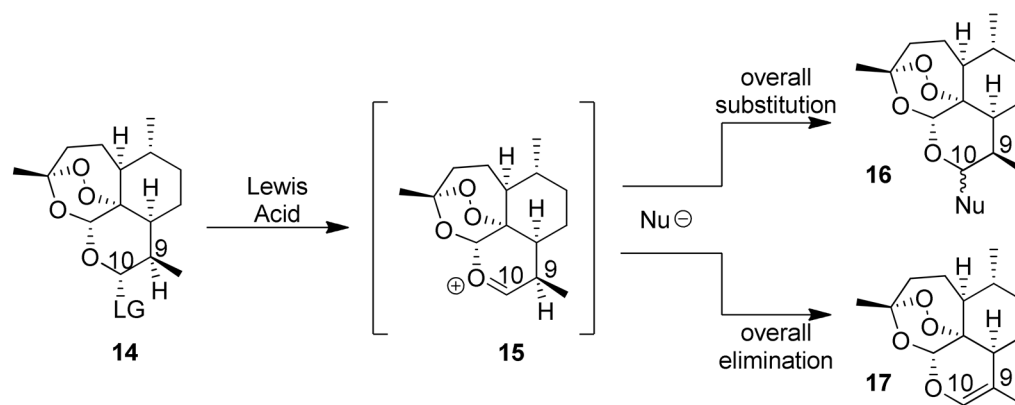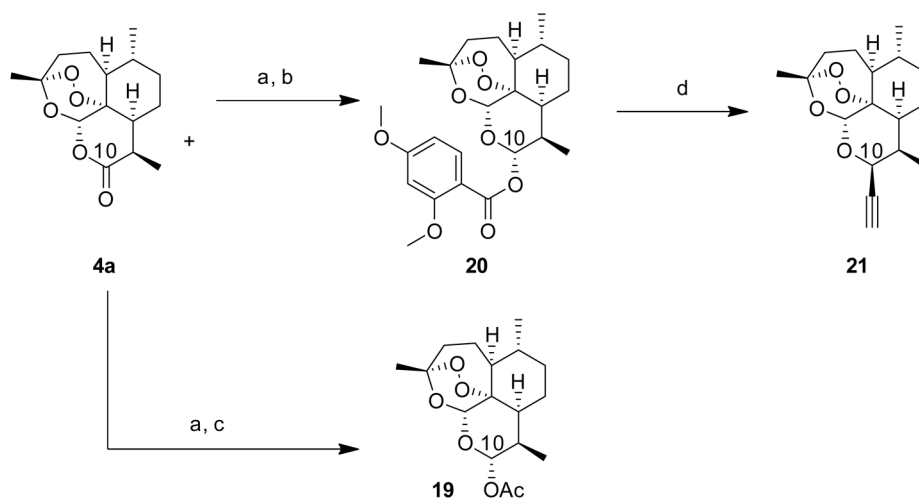Carbon numbering for proton NMR analysis

Figure 6.
a) proposed interactions in the less polar dimer methyloxime (*Z*)-**26**; b) proposed interactions in the more polar dimer methyloxime (*E*)-**26**
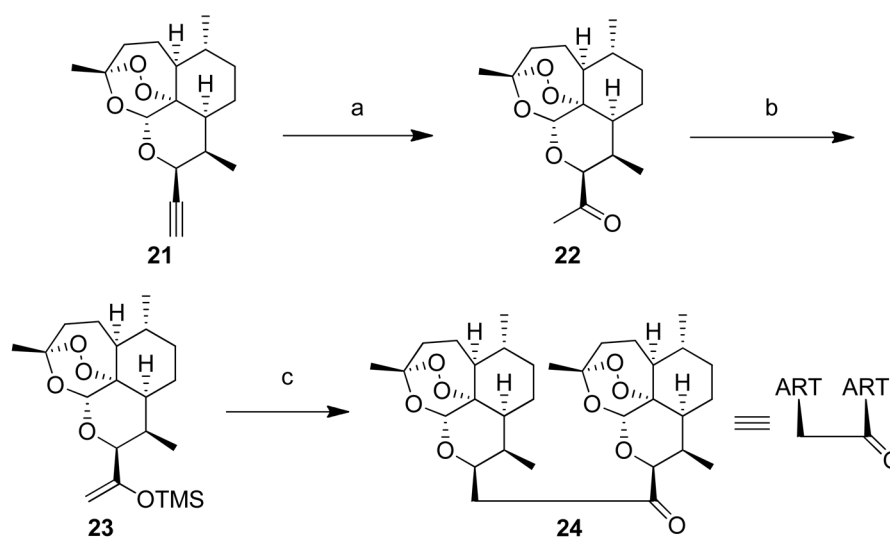
Scheme 1.
Nucleophilic substitution at C10 and competing elimination pathway
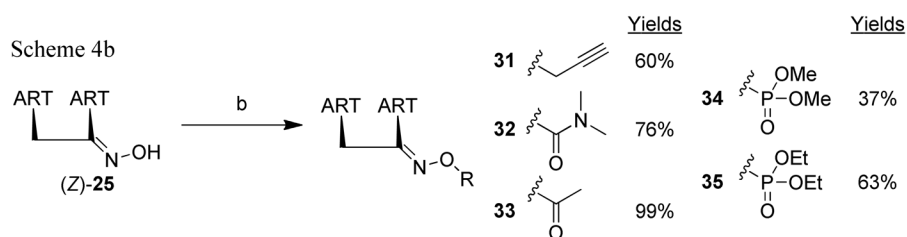
Scheme 2.
Preparation of DHA-OAc (**19**) and C10 β-alkyne (**21**)
a) DIBALH, CH$_2$Cl$_2$, −78 °C, b) DMAP, py, 2,4 dimethoxybenzoyl chloride (**18**), 98% over 2 steps; c) DMAP, py, Ac$_2$O, 98% over 2 steps; d) 1.1 eq ZnCl$_2$, 3.5 eq ethynylmagnesium chloride, Et$_2$O, 74%.

Scheme 3.
synthesis of two-carbon linked artemisinin dimer ketone (**24**)
a) pTsOH, Hg(OAc)$_2$, acetone: H$_2$O, rt, 90%; b) TMSOTf, Et$_3$N, THF, −78 °C to rt, 81%; c) DHA-OAc (**19**), SnCl$_4$, CH$_2$Cl$_2$, −78 °C, 69%.
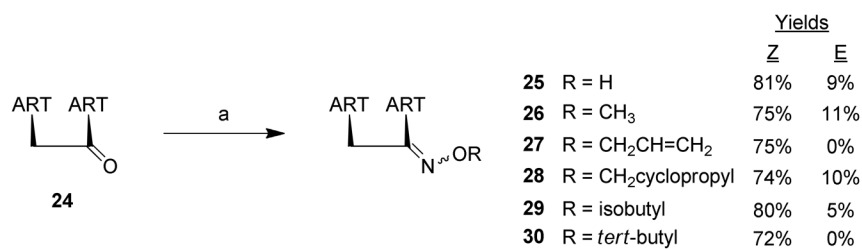
Scheme 4.
preparation of two-carbon linked dimer oxime analogs from **24**
a) RONH$_2$-HCl, py, rt; b) R-X, NaH, THF, 0 °C.

Table 1

In Vivo Antimalarial Efficacy Using a Single Oral Dose of Trioxane Combined with Mefloquine Hydrochloride in *P.berghei* Infected Mice

trioxane	single oral dose (mg/kg)		survival after infection (days)	avg survival	% parasitemia suppression[1]
	trioxane	Mef-HCl			
24	6	18	34, 39, 60, 60	48.3	>99.9
25	6	18	30, 60, 60, 60	52.5	>99.9
26	**6**	**18**	**60, 60, 60, 60**	**60.0**	**>99.9**
27	6	18	44, 60, 60, 60	56.0	>99.9
27	120	0	20, 21, 60, 60	40.3	>99.9
31	**6**	**18**	**60, 60, 60, 60**	**60.0**	**>99.9**
32	6	18	27, 60, 60, 60	51.8	>99.9
33	6	18	13, 16, 20, 60	27.3	>99.9
34	6	18	44, 60, 60, 60	56.0	>99.9
35	6	18	16, 20, 30, 30	21.5	>99.9
controls:					
vehicle (no drug)	0	0	6, 7, 8, 8	7.3	0
artemether(4b)	6	18	16, 24, 60, 60	40.0	>99.9
mefloquine	0	18	17, 20, 20, 27	21.0	>99.9

[1] determined on day 3 post infection

Table 2

In Vivo Antimalarial Efficacy Using a Single Oral Dose of Trioxane Combined with Mefloquine Hydrochloride or Lumefantrine in *P.berghei* Infected Mice

trioxane	single oral dose (mg/kg)			survival after infection (days)	avg survival	% parasitemia suppression[1]
	trioxane	adjuvant	dose			
E-26	6	mefloquine	18	16, 35, 37, 60	37.0	>99.9
26	6	mefloquine	18	16, 60, 60, 60	49.0	>99.9
26	6	lumefantrine	18	13, 16, 16, 60	26.3	>99.9
26	150	none	0	7, 7, 9, 10	8.3	98
27	6	mefloquine	18	16, 60, 60, 60	49.0	>99.9
27	6	lumefantrine	18	16, 29, 60, 60	41.3	>99.9
27	150	none	0	7, 60, 60, 60	46.8	>99
28	6	mefloquine	18	15, 29, 60, 60	41	>99.9
30	6	mefloquine	18	18, 20, 20, 37	23.8	>99.9
31	6	mefloquine	18	35, 60, 60, 60	53.8	>99.9
31	6	lumefantrine	18	60, 60, 60, 60	60.0	>99.9
31	150	none	0	7, 60, 60, 60	46.8	>99.9
controls:						
vehicle (no drug)	0		0	6, 7, 7, 15	8.8	0
artemether(**4b**)	6	mefloquine	18	29, 29, 36, 60	38.5	>99.9
artemether(**4b**)	6	lumefantrine	18	15, 16, 16, 29	19.0	>99.9
mefloquine	0	mefloquine	18	15, 16, 30, 31	23.0	>99.9
lumefantrine	0	lumefantrine	18	7, 7, 7, 35	14.0	>99.9

[1] determined on day 3 post infection