Structure−Activity Relationships of the Antimalarial Agent Artemisinin. 7. Direct Modification of (+)-Artemisinin and In Vivo Antimalarial Screening of New, Potential Preclinical Antimalarial Candidates

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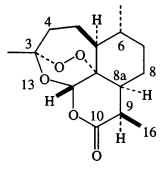
On the basis of earlier reported quantitative structure−activity relationship studies, a series of 9*β*-16-(arylalkyl)-10-deoxoartemisinins were proposed for synthesis. Several of the new compounds **7** and **10**−**14** were synthesized employing the key synthetic intermediate **23**. In a second approach, the natural product (+)-artemisinic acid was utilized as an acceptor for conjugate addition, and the resultant homologated acids were subjected to singlet oxygenation and acid treatment to provide artemisinin analogues. Under a new approach, we developed a one step reaction for the interconversion of artemisinin **1** into artemisitene **22** that did not employ selenium-based reagents and found that 2-arylethyliodides would undergo facile radical-induced conjugate addition to the exomethylene lactone of **22** in good yield. The lactone carbonyls were removed sequentially by diisobutylaluminum hydride reduction followed directly by a second reduction (BF_3-etherate/Et_3SiH) to afford the desired corresponding pyrans. Six additional halogen-substituted aromatic side chains were installed via **22** furnishing the bioassay candidates **15**−**20**. The analogues were examined for in vitro antimalarial activity in the W-2 and D-6 clones of *Plasmodium falciparum* and were additionally tested in vivo in *Plasmodium berghei*- and/or *Plasmodium yoelii*-infected mice. Several of the compounds emerged as highly potent orally active candidates without obvious toxicity. Of these, two were chosen for pharmacokinetic evaluation, **14** and **17**.

Introduction

Malaria parasites have tormented humankind throughout history. As early as 6000 B.C.E., there were recordings of lethal fevers, which were most likely malaria.[1] The Greek physician Hippocrates, in 400 B.C.E., was the first to describe the intermittent and often relapsing fever attacks followed by shaking chills, sweating, and dizziness now known to be caused by malaria. Later, the Romans described malaria as "a horrid disease, which comes every summer and kills".[1] Today, malaria is one of the deadliest diseases on the planet and the leading cause of sickness and death in the developing world. According to the World Health Organization (WHO), malaria causes approximately 500 million clinical cases per year and kills 2.7 million people.[2] It is prevalent in the young, causing one million deaths in children under the age of five each year. The disease also causes anemia in children and pregnant women and increases vulnerability to other diseases. It afflicts the underprivileged most severely, decreasing productivity and causing chronic poor health.

Medicinal use of the Chinese herb qinghao appears in several standard Chinese Materia Medica texts as a treatment for febrile illnesses.[3] The herb was specifically recommended for fevers in the *Zhou Hou Bei fi Fang* (The Handbook of Prescriptions for Emergencies) written by Ge Heng and published in 341 C.E. The most detailed description appears in the "Compendium of Materia Medica−*Ben Cao Gang Mu*", compiled in 1596, and is still printed in China today.[4,5] The antimalarial activity of qinghao was rediscovered in China in 1972, and the antimalarial active principal of qinghao was named "qinghaosu". The western name for the compound is artemisinin (**1**).

Artemisinin (**1**) is a naturally occurring peroxidic cadinane sesquiterpene. Additional names found in China for **1** include qinghaosu, huanghuahaosu, arteannuin, and artemisinine. The Chemical Abstracts adopted artemisinin as the official name; however,



1, Artemisinin

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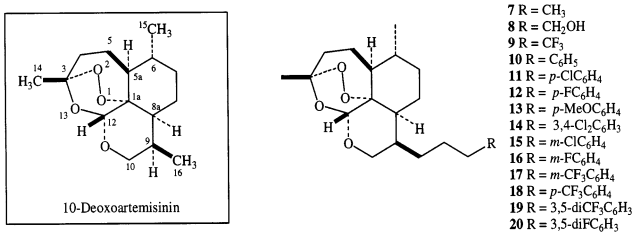


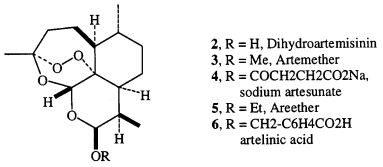
Figure 1. 10-Deoxoartemisinin analogues developed for synthesis and bioassay.

earlier entries as qinghaosu can be found. Systematically, it is named [3*R*-(3α,5a*β*,6*β*,9α,12*β*,1a*R**)]-octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-*j*]-1,2-benzodioxepin-10(3H)-one.

Chinese researchers isolated artemisinin (**1**) in 1972 from *Artemisia annua* L. (annual wormwood), and its structure was elucidated in 1979.[6] The plant now grows in many countries, although it is originally from northern China.[7] Artemisinin (**1**) yields in the plant can vary considerably, depending on plant material and growth conditions. It is present in the leaves and the flowers of the plant in 0.01−0.8% dry weight.[2]

Artemisinin (**1**) has the ability to quickly lower parasite levels, even in severe cases of cerebral malaria. Additionally, artemisinin (**1**) has demonstrated activity against drug resistant strains of *Plasmodium falciparum* such as W-2 Indochina (chloroquine resistant) and D-6 Sierra Leone (mefloquine resistant) clones. Because of this outstanding pharmacological profile in combination with its novel chemical structure, artemisinin (**1**) became a target studied worldwide.

However, problems associated with artemisinin (**1**), including a short plasma half-life, limited bioavailability, poor solubility in oil and water, and the low yield of artemisinin (**1**) from natural sources, prompted scientists to develop new syntheses of artemisinin (**1**) derivatives for more than a decade.[8] Several analogues have been obtained semisynthetically from artemisinin (**1**). For example, artemisinin (**1**) can be reduced to dihydroartemisinin (**2**), which is approximately 10 times more potent than the parent compound in vitro. Derivatives such as artemether (**3**) and sodium artesunate (**4**), obtained by simple modification to the lactol (**2**), are recognized as clinically useful drugs in Southeast Asia (Figure 2).[8]



Artesunate (**4**) has been licensed to the pharmaceutical company Sanofi, which has launched an oral preparation, Arsumax, in Western Africa, whereas Rhone Poulenc Rorer has licensed artemether (**3**) only for use in severe malaria. In the western world, the U.S. Army has expended considerable effort toward clearance of arteether (**5**), currently available only in Asia, for human use.[9] The Walter Reed Army Institute of Research has also developed a more stable, water soluble derivative related to artesunate (**4**), artelinic acid (**6**) (Figure 2). A new malaria drug, Riamet, already available in China, has been approved for sale by Novartis in Switzerland, its first Western market. This product, licensed from the Institute of Microbiology and Epidemiology in Beijing, combines artemether (**3**) with a synthetic substance, lumefantrine.

Riamet is indicated for the treatment of *P. falciparum* malaria, including emergency treatment for travelers to regions where malaria is prevalent. While the development of second-generation artemisinin analogues has been useful for the treatment of severe and complicated malaria, there are concerns about recent observations of fatal neurotoxicity of arteether (**5**) in animals. Studies have demonstrated that dihydroartemisinin (**2**), an in vitro metabolite of artemether (**3**), artesunate (**4**), and arteether (**5**), is probably the causative neurotoxic agent. To avoid metabolism to neurotoxic dihydroartemisinin (**2**), it has been suggested that future generations should avoid a hemiacetal type structure.[10,11]

The objectives we have embraced are to develop a commercially viable, nontoxic, orally potent antimalarial agent designed after artemisinin (**1**) worthy of entering a costly development process. To achieve these goals, we relied on quantitative structure−activity relationship (QSAR) studies, using comparative molecular field analysis (CoMFA) techniques to predict biological activities of new compounds and infer possible activity-enhancing modifications.[12] The set of compounds focused upon for scale-up and in vivo screening was halogenated aromatic derivatives (**11**−**20**) of our previously reported base structure **10**. Additional analogues **7**−**9** were prepared based upon our prior report of high potency of **7** in vitro.[13]

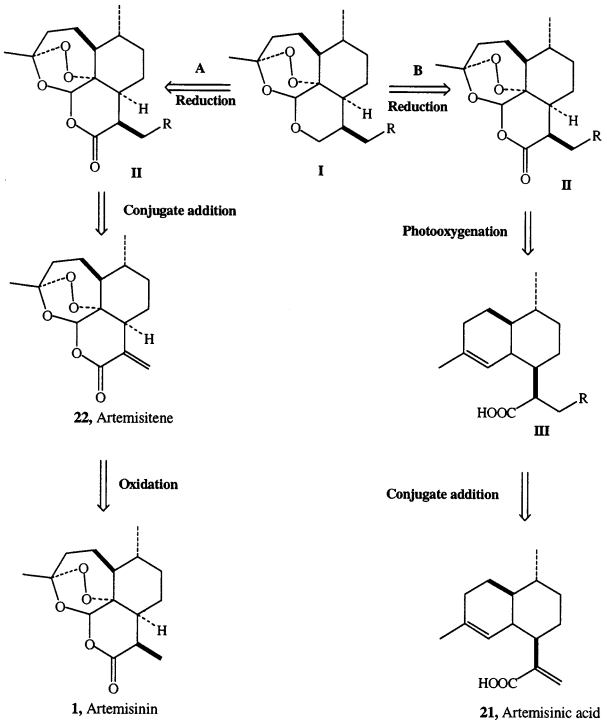
These analogues were tested in vitro against known drug resistant strains of *P. falciparum*: W-2 Indochina clone (mefloquine sensitive, chloroquine resistant), D-6 Sierra Leone (chloroquine sensitive, mefloquine resistant), K1 (chloroquine resistant), and NF54 (chloroquine sensitive). In vivo screening was conducted in *Plasmodium berghei*- or *Plasmodium yoelii*-infected mice by available methods.

Methods

Three routes were employed to synthesize the homologues substituted at the 16-position of 10-deoxoartemisinin shown in Figure 1. The first route, via total synthesis, was amenable to multigram scale-up and has been described before for milligram quantities of **7**, **10**, and **11** for in vitro screening.[13,14] The other two routes involved the conversion of co-occurring natural products, artemisitene and artemisinic acid, each utilizing unique strategies that will be discussed below.

As shown in Scheme 1, **I** may be built either from the accessible artemisinin (**1**) (route A) or from artemisinic acid (**21**) (route B), both of which are produced by *Artemisia annua* L. Structures **I** and **II** differ only in the lactonic C═O bond. The lactone carbonyl could be reduced under heterogeneous conditions with sodium borohydride ($NaBH_4$) in the presence of a Lewis acid to give the target class **I**.[15] Two independent solution reactions may also be employed to solve the same problem with better reproducibility. Thus, reduction of the lactone **II** to the respective lactol with diisobutylaluminum hydride (DIBAL) can be followed by treatment with triethylsilane (Et_3SiH) and boron trifluoride

Scheme 1



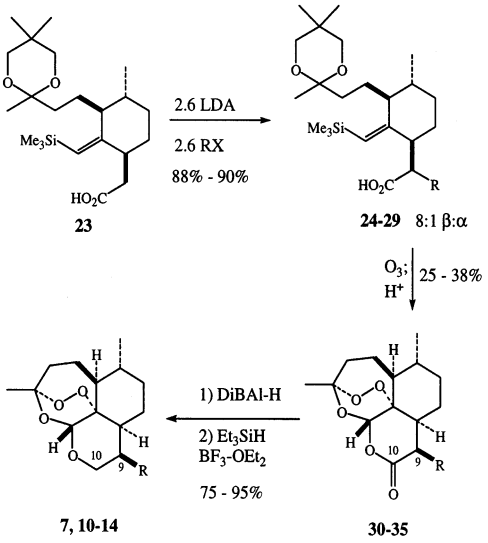
diethyl etherate complex (BF_3·OEt_2) to afford the pyran adduct **I**. Exposure of **III** to light and oxygen, followed by in situ acidification, serves to catalyze the photooxygenation reaction to yield the adduct **II**. Starting with artemisinic acid (**21**), a one pot protection, conjugate addition, and deprotection can be applied. This route has been developed in early studies, in which in four chemical reactions the conversion of artemisinic acid (**21**) into our lead compound was achieved. Another approach is to use artemisitene (**22**), the oxidized form of the natural product artemisinin (**1**), for conjugate addition (route A).

Synthetic Approaches

Total Synthesis. Starting with the synthetic intermediate, ketal-acid **23**,[16] alkylation was facilitated by treatment with 2.6 equiv of lithium diisopropylamine (LDA) and heated to 50 °C. The orange-colored solution that resulted was indicative of dianion formation. After it was cooled to 0 °C, the appropriate alkylating agent was added giving homologated ketal-acids **24**−**29** (Scheme 2). After silica gel, purification yields ranged from 80 to 90%. The alkylating agents were either purchased (**7** and **10**) from Aldrich or were prepared by known procedures.[13,17−20] Ozonolysis of a solution of the ketal-acids **24**−**29**, until a faint blue color appeared, resulted in the primary ozonide. The transformation to artemisinin analogues was accomplished by subsequent acid-catalyzed rearrangement/cyclization giving **30**−**35** in 25−38% yields after purification.[13,16,21]

We investigated the transformation of artemisinin to 10-deoxoartemisinin for various artemisinin analogues such as **30**−**35** following the procedure of Jung using BF_3·OEt_2 and sodium borohydride ($NaBH_4$) in tetrahydrofuran (THF)/MeOH.[14,15] It was found that the yields were variable and somewhat lower than those reported for artemisinin (**1**) when using this reagent

Scheme 2

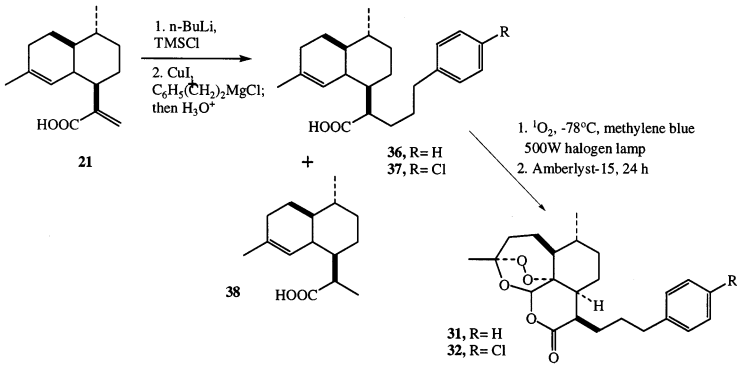


combination. We found that reduction of these lactones to lactols with DIBAL, followed by treatment of the crude lactol with triethylsilane and borontrifluoride etherate, gave the desired pyrans **7** and **10**−**14** in 75−95% yield after purification.[14,22]

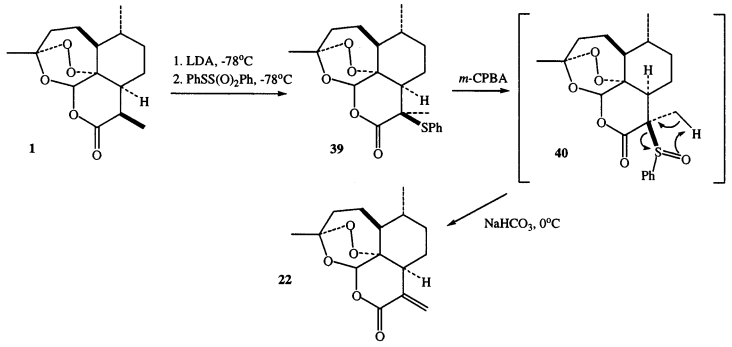
In the attempted optimization of this reduction methodology, numerous conditions were explored attempting to find a one pot conversion. Several different aluminum-based Lewis acids were investigated, which did in fact produce the desired products. However, a high-yielding reproducible one pot procedure that was an improvement over the current two step methods could not be found readily. For example, a one pot procedure using artemisinin as the substrate gave 10-deoxoartemisinin in 59% as compared to 95% with the two step sequence.[14]

Semisynthesis from Artemisinic Acid. The methodology for the conversion of artemisinic acid (**21**) into the C-16-substituted-10-deoxo analogues was developed in earlier studies (Scheme 2).[23,24] Transient protection of the acid moiety of **21** by a silicon protecting group furnishes an acrylate ester capable of conjugate addition. With this methodology, the conversion can be accomplished in one pot. Artemisinic acid (**21**) is deprotonated with *n*-butyllithium, and the silyl ester is formed after the addition of trimethylsilyl chloride (TMSCl) at 0 °C. Upon addition of 10 mol % of copper(I) iodide, a Grignard reagent could then be added leading to 1,4-addition products. Unfortunately, we found that an unexpected reaction occurs, providing along with the desired conjugate addition product **36**, the reduced artemisinic acid (**38**). Furthermore, singlet oxygenation of the phenylpropylartemisinic acid derivative (**36**), followed by acid treatment with Amberlyst-15, gave the 9β-phenylpropyl artemisinin (**31**) in a modest yield of 36% (Scheme 3) and suffered from reproducibility problems on scale-up. To prepare the 9β-*p*-chlorophenylpropyl artemisinin analogue (**32**), the same procedure was applied for the *p*-chlorophenylpropylartemisinic acid (**37**). In this case, photolysis did not produce the desired product **32**. Instead, several compounds were obtained in which the aryl ring appeared to have undergone oxidation. Thus, while this method worked well for **31** and other robust unsubsti-

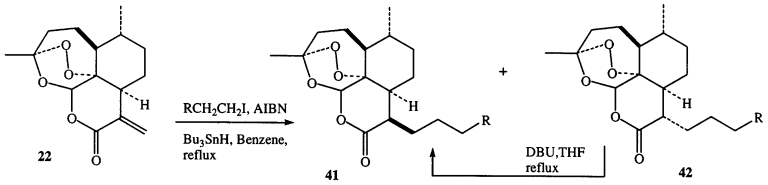
Scheme 3



Scheme 4



Scheme 5



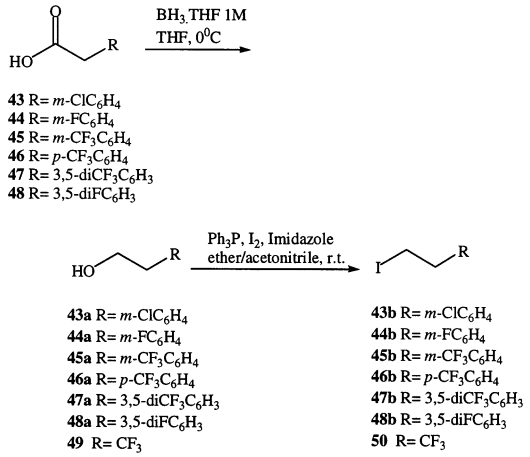
tuted substrates, it failed with halogenated substrates and was not used further to prepare analogues.

Semisynthesis from Artemisinin. Artemisitene (**22**), an oxidized form of artemisinin (**1**), presents an opportunity for conjugate addition by a radical or nucleophilic mechanism. Others have added various nucleophiles to artemisitene,[25,26] but we have had limited success with cuprate and Grignard chemistry on artemisitene. On the other hand, a radical addition could in principle avoid the problematical peroxide reductions effected by Cu(I) upon attempted Normant coupling of artemisitene. Early success with additions of primary radicals to artemisitene prompted the search for a nontoxic, inexpensive route to artemisitene. Artemisinin (**1**) is commercially available in kilogram quantities from Vietnam and has been converted by others to artemisitene as follows. The semisynthesis of artemisitene (**22**) from **1** was accomplished by El-Feraly in 1990 in four steps, using a photochemical approach.[27] Later, Paitayatat developed a short and convenient method for the conversion of artemisinin (**1**) to artemisitene (**22**) using a one pot selenoxide elimination reaction.[26] However, the selenium reagent employed in this conversion has a high level of toxicity and would not be suitable for large-scale production. The above conversion was an important first step, but the development of a better method was necessary.

We adopted a new procedure for the synthesis of artemisitene **22**, which avoided the use of selenium, by concentrating on sulfoxide eliminations instead. In an unsuccessful attempt to introduce the 9α-thiophenyl moiety for subsequent oxidation and elimination, we treated artemisinin with LDA followed by PhSSPh. This reaction might be slow due to the relative unreactivity of PhSSPh; therefore, a more reactive reagent capable of transferring the PhS moiety was investigated. Studies of LDA/$PhSS(O)_2Ph$ were conducted with artemisinin (**1**). Upon treatment of artemisinin (**1**) with LDA in THF at −78 °C followed by addition of $PhSS(O)_2Ph$, the desired thioether **39** was obtained in 67% yield. The intermediate **39** was oxidized by *m*-CPBA in CH_2Cl_2 at −78 °C to the sulfoxide **40**, which underwent "Cope type" elimination at 0 °C, providing artemisitene (**22**) as the major product isolated in 78% yield (Scheme 4).

We next focused on radical-induced Michael additions for the synthesis of the new structures from Scheme 1 (Scheme 5). Reaction of **22** with arylethyl iodides, 2,2′-azobisisobutyronitrile (AIBN), and tributyltin hydride resulted in an almost equal mixture of β and α epimers **41** and **42**. A more efficient process for the conversion of 9α-substituted analogues to their 9β congeners was achieved, by refluxing the 9α epimer with DBU in THF for 12 h. Previously, this conversion had been performed by deprotonation of the α epimer at low temperatures

Scheme 6



43 R= *m*-ClC_6H_4
44 R= *m*-FC_6H_4
45 R= *m*-$CF_3C_6H_4$
46 R= *p*-$CF_3C_6H_4$
47 R= 3,5-di$CF_3C_6H_3$
48 R= 3,5-diFC_6H_3

43a R= *m*-ClC_6H_4
44a R= *m*-FC_6H_4
45a R= *m*-$CF_3C_6H_4$
46a R= *p*-$CF_3C_6H_4$
47a R= 3,5-di$CF_3C_6H_3$
48a R= 3,5-diFC_6H_3
49 R= CF_3

43b R= *m*-ClC_6H_4
44b R= *m*-FC_6H_4
45b R= *m*-$CF_3C_6H_4$
46b R= *p*-$CF_3C_6H_4$
47b R= 3,5-di$CF_3C_6H_3$
48b R= 3,5-diFC_6H_3
50 R= CF_3

with LDA and subsequent kinetic quench in very poor yields.[13] Using the new approach, a combined overall yield of 60% of the adduct **41** could be obtained.

Iodides required for each synthesis were prepared in a straightforward manner (Scheme 6) from commercial available carboxylic acids. Attempted reduction with lithium aluminum hydride ($LiAlH_4$) led to overreduction of trifluoromethyl arylacetic acids, giving toluene derivatives by displacement of fluorine atoms with hydride. Borane evolved as a particularly useful method for the reduction of these carboxyl groups and permitted selective reduction in the presence of carbon−halogen bonds.

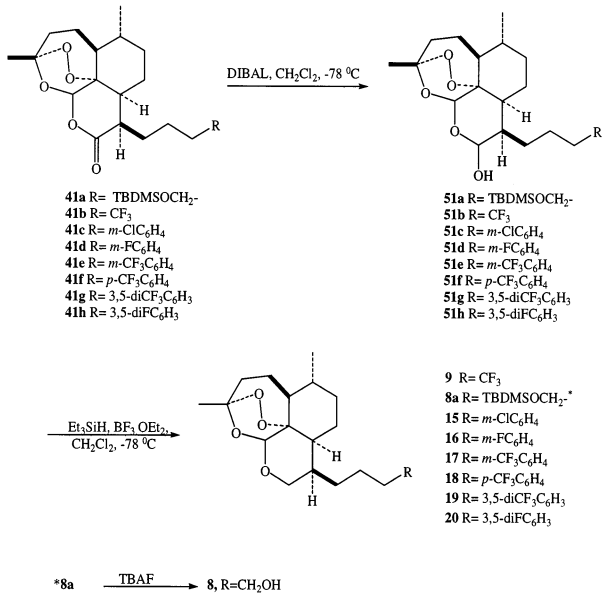
Triphenylphosphine−iodine was used to transform the alcohols into the iodides, furnishing the radical precursors. Again, the alcohol **49** was commercially available and was converted to **50** by the same method.

For the formation of the primary radical and trapping with artemisitene (**22**), a very dilute solution of tributyltin hydride (Bu_3SnH) was added slowly over 8 h via a syringe pump, to a refluxing solution of **22**, AIBN, and the corresponding iodide. The toxic byproduct, tributyltin iodide (Bu_3SnI), was precipitated as the fluoride (Bu_3SnF) by treatment of the reaction mixture with a saturated solution of potassium fluoride (KF). The resulting solution was filtered, and the product was chromatographed to provide the readily separable α and *β* lactone adducts. The α adducts were isomerized to the *β* adducts by heating with DBU and THF and monitoring the reaction for completion by thin-layer chromatography (TLC). A second chromatography provided another batch of *β* isomer, and the combined isomers were then used for the ensuing reactions.

The various lactones from these radical additions and isomerizations **41a**−**h** were reduced to lactols **51a**−**h**, as discussed above, and then reduced with triethylsilane and borontrifluoride etherate to furnish the targets **8a**, **9**, and **15**−**20** (Scheme 1, Scheme 7).

In the present work, no evidence of the products **41a** and **41b** arising from the radical-induced Michael addition was obtained (Scheme 8). The trifluoromethylbutyl derivative (**41b**) was designed to restore the oral activity to the butyl parent compound, which had very high subcutaneous potency in vivo but poor oral activity. Nonetheless, drug development requires identifying the drug candidate's metabolites. Because metabolites are frequently hydroxylated analogues, we have continuing

Scheme 7



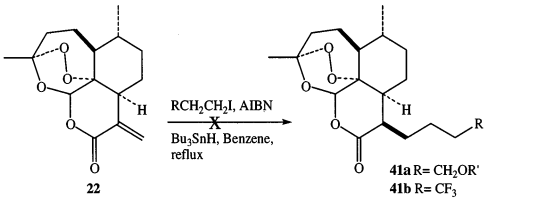
41a R= $TBDMSOCH_2$-
41b R= CF_3
41c R= *m*-ClC_6H_4
41d R= *m*-FC_6H_4
41e R= *m*-$CF_3C_6H_4$
41f R= *p*-$CF_3C_6H_4$
41g R= 3,5-di$CF_3C_6H_3$
41h R= 3,5-diFC_6H_3

51a R= $TBDMSOCH_2$-
51b R= CF_3
51c R= *m*-ClC_6H_4
51d R= *m*-FC_6H_4
51e R= *m*-$CF_3C_6H_4$
51f R= *p*-$CF_3C_6H_4$
51g R= 3,5-di$CF_3C_6H_3$
51h R= 3,5-diFC_6H_3

9 R= CF_3
8a R= $TBDMSOCH_2$-*
15 R= *m*-ClC_6H_4
16 R= *m*-FC_6H_4
17 R= *m*-$CF_3C_6H_4$
18 R= *p*-$CF_3C_6H_4$
19 R= 3,5-di$CF_3C_6H_3$
20 R= 3,5-diFC_6H_3

*8a → **8**, R=CH_2OH

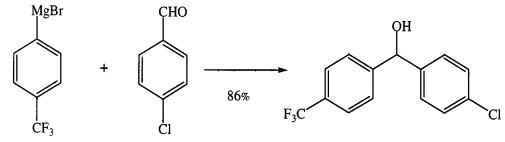
Scheme 8



22

41a R= CH_2OR'
41b R= CF_3
R'= THP, TIPS, TBDMS

Scheme 9



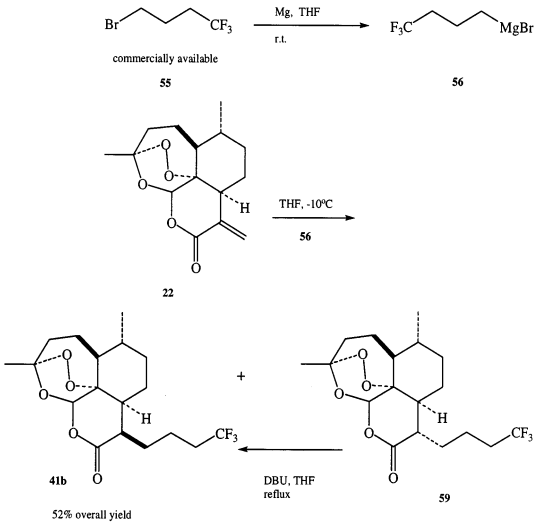
52 **53** **54**

interest in obtaining the proposed metabolite **8**. In this context, we noted that it was necessary to develop an alternate conjugate addition methodology. It should be possible to prepare the new compound using Grignard reagents. We know that trifluoromethylphenyl Grignard behaves as expected, undergoing 1,2-addition to aldehydes to provide the secondary alcohols (Scheme 9). Thus, it may be possible to add the corresponding Grignard simply using copper(I) iodide (CuI).

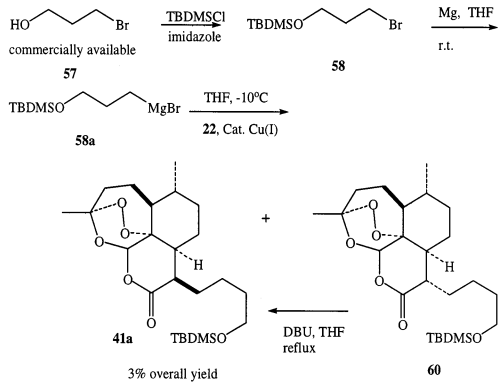
Reaction of artemisitene (**22**) and a catalytic amount of CuI in THF at −10 °C under inert atmosphere with the trifluoromethylpropylmagnesium bromide afforded diastereomeric mixtures of 9-trifluoromethylbutyl artemisinin in 69% yield (Scheme 10). The mixture was purified by flash chromatography (SiO_2) to furnish the desired 9*β*-trifluoromethylbutyl artemisinin (**41b**) as a white solid in 28% yield. The conversion of the α epimer was performed using DBU, and an overall yield of 52% of the adduct **41b** was obtained.

Reduction of compound **41b** was carried out with DIBAL producing the lactol **51b** in 89% yield. Treatment of the lactol **51b** with Et_3SiH and $BF_3{\cdot}OEt_2$ gave the desired pyran **9** (78% yield after purification).

Scheme 10



Scheme 11



One can readily envisage the synthesis of **41a** by Grignard conjugate addition as described above for **41b**. Protection of the hydroxyl group of the 3-bromo-1-propanol (**57**) was necessary for the preparation of the Grignard reagent. Silylation of **57** using *tert*-butyldimethylsilyl (TBDMS) chloride under standard conditions proved successful (Scheme 11) providing **58**.[28] The TBDMS group was our first choice as a protecting group since it can be removed under mild conditions without affecting the core structure. While this approach yielded the desired product **41a**, the very poor yield of 3% inspired the use of different protecting groups. We felt that it would be worthwhile to explore the same reaction using the tetrahydropyranyl (THP) ether derivative of **57** and trimethylsilyl (TMS) derivative of **57**. However, compound **41a** was not observed. Optimization studies, which improve the yield of compound **41a**, remain a project for further investigation.

This work demonstrates that artemisitene can be used as the precursor to produce artemisinin derivatives with new functionality at C-16. The routes described here are reasonably simple pathways to (9β)-16-substituted artemisinin.

Biological Activity

In vivo testing of **7** and **10**−**14** was conducted in the Thompson test at various concentrations, both subcutaneously and orally, in mice infected with *P. berghei*.[29] The concentrations tested subcutaneously were 128, 32, and 8 mg/kg/day. The drug was administered on days 3−5 postinfection. The results were compared to mice treated both at the same concentration with artemisinin and to a control group in which no treatment for the disease was administered.

The most potent analogue from the subcutaneous in vivo studies was 16-propyl-10-deoxoartemisinin **7** having an IC_{100} = 8 mg/kg/day conducted in *P. berghei*-infected mice, with the drug administered on days 3−5 postinfection. However, when **7** was administered orally, the results were unsatisfactory. Excellent oral potency was found when the arylpropyl analogues **10**−**14** were administered in peanut oil. Moreover, the subcutaneous activity observed for these compounds could be reproduced with oral administration using the oil vehicle. It is presumed that the oil vehicle aids solubilization of the drug and hence leads to better absorption in the stomach. Thus, more of the compound would enter the blood stream with an intact endoperoxide moiety, which is essential for antimalarial activity. The improvement of 16β-propyl-10-deoxoartemisinin **7** over the parent compound artemisinin can be seen by focusing on the 8 mg/kg/day treatment regiment. After 60 days postinfection, 100% of the mice treated with **7** were alive, whereas 0% of the mice treated with artemisinin **1** were alive. The parasitemia count after 20 days was an average of 3.6 in mice treated with **7** as compared to 78 for an average in mice treated with artemisinin **1**. The parasitemia count was undetectable in 80% of the mice treated with **7** after day 20, whereas 80% of the mice treated with artemisinin had a detectable level of parasitemia by day 10. Without treatment, 100% of the mice had a detectable level of parasitemia by day 6, and 100% of the mice had perished by day 10 of the test.

At the higher concentration of 128 mg/kg/day dosage, both **7** and artemisinin were equally effective at controlling the disease with a 100% survival rate. At the 32 mg/kg/day dosage, **7** was again able to completely eradicate the disease whereas artemisinin **1** was unable to protect 20% of the mice. This 20% of the mice had a detectable level of parasitemia by day 20 and death occurred by day 21 postinfection. At the lower concentrations of 2 and 0.5 mg/kg/day dosages, both **7** and artemisinin were inadequate at protecting the mice against the disease with a 0% survival rate.

We were encouraged by the improved potency as compared to artemisinin by subcutaneous administration; yet, we were disappointed by the marginal improvement in potency via oral administration. At all of the concentration levels of 128, 32, and 8 mg/kg/day, there was a 0% survival rate with both **7** and artemisinin **1** as the treatment method. At the highest concentration of 128 mg/kg/day, **7** did lower the parasitemia level at day 10 when compared to artemisinin **1** (3 vs 23.2) and also extended the life of the mice by an average of 3 days. At the lower concentrations of 32 and 8 mg/kg/day, oral administration of the drugs **7** and artemisinin **1** was equally ineffective at treating the disease with a 0% survival rate. Analogue **7** was retested orally, this time administered in oil; yet, this failed to give improvement in protection from the disease. It is postulated that first pass metabolism is occurring, presumably at the ω position (or ω-1), to an

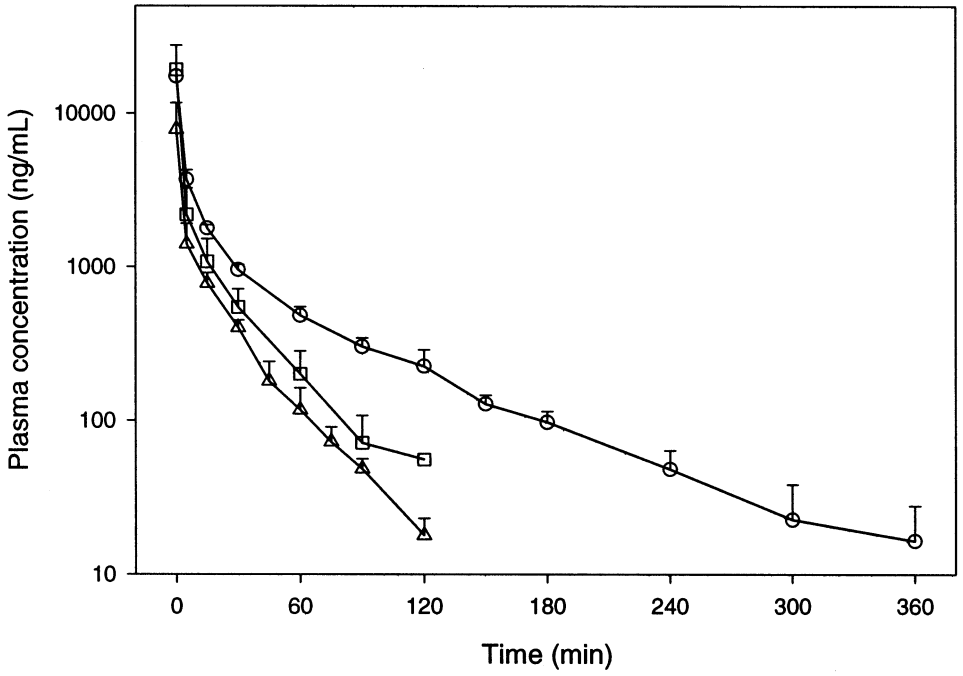


Figure 2. Comparison of the mean pharmacokinetic profiles of **17** (circles) and **14** (squares) and the reported profile for DQHS (triangles, previous study of Bloodworth et al., 1999) following iv administration.

inactive metabolite **8**. Attempts were made to synthesize this suspected metabolite (vide supra) for subcutaneous in vivo testing. If this compound was inactive subcutaneously, it would lend support to the metabolism hypothesis.

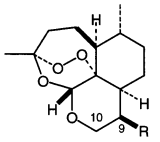
An excellent in vitro activity exhibited by two C-9-modified analogues [R = $-(CH_2)_3C_6H_5$ (**10**) and $-(CH_2)_3$-C_6H_4(4′-Cl) (**11**)][14] prompted us to explore the functionality at the benzene ring. Initially, we focused upon the five C-9-modified analogues **10**−**14**. Considering the significant in vitro activity of **10**, R = $-(CH_2)_3C_6H_5$, and **11**, R = $-(CH_2)_3C_6H_4$(4′-Cl), compounds **12**−**14** were of great interest for in vivo study. The fluoro analogue, for example, would be expected to reduce aromatic metabolism thus improving potency. Other modifications also intended to provide in vivo SAR and required a large scale synthesis of **10**−**14**, which was successfully accomplished via the synthetic route outlined (Scheme 2).

The 16β-propyl-10-deoxoartemisinin analogue **7** is the best analogue tested via subcutaneous administration. All of the analogues containing a phenyl ring **10**−**14** gave complete protection at 32 mg/kg/day in the subcutaneous test. The *p*-Cl derivative **11** showed a slight advantage over the other analogues giving a 40% survival rate at the 8 mg/kg/day in the subcutaneous test. The real advantage of the phenyl derivatives became apparent in the oral test. Analogues **11**−**14** possess IC_{100} values less than or equal to 32 mg/kg/day in the oral test, and the unsubstituted phenyl derivative **10** gave 80% protection at this dosage. The oral test was also conducted in *P. berghei*-infected mice, with the drug administered on days 3−5 postinfection. The *p*-Cl **11** and the *p*-F **12** even showed marginal protection (20%) at the 8 mg/kg/day dosage in the oral test. While the *p*-F analogue **12** (relative activity = 490, where artemisinin **1** = 100) was notably less active than the *p*-Cl analogue **11** (relative activity = 3317, where artemisinin **1** = 100) in vitro, it performed as well orally. These results demonstrate a lack of correlation between the in vitro and the in vivo assays.

While these analogues may not be the "cure-all" for malaria, they are a definite improvement over the parent compound, artemisinin **1**. They are also comparable to other artemisinin analogues that have been tested in vivo such as artemether **3**, arteether **5**, dihydroartemisinin **2**, and sodium salt of artelinic acid **6**. The above analogues have shown promise as antimalarial agents; thus, the in vivo analysis was pursued. Later, it was discovered that **5** was fatally neurotoxic.[10,11] The major metabolite of ethers at the 10-position is dihydroartemisinin **2**, which is highly neurotoxic both in vivo and in vitro ($IC_{50} = 10^{-6}$ M).[30,31] Unlike the previous analogues, the 10-deoxo analogues are postulated not to be metabolized to lactol type compounds, which is presumed to be the culprit, at least in part, for the fatal neurotoxicity. The analogues **7** and **10**−**14** have also undergone in vitro neurotoxicity screening and have not shown substantial neurotoxicity as compared to dihydroartemisinin **2**.[32]

The WHO/TDR in vitro antimalarial screen was conducted using modifications of the procedures of Desjardins et al.,[33] to assess the intrinsic activity of the new analogues **12**−**19** relative to known controls such as quinine, chloroquine, mefloquine, and sodium artesunate (**4**). This method can provide quantitative measurements of the antimalarial activity, based on the inhibition of uptake of radiolabeled nucleic acid precursor by the parasite during short-term cultures in microtitration plates.[33] Pure samples were tested for antimalarial activity in vitro in parasitized human red blood cells (RBC) against four *P. falciparum* clones: mefloquine resistant and chloroquine, pyrimethamine, and sulfadoxine sensitive African Sierra Leone (D6); chloroquine sensitive NF54; chloroquine resistant K1; and chloroquine resistant, mefloquine sensitive, and chloroquine, pyrimethamine, and sulfadoxine resistant

Table 1. In Vivo Antimalarial Activity of Substituted Analogues of Artemisinin against *P. berghei*[a]



structure	R	survival (SC)[b] dose (mg/kg/day) 128	32	8	survival (PO)[b,c] dose (mg/kg/day) 128	32	8
1	(artemisinin)	5/5	3/5	0/5	0/5	0/5	0/5
7	$CH_3(CH_2)_3$	5/5	5/5	5/5		2/5	0/5
10	$C_6H_5(CH_2)_3$	5/5	5/5	0/5	5/5	4/5	0/5
11	p-$ClC_6H_4(CH_2)_3$	5/5	5/5	2/5	5/5	5/5	1/5
12	p-$FC_6H_4(CH_2)_3$	5/5	5/5	0/5	5/5	5/5	1/5
13	p-$MeOC_6H_4(CH_2)_3$	5/5	5/5	0/5	5/5	5/5	0/5
14	3,4-$Cl_2C_6H_4(CH_2)_3$	5/5	5/5	0/5	5/5	5/5	0/5

[a] Drug administered on days 3−5 postinfection. [b] Parasitemia levels were 0 after 60 days on survivors. [c] Administered in peanut oil by gavage.

Indochina (W2). All test compounds were solubilized in dimethyl sulfoxide (DMSO) and diluted 400-fold (to rule out a DMSO effect) in culture medium with plasma for a starting concentration of at least 12 500 ng/mL. Drugs were subsequently diluted 5-fold using the Cetus Pro/Pette system utilizing a range of concentrations from 0.8 to 12 500 ng/mL. Fifty percent inhibitory concentrations were reported in nanograms per milliliter.

The results of the in vivo Peters' 4 day antimalarial testing[34] of our compounds revealed that the new analogues have good oral activities (Tables 3−5). Factors including stability, synthesis cost, and activity led to the selection of compounds **17** (the most potent) and **14** (the most economical) for synthesis of 5 g scale-up for pharmacokinetic analysis (PK).

Preliminary data for the PK results of compounds **17** and **14** have been completed with the objective to evaluate the key pharmacokinetic parameters of the artemisinin derivatives **17** and **14** in rats after intravenous administration and to then compare these data to dihydroartemisinin, which is being used as a reference compound. The absolute oral bioavailability of **17** and **14** was also evaluated. Because of their very low aqueous solubility, the absolute oral bioavailability of each compound was low. The low value is partially a function of the high oral dose (∼120 mg/kg), but even with these high doses, the plasma concentrations are still relatively low.

The ED_{90} data indicated that doses of 10 mg/kg or less are effective in the mouse models for these compounds, so in the future, lower oral doses will be considered. The reason for the use of high oral doses is that the liquid chromatography (LC)/mass spectrometry (MS) assay has different intrinsic sensitivities for the different series of peroxides under PK analysis in the Charman lab. Also, to compare all compounds from the different series at the same oral molar dose (dictated by the compound with the highest LOQ in the assay), it was necessary to use a consistent dose.

Compounds **17** and **14** had a reasonably low plasma LOQ of about 10 ng/mL and will be tested with lower oral doses in the future. If it is assumed that it is only the fraction of drug in solution that is absorbed, then the oral bioavailability may well increase with lower doses. However, these two compounds still have very low aqueous solubilities (pH 6.5, phosphate buffer) of approximately 0.1 μg/mL; hence, this remains a key issue.

In terms of the IV PK data, these two compounds have clearance values of 20−30 mL/min/kg for **17** and **14**. The half-life for **17** is also reasonably good at 73 min, with the shorter value for **14** being an issue. Solubility limitations meant that **14** could only be dosed IV at approximately 2 mg/kg (as compared with the 12 mg/kg dose for **17**). It is possible that the shorter measured half-life for **14** was a function of lower plasma concentrations, which have reflected part of the distribution phase rather than a true elimination phase. Future studies should explore the plasma protein binding of this series, as this may be an important factor governing the changes in their pharmacokinetic profile relative to dihydroartemisinin. Furthermore, it is possible that the *p*-substituted phenyl ring is the key in the longer half-life for **17**. As a rule of thumb, high plasma protein binding of a drug has the potential to decrease the free fraction present in plasma. This could have a negative impact on activity and tissue distribution. The assessment of structural changes on the intrinsic clearance of a compound can only be determined by correcting for changes in the plasma free fraction. In attempting to address these issues, the very low aqueous solubility of **17** and **14** becomes a significant limitation that needs to be addressed as further compounds are explored.

Table 2. In Vitro IC_{50} Data for Various Artemisinin Analogues and Control Drugs against Four Strains of *P. Falciparum*

compd[a]	MW	*P. falciparum* (IC_{50}) D6 (ng/mL)[b]	W2 (ng/mL)[b]	K1 (ng/mL)[b]	NF54 (ng/mL)[b]
chloroquine	319.88	7.58 (4.10)[c]	118.04 (102.5)[c]	79.01	7.36
mefloquine	442.28	45.55 (8.35)[c]	34.06 (2.78)[c]	20.34	35.38
quinine	342.42	159.61 (129.6)[c]	149.88	99.60	9.08
10 (H)	372.50	0.90 (1.1)[c]	1.35 (0.8)[c]		
11 (p-Cl)	406.94	1.90 (1.9)[c]	2.60 (1.6)[c]	NT	NT
9 (n-$C_4H_6F_3$)	364.40	12.46	8.87		
15 (m-Cl)	406.94	7.55 (0.46)[c]	21.60 (0.38)[c]	10.07	18.27 (40.19)[c]
16 (m-F)	390.49	8.28	26.83	11.56	41.76 (27.33)[c]
17 (m-CF_3)	440.50	8.48	30.32	12.00	35.60 (30.76)[c]
18 (p- CF_3)	440.50	28.13 (0.47)[c]	58.01 (0.45)[c]	39.55	101.52 (48.78)[c]
19 (3,5-CF_3)	508.49	0.78	7.11 (0.58)[c]	4.06	NT
20 (3,5-F_2)	408.48	0.82	6.50 (0.45)[c]	3.20	NT

[a] Compounds **11**−**20** are all 10-deoxoartemisinins with a 9-$(CH_2)_3$PhX (Figure 1), whereas compound **9** has a n-$CH_2CH_2CH_2CF_3$ side chain. [b] Assay performed by TIBOTEC. [c] Assay performed by Walter Reed Army Institute of Research (WRAIR).

Table 3. In Vivo ED_{50} and ED_{90} Data in a Mouse Malaria Model (*P. berghei* N) for Artemisinin Analogues and Control Drugs

	P. berghei N (SC)		*P. berghei* N (PO)	
compd[a]	ED_{50}	ED_{90}	ED_{50}	ED_{90}
chloroquine	1.5	3	1.8	3.4
mefloquine	1.1	2.3	3.3	5.1
quinine	83.0	1050	71	118
sodium artesunate	0.8	2.5	2.4	13.0
10 (H)	0.5	1.0	1.4	3.4
11 (*p*-Cl)	0.7	1.2	1.0	2.1
15 (*m*-Cl)	0.5	0.9	2.0	8.3
16 (*m*-F)	0.46	0.85	0.9	3.4
17 (*m*-CF_3)	0.4	0.75	1.25	4.4
18 (*p*-CF_3)	0.44	0.8	0.63	2.1
19 (3,5-CF_3)	0.9	1.7	2.6	5.0
20 (3,5-F_2)	1.3	2.0	1.8	5.7

[a] Compounds **10**, **11-20** are all 10-deoxoartemisinins with a 9-$(CH_2)_3$PhX (Figure 1).

Table 4. In Vivo ED_{50} and ED_{90} Data in Two Mouse Malaria Models (*P. yoelli* ssp. NS and *P. berghei* N) for Artemisinin Analogue **9** and Control Drugs[a]

			mg/kg × 4		%
compd	strain	route	ED_{50}	ED_{90}	suppression
9 (*n*-$C_4H_6F_3$)	N	PO	>10	>10	6.5
9 (*n*-$C_4H_6F_3$)	NS	PO	NA 10	NA 10	0.3
sodium artesunate	N	PO	>10	>10	77.2
sodium artesunate	NS	PO	>10	>10	56.5

[a] Compound **9** is a 10-deoxoartemisinins with a 9-$(CH_2)_3CF_3$ (Figure 1).

Table 5. In Vivo Data (*P. yoelli* ssp. NS)

	P. yoelli ssp. NS (SC)		*P. yoelli* ssp. NS (PO)	
compd[a]	ED_{50}	ED_{90}	ED_{50}	ED_{90}
chloroquine	1.6	28		
mefloquine	2.3	6.0		
quinine	120	56	128	29
sodium artesunate (SSV)	1.8	22.0	1.6	100
10 (H)	0.5	0.9		
11 (*p*-Cl)	0.6	1.0		
15 (*m*-Cl)	0.6	1.00		
16 (*m*-F)	0.58	1.00		
17 (*m*-CF_3)	0.6	1.00		
18 (*p*- CF_3)	0.67	1.25		
19 (3,5-CF_3, SSV*)			4.2	12.0
19 (DMSO*)			1.5	3.6
20 (3,5-F_2, SSV*)			2.2	6.5
20 (DMSO*)			2.3	7.5

[a] Compounds **10**−**20** are all 10-deoxoartemisinins with a 9-$(CH_2)_3$PhX (Figure 1). *SSV and DMSO refer to formulations for oral administration; see Experimental Section.

Conclusions

In summary, we have developed a short and efficient methodology from the easily accessible natural product artemisinin (**1**) to artemisitene (**22**). Because the syntheses of artemisitene (**22**) can be carried out in a straightforward and nontoxic procedure, synthetic application of this methodology as a key step in the synthesis of new artemisinin derivatives was achieved.

Our four step synthesis of the 9β-16-substituted 10-deoxyartemisinin derivatives compares favorably in terms of the use of simple reagents and transformations, as well as better yields with the previously reported synthesis.

The new compounds were fully characterized and evaluated for in vitro and in vivo antimalarial activity. On the basis of the data from the in vivo antimalarial experiment (Peters' 4 day) and factors such as stability and synthesis cost, we chose two compounds, **17** (the most potent) and **14** (the most economical), for synthesis of 5 g scale-up for PK studies. The preliminary PK data led us to believe that **17** has the better PK profile, although the series as a whole still suffers from limiting solubility issues. Future chemistry needs to address these limitations, as the potency seems reasonable, considering that absorption is limited.

Experimental Section

All solvents were purchased as reagent grade, dried appropriately, and stored over dry 4 Å molecular sieves. Solvent and reagent transfers were accomplished either via dried syringe or via cannula, and all reactions were routinely conducted under argon atmosphere unless otherwise indicated. Flash chromatography was accomplished using silica gel (Whatman 60, 230−400 mesh). Unless otherwise noted, all NMR analyses were conducted in $CDCl_3$ on a Bruker Avance DPX-400 and were referenced to chloroform at δ 7.27. IR spectra were obtained using a Digilab FTS-40 FT-IR or a ATI Mattson Genesis Series FT-IR and run neat. Melting points were obtained on a MelTemp. Mass spectral data was obtained on a high-resolution ESI FT-ICR or VG 7070E-HF mass spectrometer. Elemental analyses were within 0.35 as determined by QTI, Whitehouse, NJ, or by Desert Analytics, Tucson, AZ.

General Procedure for Preparation Ketal-Acids 24−29. To a solution of ketal-acid **23** (2 mmol) in THF (5 mL) at −78 °C was added, via cannula, a freshly prepared solution of LDA (2.6 mmol). After 10 min, the resultant solution was allowed to warm to room temperature over a 30 min period. The solution was then heated at 50 °C for 2 h, an orange-colored solution being indicative of dianion formation. The solution was then cooled to 0 °C, and 2.6 equiv of the appropriate alkylating agent was added via syringe. After 2.5 h, TLC showed that no starting material remained. The reaction was quenched with saturated NH_4Cl solution (15 mL) and then extracted with $CHCl_3$ (3 × 15 mL). The combined organic layers were then washed with brine (2 × 30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified via flash chromatography (200:1; silica gel: compound) eluting with 50% EtOAc/hex + 1% HOAc. Pure products **24**−**29** were obtained as yellow oils in 80−90% yield.

General Procedure for Preparation of Target Artemisinin Analogues. Ozonolysis and Acid-Catalyzed Cyclization of Ketal-Acids to Yield Analogues 30−35. O_3/O_2 (7 PSI, 0.5 L/min, 80 V) was bubbled into a solution of ketal-acid **24**−**29** (1 mmol) in dry CH_2Cl_2 (125 mL) at −78 °C, until the reaction mixture had turned faint blue. After the reaction mixture was purged with O_2 followed by Ar, silica gel (750 mg) and 15% aqueous H_2SO_4 (75 μL) were added. The mixture was allowed to come to ambient temperature and stirred overnight, and the solids were filtered and washed with CH_2Cl_2 (2 × 10 mL) and then EtOAc (2 × 10 mL). The resulting filtrate was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and evaporated in vacuo to give crude products, which were easily purified by preparative TLC (PTLC) on silica gel. Pure products **30**−**35** were recovered as white crystalline solids in 25−39% yield.

Procedure for Reduction of Artemisinin Analogues to 10-Deoxoartemisinin Analogues 7 and 10−14 using Et_3SiH/BF_3. To a stirred solution of artemisinin analogues **30**−**35** (0. 320 mmol or 1.0 equiv) in dry CH_2Cl_2 (3 mL) at −78 °C was added DIBAL (335 μL or 0.335 mmol, 1.0 M in hexanes). The dry ice/acetone bath was removed long enough to allow complete dissolution of the substrate (about 1 min) and then replaced. After 1.25 h, the reaction was quenched with saturated $NaHCO_3$ (1 mL), diluted with CH_2Cl_2 (3 mL),

and allowed to warm to ambient temperature with stirring. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with 10% HCl/saturated NH_4Cl (1:15 v/v) (1 × 10 mL). The CH_2Cl_2 layer was then dried ($MgSO_4$), filtered, and concentrated in vacuo to give 90 mg (100%) of a white solid, which was pure enough for the next step. The 90 mg was dissolved in dry CH_2Cl_2 (3 mL) at −78 °C. Et_3SiH (210 μL or 1.31 mmol) was added. The reaction was stirred for 10 min, and then, BF_3-OEt_2 (45 μL or 0.366 mmol) was added. The resultant solution was allowed to stir 3 h at −78 °C. After 3 h, the reaction was quenched at −78 °C with pyridine (150 μL or 1.85 mmol) and was allowed to warm to ambient temperatures. The reaction mixture was poured into aqueous saturated NH_4Cl (10 mL) and extracted with EtOAc (3 × 30 mL). The combined EtOAc layers were washed with NH_4Cl (2 × 15 mL), dried ($MgSO_4$), and concentrated in vacuo to give a white solid, which was purified by PTLC (silica gel) eluting with 20:80 EtOAc/hexanes to give pure products **7** and **10**−**14** as white crystalline solids in 75−90% yield.

In Vivo Testing. The in vivo antimalarial testing was performed at the University of Miami, Department of Microbiology, Miami, FL, by Dr. Arba Ager and co-workers. The mice used in this testing were CD-1 mice, 16−18 g in size. The mice were infected with *P. berghei* after a 2 week quarantine. The treatment regiment began on day three postinfection and lasted for 3 days (days 3−5 postinfection). The subcutaneous administration route was by single injection, and the oral administration was accomplished by using the gavage method with the drug suspended in peanut oil.[35,36]

In the in vivo Peters' 4 day antimalarial testing[34] of our compounds conducted by Dr. Wallace Peters, two batches of five male random-bred Swiss albino mice weighing 18−22 g were inoculated intravenously with 2×10^6 RBC parasitized with *P. berghei* N. Animals were then given a fixed dose of 30 mg/kg once a day for four consecutive days. Compounds were first dissolved in DMSO (due to lower water solubility), and subsequently, an aqueous dilution was prepared for use and administered subcutaneously to one group and orally to a second group. The parasitemia was determined on the day following the last treatment, day 4, to determine qualitatively the presence and degree of activity at the screening dose.

SSV is a formula used by Roche for biological, toxicological, and PK studies whose components are Na-carboxymethylcellulose (CMC), medium viscosity (Merck), benzyl alcohol p.a. (Merck), Tween 80 (Fluka), NaCl 0.9% (Braun) and whose composition is Na-CMC, 5 g; benzyl alcohol, 5 mL; Tween 80, 4 mL; NaCl, 0.9% at 1000 mL.

Pharmacokinetic Studies. All experimental procedures were approved and performed in accordance with the guidelines of the Institutional Animal Experimentation Ethics Committee. The studies were conducted in fasted male Sprague−Dawley rats, and the treatments included IV administration of **14** or **17**, and oral administration of **14** or **17** in the SSV suspension, which is a formulation amenable to use in later developmental and toxicology studies.

Surgical Procedures. On the day prior to dosing, polyethylene cannulae were inserted into the right jugular vein and/or right carotid artery of rats (280−350 g) under isoflurane anaesthesia. Once they were implanted, the cannulae were flushed with heparinised saline (2 U/mL). The cannulae were then exteriorized by tunneling subcutaneously to emerge above the scapulae. Rats were housed in cages fitted with swivel attachments to enable free movement about the cages while protecting the integrity and patency of the cannulae. All rats returned to normal grooming, drinking, and sleeping behavior within 1 h of surgery. Animals were fasted but had free access to water for the duration of the study. At the conclusion of each experiment, rats were euthanized by a lethal injection of pentobarbitone.

Drug Formulations. *Intravenous Formulation and Administration.* The IV dose of **14** and **17** comprised an aqueous solution comprising 20% dimethylsulfoxide (DMSO)/5% Cremophore EL. This formulation provided for **17** to be administered at a 10 mg/kg dose (i.e., 2 mL dose of a 1.75 mg/mL solution) and **14** to be administered at a 2 mg/kg dose (i.e., 2 mL dose of a 0.3 mg/mL solution). IV formulations were prepared on a day of dosing and sterile-filtered through a 0.2 μm syringe filter immediately prior to use. An aliquot of the formulation was retained and assayed for drug content to confirm the concentration of the administered dose. For administration of the dose, the IV solution was administered as a 5 min infusion (2 mL volume) via the jugular vein cannula, after which the cannula was flushed with heparinized saline (2 U/mL) to ensure complete administration of the dose.

Oral Formulation and Administration. The oral formulations of **14** and **17** were prepared as standard aqueous suspensions in 1 mL of 1% carboxymethylcellulose (medium viscosity, Sigma), with 50 μL of 1% Tween 80 (Sigma), at a nominal dose of 120 mg/kg (0.3 mmol/kg) in the expectation that it would provide quantifiable plasma concentrations. The exact amount of **14** and **17** to be administered was weighed into 12 mL polypropylene tubes and then suspended in the total administration volume. Any drug-related material remaining in the tube after dosing was dissolved in a known volume of acetonitrile and assayed by LC-MS to enable determination of the administered dose. For administration of the dose, rats were lightly anaesthetized with isofluorane to facilitate insertion of a gavage tube. The suspension formulation was taken up into a syringe, attached to the gavage tube, and the dose was administered into the stomach. The polypropylene tube (previously containing the suspension) was then rinsed with 1 mL of water, which was also then administered to each rat.

Plasma Sample Collection and Processing. Blood samples were taken from the indwelling carotid cannula. An initial blood volume of 0.2 mL was withdrawn to clear the line of heparinized saline. A fresh syringe was then used to withdraw a 0.3 mL blood sample that was placed in a clean eppendorf tube containing approximately 20 μL of a 1 U/μL heparin solution to prevent clotting. Blood samples were stored on ice until centrifuged to separate plasma from red blood cells, and a 150 μL aliquot of plasma was transferred to a fresh eppendorf tube and then stored frozen until analysis. The blood sample that was initially drawn to clear the cannula was then readministered via the carotid cannula, followed by a small volume (0.5 mL) of heparinized saline (2 U/mL). This procedure facilitated patency of the cannula and minimized any possible changes to blood haematocrit and blood volume that can occur with serial blood sampling.

Blank blood samples were collected from each rat prior to dosing. After IV administration of **14** or **17**, blood samples were collected at 0 (end of infusion), 5, 15, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min postdosing. After oral administration of each compound as the SSV oral suspension formulations, blood samples were collected at 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min postdosing.

Analysis of Plasma Concentrations of 14 and 17. *Preparation of Plasma Standards.* Calibration standards for **14** and **17** were prepared separately by spiking 1 mL of blank rat plasma with 10 μL of a methanol solution containing 1 mg/mL of compound. This process provided a plasma standard with a concentration of 10 000 ng/mL. Further dilutions were made from this stock with blank plasma to afford plasma standards in the range of 5−10 000 ng/mL for **17** and 10−10 000 ng/mL for **14**. Aliquots of each plasma standard (150 μL) were then transferred to eppendorf tubes for processing. Acetonitrile (300 μL) was added to precipitate proteins, and the tubes were vortexed and centrifuged for 10 min at 3500 rpm (Beckman G5-6R refrigerated centrifuge). A 300 μL aliquot of the supernatant was then transferred to a glass autosampler vial, and 15 μL of the internal standard solution (100 μg/mL TDR 7551/2) was added. An aliquot of the solution (50 μL) was then injected onto the LC-MS system. For each conpound, calibration curves were linear over the stated concentration range, and correlation coefficients typically exceeded 0.999.

Plasma Sample Preparation. A simple plasma sample preparation protocol was developed, which involved acetoni-

trile precipitation of plasma proteins, addition of an internal standard, and injection onto the LC-MS system. To a 150 μL aliquot of rat plasma was added 300 μL of acetonitrile to precipitate plasma proteins. All samples were then processed as described above for the preparation of plasma standards.

Sample Analysis. Samples were analyzed on a Waters Alliance 2690 HPLC interfaced with a Micromass LCZ mass spectrometer operating under atmospheric pressure chemical ionization conditions (APCI). The full scan spectrum of **17** resulted in a substantial parent ion, MH^+ (441 amu), which was chosen as the analytical species. The full scan spectrum of **14** resulted in a substantial parent ion, MH^+ (407 amu), with the expected isotopic ratio resulting from a monochlorinated molecule, and this was chosen as the analytical species.

The assay validation criteria were chosen to facilitate rapid assay development while still providing confidence in data integrity. The validation of the assay included linearity of response over the concentration range analyzed, interday variability, system suitability, and replicate analysis of samples at the limit of quantitation (LOQ). Calibration curves were accepted provided that not more than two standards on each day had a back-calculated value that deviated by more than 20% from the nominal concentration. All calibration curves run during the course of assay validation and analysis of plasma samples met this criterion and were linear over the stated concentration range. Individual calibration curves were weighted ($1/x^2$) given the range of plasma concentrations. The LOQ was determined as the lowest standard for which at least three out of four back-calculated concentrations were within 30% of the nominal value. The LOQ was determined to be 5 ng/mL for **17** and 10 ng/mL for **14**.

Suitability of storage and sample processing was investigated by spiking blank plasma to a concentration of 5 μg/mL and then comparing the measured value with a sample that had previously been frozen and then thawed. Both **14** and **17** were shown to be unaffected by the freezing process, and the sample preparation procedure resulted in measured concentrations in agreement with nominal concentrations.

Reproducibility and on-system stability after sample processing was assessed by reinjecting samples from the calibration curves following storage in the autosampler (10 °C) overnight. The consistency of the slope of the calibration curves from day-to-day was used as a simple measure of interday variability. The %CV of the slopes of the calibration curves remained within acceptable limits and did not vary significantly from the slope of a solvent standard curve, indicating that samples are stable if stored overnight in the autosampler and that there were no matrix effects associated with the injection of plasma samples.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxypentyl)-6″-methyl-2″-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (24). Yield 88%. ^{1}H NMR ($CDCl_3$): δ 5.32 (s, 1H), 3.55 (dd, 2H, J = 1.4, 11.5 Hz), 3.44 (dt, 2H, J = 1.4, 11.5 Hz), 2.72 (br dd, 1H, J = 11.2, 11.2 Hz), 2.40 (br dd, 1H, J = 5.0, 11.9 Hz), 2.11 (br dd, 1H, J = 1.9, 10.5 Hz), 1.36 (s, 3H), 1.03 (s, 3H), 0.94 (d, 3H, J = 7.1 Hz), 0.89 (s, 3H), 0.88 (dd, 3H, J = 7.2, 7.2 Hz), 0.13 (s, 9H). IR (CH_2CL_2): 3600−2500, 1734, 1703, 1600, 1456, 1395, 1374, 1246, 1212, 1164, 1091, 845 cm^{-1}. EIMS: m/z 452 (M^+), 437 (M − Me), 348, 338, 296. Anal. ($C_{25}H_{46}O_4Si$) C, H.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxy-4‴-phenylbutyl)-6″-methyl-2″-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (25). Yield 91%. ^{1}H NMR ($CDCl_3$): δ 7.10−7.30 (m, 5H), 5.29 (s, 1H), 3.55 (dd, 2H, J = 3.1, 11.5 Hz), 3.41 (dt, 2H, J = 1.4, 11.0 Hz), 2.74 (br dd, 1H, J = 11.5, 11.5 Hz), 2.59 (br dd, 2H, J = 6.8, 6.8 Hz), 2.39 (br dd, 1H, J = 4.8, 12.0 Hz), 2.08 (br dd, 1H, J = 2.8, 11.5 Hz), 1.40−2.00 (m, 12H), 1.37 (m, 2H), 1.02 (s, 3H), 0.93 (d, 3H, J = 7.1 Hz), 0.87 (s, 3H), 0.10 (s, 9H). IR (Nujol): 3600−2400, 1700, 1600, 1450, 1380, 1250, 1220, 1130, 1100, 850, 750, 710 cm^{-1}. DCIMS−NH_3: m/z 587 (M + TMS), 515 (M + H), 446, 428, 411, 359, 339. Anal. ($C_{31}H_{50}O_4Si$) C, H.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxy-4‴-*p*-chlorophenylbutyl)-6″-methyl-2″-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (26). Yield 74%. ^{1}H NMR ($CDCl_3$): δ 7.23 (d, 2H, J = 8.3 Hz), 7.10 (d, 2H, J = 8.4 Hz), 5.26 (s, 1H), 3.54 (dd, 2H, J = 2.3, 11.6 Hz), 3.38−3.48 (m, 2H), 2.71 (br dd, 2H, J = 10.5, 10.5 Hz), 2.56 (m, 1H), 2.06 (br dd, 1H, J = 2.9, 10.7 Hz), 1.72−1.94 (m, 3H), 1.31 (s, 3H), 1.15 (m, 1H), 1.01 (s, 3H) 0.86−0.94 (m, 6H), 0.09 (s, 9H). IR (CH_2Cl_2): 3400−2500, 1700, 1598, 1491, 1454, 1246, 1091, 861, 748 cm^{-1}. DCIMS−NH_3: m/z 566 (M + NH_4), 549 (M^+). Anal. [$C_{31}H_{49}O_4ClSi$ (as hydrate, 1/4H_2O)] C, H.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxy-4‴-*p*-fluorophenylbutyl)-6″-methyl-2″-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (27). Yield 75%. ^{1}H NMR ($CDCl_3$): δ 7.04 (d, 2H, J = 5.5 Hz), 6.90 (d, 2H, J = 8.8 Hz), 5.26 (s, 1H), 3.53 (d, 2H, J = 11.5 Hz), 3.40 (ddd, 2H, J = 2.7, 7.6, 10.3 Hz), 2.53 (m, 1H), 2.06 (br dd, 1H, J = 2.9, 10.7 Hz), 1.72−1.94 (m, 3H), 1.30 (s, 3H), 1.15 (m, 1H), 1.00 (s, 3H), 0.91 (d, 3H, J = 6.8 Hz), 0.89 (s, 3H), 0.08 (s, 9H). IR (CH_2Cl_2): 3400−2700, 1705, 1506, 1456, 1376, 1259, 1093 cm^{-1}. DCIMS−NH_3: m/z 550 (M + NH_4), 533 (M + H), 464, 357, 110. Anal. ($C_{31}H_{49}O_4SiF$) C, H.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxy-4‴-*p*-methoxyphenylbutyl)-6″-methyl-2″-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (28). Yield 88%. ^{1}H NMR ($CDCl_3$): δ 7.03 (d, 2H, J = 8.5 Hz), 6.78 (d, 2H, J = 8.6 Hz), 5.28 (s, 1H), 3.76 (s, 3H), 3.55 (dd, 2H, J = 2.3, 11.5 Hz), 3.38−3.44 (m, 2H), 2.52 (dd, 2H, J = 6.1, 6.1 Hz), 2.07 (br dd, 1H, J = 2.9, 10.5 Hz), 1.70−1.95 (m, 3H), 1.31 (s, 3H), 1.15 (m, 1H), 1.02 (s, 3H), 0.92 (dd, 3H, J = 3.3, 3.3 Hz), 0.88 (s, 3H), 0.09 (s, 9H). IR (CH_2Cl_2): 3400−2500, 1736, 1704, 1611, 1513, 1462, 1376, 1300, 1247, 1092, 1041, 848 cm^{-1}. FABMS: m/z 545 (M + H), 441, 383, 369, 323, 157, 121. Anal. ($C_{32}H_{50}O_5Si$) C, H.

(1″*S*,1‴*S*,3″*S*,6″*R*)-(2″*E*)-2-[2′-[3″-1‴-Carboxy-4‴-3,4-dichlorophenylbutyl)-6″-methyl-2″-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (29). Yield 90%. ^{1}H NMR ($CDCl_3$): δ 7.23 (dd, 2H, J = 2.0, 20.1 Hz), 6.94 (dd, 1H, J = 2.0, 8.2 Hz), 5.27 (s, 1H), 3.55 (dd, 2H, J = 1.8, 11.5 Hz), 3.36−3.43 (m, 2H), 2.53 (dd, 2H, J = 6.8, 6.8 Hz), 2.07 (br dd, 1H, J = 2.9, 10.5 Hz), 1.70−1.95 (m, 3H), 1.32 (s, 3H), 1.18 (m, 1H), 1.01 (s, 3H), 0.92 (d, 3H, J = 6.8 Hz), 0.87 (s, 3H), 0.09 (s, 9H). IR (CH_2Cl_2): 3440−2600, 1732, 1705, 1599, 1469, 1396, 1373, 1248, 1093, 1032, 848 cm^{-1}. Anal. ($C_{31}H_{48}O_4SiCl_2$) C, H.

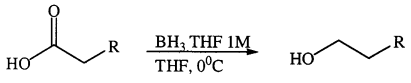
Preparation of 39 from Artemisinin (1). In a flame-dried, 10 mL round-bottom flask equipped with an argon line, **1** (282 mg, 1 mmol, 1 equiv) was placed and was dissolved in THF (2 mL). This solution was added via cannula to a solution of LDA (1.2 equiv, 1.2 mmol) in dry THF (2 mL) at −78 °C. After 30 min, a solution of *S*-phenylbenzenethiosulfonate (325 mg, 1.3 mmol, 1.3 equiv) in THF was added. The reaction mixture was left stirring for another 30 min at a temperature of −78 °C. The reaction was quenched with distilled water (2 mL) and then allowed to warm to room temperature. The reaction mixture was diluted with H_2O (5 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were then washed with brine (1 × 25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude oil was purified via flash column chromatography eluting with 20% ethyl acetate/hexanes. Compound **39** (255 mg) was isolated in 67% yield.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9*β*-(phenylthio)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (39). Yield 67%; mp 171−174 °C. ^{1}H NMR ($CDCl_3$): δ 7.47 (d, 2H J = 8 Hz), 7.38 (d, 1H J = 1.2 Hz), 7.32 (t, 2H, J = 7.28 Hz), 6.02 (s, 1H), 2.42 (d, 1H J = 2.92 Hz), 2.34 (m, 1H), 2.06 (d, 1H J = 3.6 Hz), 2.03 (d, 1H J = 3.8 Hz), 1.92 (d, 1H J = 3.9 Hz), 1.81−1.74 (m, 2H), 1.53 (s, 3H), 1.43 (s, 3H), 1.00 (m, 1H), 0.99 (d, 3H, J = 5.0 Hz). ^{13} CNMR ($CDCl_3$): δ 171.74, 138.55, 130.35, 128.99, 105.44, 94.60, 82.71, 54.28, 51.51, 50.30, 37.67, 36.38, 34.75, 29.67, 26.19, 26.02, 25.05, 20.31. FABMS: m/z 391.2 (M + H). Anal. ($C_{21}H_{26}O_5S$) C, H.

Preparation of Artemisitene (22) from 39. In a flame-dried 10 mL round-bottom flask equipped with an argon line, **39** (30 mg, 0.1 mmol, 1 equiv) was placed and was dissolved

in CH_2Cl_2 (2 mL). This solution was added via cannula to a solution of *m*-CPBA (1.05 equiv, 0.11 mmol) in dry CH_2Cl_2 (2 mL) at −78 °C. After 6 h at −78 °C, the reaction was allowed to warm to room temperature. The reaction mixture was diluted with H_2O (5 mL), washed with a saturated solution of $NaHCO_3$, and extracted with EtOAc (3 × 25 mL). The combined organic layers were then washed with brine (1 × 25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude oil was purified via flash column chromatography eluting with 20% ethyl acetate/hexanes. Artemisitene (**22**) (16.8 mg) was isolated in 78% yield.

General Procedure for Preparation of the Alcohols 43a−48a from 43−48. In a flame-dried three neck 1000 mL round-bottom flask equipped with a reflux condenser and an argon line, the appropriate carboxylic acid (**43**−**48**) (1 equiv) was placed and was dissolved in THF. To this solution, a solution of 1 M BH_3·THF was added via cannula (2 equiv) at 0 °C. The reaction mixture was kept at 0 °C for 4 h and monitored by TLC. The reaction mixture was diluted with cold H_2O, washed with a saturated solution of $NaHCO_3$, and extracted with ether. The combined organic layers were then washed with brine (1 × 25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via flash chromatography on silica gel eluting with 20% ethyl acetate/hexanes to give **43a**−**48a** in 58−88% yield.



43 R= *m*-ClC_6H_4
44 R= *m*-FC_6H_4
45 R= *m*-$CF_3C_6H_4$
46 R= *p*-$CF_3C_6H_4$
47 R= 3,5-di$CF_3C_6H_3$
48 R= 3,5-diFC_6H_3

43a R= *m*-ClC_6H_4
44a R= *m*-FC_6H_4
45a R= *m*-$CF_3C_6H_4$
46a R= *p*-$CF_3C_6H_4$
47a R= 3,5-di$CF_3C_6H_3$
48a R= 3,5-diFC_6H_3

***m*-Chlorophenethyl Alcohol (43a).** ^{1}H NMR ($CDCl_3$): δ 7.21−7.19 (q, 3H), 7.12 (t, 1H), 3.76 (t, 2H), 2.78 (t, 2H). ^{13}CNMR ($CDCl_3$): δ 140.90, 134.14, 129.69, 129.06, 127.02, 126.45, 62.96, 38.69. IR (liquid film): 3464, 2948, 2879, 1598,-1574, 1429, 1096, 1047, 958. FT-ICR MS: *m*/*z* 157 (M + H).

***m*-Fluorophenethyl Alcohol (44a).** ^{1}H NMR ($CDCl_3$): δ 7.19−6.89 (q, 3H), 6.83 (t, 1H), 3.76 (t, 2H), 2.74 (t, 2H). ^{13}CNMR ($CDCl_3$): δ 162.52, 141.90, 130.14, 127.59, 114.70, 112.70, 63.46, 38.29. IR (liquid film): 3343, 2942, 2878, 1510, 1436, 1416, 1098, 1016, 958. FT-ICR MS: *m*/*z* 141 (M + H).

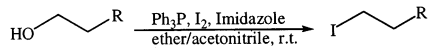
***m*-(Trifluoromethyl)phenethyl Alcohol (45a).** ^{1}H NMR ($CDCl_3$): δ 7.30−7.28 (q, 3H) 7.12 (t, 1H), 3.86 (t, 2H), 2.74 (t, 2H). ^{13}CNMR ($CDCl_3$): δ 139.95, 132.58, 130.59, 129.00, 127.11, 125.75, 123.30, 63.11, 38.90. IR (liquid film): 3323, 2948, 2879, 1598, 1574, 1429, 1080, 1047, 1000, 968. FT-ICR MS: *m*/*z* 191.02 (M + H).

***p*-(Trifluoromethyl)phenethyl Alcohol (46a).** ^{1}H NMR ($CDCl_3$): δ 7.26 (d, 2H J = 8.6 Hz), 7.04 (d, 2H J = 7.8 Hz), 3.91 (t, 2H, J = 7.9 Hz), 2.76 (t, 2H, J = 8.0 Hz). ^{13}CNMR ($CDCl_3$): δ 139.95, 132.58, 130.59, 129.00, 127.11, 125.75, 123.30, 63.11, 38.90. IR (liquid film): 3463, 2948, 2879, 1598, 1574, 1429, 1080, 1047, 1000, 968. FT-ICR MS: *m*/*z* 191.02 (M + H).

3,5-Di(trifluoromethyl)phenethyl Alcohol (47a). ^{1}H NMR ($CDCl_3$): δ 7.38 (s, 1H), 7.23 (s, 2H), 3.82 (t, 2H, J = 8.2 Hz), 2.75 (t, 2H J = 8.7 Hz). ^{13}CNMR ($CDCl_3$): δ 140.90, 131.3, 128.05, 119.70, 65.76, 39.31. IR (liquid film): 3468, 2947, 2878, 1530, 1436, 1416, 1098, 1010, 958. FT-ICR MS: *m*/*z* 257.18 (M + H).

3,5-Difluorophenethyl Alcohol (48a). ^{1}H NMR ($CDCl_3$): δ 6.63 (s, 2H), 6.03 (s, 1H), 3.88 (t, 2H, J = 8.0 Hz), 2.78 (t, 2H J = 8.8 Hz). ^{13}CNMR ($CDCl_3$): δ 163.52, 142.90, 110.70, 99.70, 65.46, 38.71. IR (liquid film): 3453, 2942, 2878, 1510, 1436, 1416, 1098, 1010, 958. FT-ICR MS: *m*/*z* 159.23 (M + H).

General Procedure for the Preparation of the Iodides 43b−48b and 50 from 43a−48a and 49. In a flame-dried 250 mL round-bottom flask equipped with an argon line, the appropriate alcohol (**43a**−**48a** and **49**) (1 equiv) was placed and was dissolved in ether/acetonitrile (3:1). To this solution, triphenylphosphine (3 equiv), imidazole (3 equiv), and iodine (3 equiv) were added in this order. The reaction mixture was kept at room temperature for 1 h and monitored by TLC. The reaction mixture was filtered and washed with ether. The organic layers were then washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via flash chromatography on silica gel eluting with 1% ethyl acetate/hexanes to give **43b**−**48b** and **50** in 58−88% yield.



43a R= *m*-ClC_6H_4
44a R= *m*-FC_6H_4
45a R= *m*-$CF_3C_6H_4$
46a R= *p*-$CF_3C_6H_4$
47a R= 3,5-di$CF_3C_6H_3$
48a R= 3,5-diFC_6H_3
49 R= CF_3

43b R= *m*-ClC_6H_4
44b R= *m*-FC_6H_4
45b R= *m*-$CF_3C_6H_4$
46b R= *p*-$CF_3C_6H_4$
47b R= 3,5-di$CF_3C_6H_3$
48b R= 3,5-diFC_6H_3
50 R= CF_3

***m*-Chlorophenethyl Iodine (43b).** ^{1}H NMR ($CDCl_3$): δ 7.21−7.19 (q, 3H), 7.12 (t, 1H), 3.52 (t, 2H), 3.28 (t, 2H). ^{13}-CNMR ($CDCl_3$): δ 140.90, 134.14, 129.69, 129.06, 127.02, 126.45, 38.69, 11,27. IR (liquid film): 2948, 2879, 1598, 1574, 1429. FT-ICR MS: *m*/*z* 267.52 (M + H).

***m*-Fluorophenethyl Iodine (44b).** ^{1}H NMR ($CDCl_3$): δ 7.19−6.89 (q, 3H), 6.79 (t, 1H), 3.47 (t, 2H), 3.16 (t, 2H). ^{13}CNMR ($CDCl_3$): δ 162.02, 141.90, 130.14, 127.39, 114.70, 112.70, 38.05, 11.29. IR (liquid film): 2942, 2878, 1510, 1436, 1416, 1098. FT-ICR MS: *m*/*z* 251 (M + H).

***m*-(Trifluoromethyl)phenethyl Iodine (45b).** ^{1}H NMR ($CDCl_3$): δ 7.30−7.28 (q, 3H), 7.12 (t, 1H), 3.56 (t, 2H), 3.24 (t, 2H). ^{13}CNMR ($CDCl_3$): δ 139.95, 132.58, 130.59, 129.00, 127.11, 125.75, 123.32, 38.90, 10.89. IR (liquid film): 2948, 2879, 1598, 1574, 1429, 1080, 1047. FT-ICR MS: *m*/*z* 301 (M + H).

***p*-(Trifluoromethyl)phenethyl Iodine (46b).** ^{1}H NMR ($CDCl_3$): δ 7.46 (d, 2H J = 8.6 Hz), 7.14 (d, 2H J = 7.8 Hz), 3.41 (t, 2H, J = 7.7 Hz), 3.22 (t, 2H, J = 8.0 Hz). ^{13}CNMR ($CDCl_3$): δ 139.95, 132.58, 130.59, 129.00, 127.11, 125.75, 123.30, 38.93, 11.02. IR (liquid film): 2948, 2879, 1598, 1574, 1429, 1080, 1047. FT-ICR MS: *m*/*z* 301.02 (M + H).

3,5-Di(trifluoromethyl)phenethyl Iodine (47b). ^{1}H NMR ($CDCl_3$): δ 7.46 (s, 1H), 7.31 (s, 2H), 3.46 (t, 2H, J = 7.2 Hz), 3.12 (t, 2H J = 7.6 Hz). ^{13}CNMR ($CDCl_3$): δ 140.97, 131.37, 128.65, 119.72, 39.31, 11.5. IR (liquid film): 2947, 2878, 1530, 1436, 1416, 1098, 1010, 958. FT-ICR MS: *m*/*z* 368.08 (M + H).

3,5-Difluorophenethyl Iodine (48b). ^{1}H NMR ($CDCl_3$): δ 6.60 (s, 2H), 6.30 (s, 1H), 3.48 (t, 2H, J = 7.8 Hz), 3.16 (t, 2H J = 7.9 Hz). ^{13}CNMR ($CDCl_3$): δ 166.62, 143.40, 110.50, 99.70, 39.71, 12.5. IR (liquid film): 2942, 2878, 1510, 1436, 1416, 1098. FT-ICR MS: *m*/*z* 269.06 (M + H).

General Procedure for the Radical-Induced Michael Addition Approach. Preparation of 9β-Artemisinin Analogues 41c−41h from Artemisitene (22). To a solution of artemisitene (**22**) (1 mmol) in dry benzene (40 mL) was added the iodo derivative **43b**−**48b** (1.5 mmol) and AIBN (0.1 mmol) at 25 °C. The reaction mixture was heated to reflux temperature, and tributyltin hydride (1.4 mmol) was added as a benzene solution (25 mL) over a period of 8 h via syringe pump. After the mixture was completely added, the reaction mixture was cooled to room temperature. The solvent was evaporated to dryness, and diethyl ether (15 mL) followed by a saturated solution of potassium fluoride (4 mL) was added. The solution was stirred at 25 °C for 12 h and was then filtered, washed with water, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel using a mixture of 15% ethyl acetate/hexanes as eluent to give a mixture of β-isomer **41c**−**41h** in 35−40% yield and α-isomer in 60−65% yield.

Conjugate Addition of 1,1,1-Trifluoropropanylmagnesium Bromide to Artemisitene (22). A solution of arte-

misitene (1 equiv) and CuI (catalytic) in THF at −10 °C under argon was treated dropwise with the appropriate Grignard reagents (1.10 equiv). The mixture was at −10 °C for a further 1 h after which saturated aqueous NH_4Cl solution was added and the mixture was allowed to warm to room temperature. After the organic layer was separated, the aqueous solution was extracted with Et_2O. The combined organic layer was washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was then purified by flash chromatography on silica gel using a mixture of 15% ethyl acetate/hexanes as eluent to afford a mixture of β isomer **41b** in 38% yield and α isomer in 58% yield.

General Procedure for the Conversion of α Epimers to the 9β-Artemisinin Analogues 30−35 and 45−52 using DBU. To a solution of 9α-artemisinin analogues (1 equiv) in dry THF was added DBU (2 equiv) at 25 °C. The reaction mixture was refluxed for 24 h. After it was cooled to room temperature, water was added, and the organic material was extracted with ethyl acetate. The solution was dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by silica gel column chromatography using 15% ethyl acetate/hexanes as eluent to give the β isomer **7**−**20** in 50−70% yield.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(butyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (30). Yield 27%; mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ 5.86 (s, 1H), 3.20 (ddd, 1H, *J* = 5.7, 5.7, 8.2 Hz), 2.43 (ddd, 1H, *J* = 3.9, 13.0 14.4 Hz), 2.04 (m, 3H), 1.80 (m, 3H), 1.20−1.60 (m, 8H), 1.46 (s, 3H), 1.09 (m, 2H), 1.01 (d, 3H, *J* = 6.0 Hz), 0.92 (dd, 3H, *J* = 7.1, 7.1 Hz). IR (CH_2Cl_2): 3054, 2958, 1736, 1421, 1274, 1254, 1114, 1001, 895, 740 cm^{-1}. DCIMS−NH_3: *m*/*z* 342 (M + NH_4), 325 (M + H), 307, 289, 274, 261, 251.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-phenylpropyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (31). Yield 28%; mp 137−138 °C. ^{1}H NMR ($CDCl_3$): δ 7.10−7.35 (m, 5H), 5.85 (s, 1H), 3.25 (ddd, 1H, *J* = 5.8, 5.8, 8.8 Hz), 2.71 (ddd, 1H, *J* = 5.6, 8.4, 13.8 Hz), 2.61 (ddd, 1H, *J* = 7.2, 8.4, 13.8 Hz), 2.44 (ddd, 1H, *J* = 4.4, 13.5, 5.1 Hz), 1.50−2.20 (m, 10H), 1.45 (s, 3H), 1.20−1.50 (m, 4H), 1.05 (m, 1H), 0.99 (d, 3H, *J* = 5.9 Hz). IR (CH_2Cl_2): 1740, 1200, 1040, 1010, 890, 840 cm^{-1}. DCIMS−NH_3: *m*/*z* 404 (M + NH_4), 387 (M + H), 369, 351, 341, 323, 313.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-chlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (32). Yield 39%; mp 154−155 °C. ^{1}H NMR ($CDCl_3$): δ 7.23 (d, 2H, *J* = 8.2 Hz), 7.09 (d, 2H, *J* = 8.3 Hz), 5.83 (s, 1H), 3.14−3.24 (m, 1H), 2.52−2.72 (m, 2H), 2.34−2.48 (m, 1H), 1.96−2.09 (m, 3H), 1.67−1.82 (m, 4H), 1.44 (s, 3H), 0.98 (d, 3H, *J* = 5.7 Hz). IR (KBr): 2951, 2925, 1740, 1491, 1112, 1031, 1000 cm^{-1}. CIMS: *m*/*z* 459 (M + K^+), 443 (M + Na^+), 409, 347. Anal. ($C_{23}H_{29}O_5Cl$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-fluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (33). Yield 25%; mp 152−153 °C. ^{1}H NMR ($CDCl_3$): δ 7.15−7.10 (m, 2H), 6.99−6.93 (m, 2H), 5.83 (s, 1H), 3.22 (ddd, 1H, *J* = 5.5, 2.7, 7.0 Hz), 2.65 (ddd, 1H, *J* = 6.2, 8.0, 10.4 Hz), 2.58 (ddd, 1H, *J* = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, *J* = 4.4, 13.0, 15.1 Hz), 1.60−2.19 (m, 9H), 1.44 (s, 3H), 1.25−1.50 (m, 4H), 1.05 (m, 1H), 0.98 (d, 3H, *J* = 5.8 Hz). IR (KBr): 1736, 1382, 1261, 1184, 1116, 881, 850, 831, 798 cm^{-1}. LRMS *m*/*z*: 422 (M + NH_4), 405 (M + H). Anal. ($C_{23}H_{29}O_5F$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-methoxyphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (34). Yield 34%; mp 161−162 °C. ^{1}H NMR ($CDCl_3$): δ 7.08 (d, 2H, *J* = 8.6 Hz), 6.82 (d, 2H, *J* = 8.7 Hz), 5.84 (s, 1H), 3.79 (s, 3H), 3.19−3.25 (m, 1H), 2.51−2.75 (m, 2H), 2.34−2.45 (m, 1H), 2.01−2.09 (m, 3H), 1.67−1.82 (m, 4H), 1.44 (s, 3H), 0.99 (d, 3H, *J* = 5.8 Hz). IR (KBr): 2958, 2920, 2859, 1741, 1511, 1247, 1114, 1034, 997, 810 cm^{-1}. FABMS: *m*/*z* 417 (M + H), 399, 371, 343, 201, 147, 121. Anal. ($C_{24}H_{32}O_6$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,4-dichlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (35). Yield 31%; mp 135−136 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 1H, *J* = 8.2 Hz), 7.26 (s, 1H), 7.01 (dd, 1H, *J* = 2.0, 8.2 Hz), 5.27 (s, 1H), 3.18−3.24 (m, 1H), 2.52−2.72 (m, 2H), 2.34−2.48 (m, 1H), 1.96−2.09 (m, 3H), 1.67−1.82 (m, 4H), 1.44 (s, 3H), 0.99 (d, 3H, *J* = 5.8 Hz). IR (KBr): 2993, 2925, 2868, 1738, 1474, 1394, 1184, 1115, 1032, 993, 885, 815 cm^{-1}. Anal. ($C_{23}H_{28}O_5Cl_2$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(trifluoromethyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41b). Yield 58%; mp 93−94 °C. ^{1}H NMR ($CDCl_3$): δ 5.91 (s, 1H), 2.23 (dd, 2H *J* = 1.5 Hz), 2.11−2.03 (m, 6H), 1.74−1.66 (m, 6H), 1.47 (s, 3H), 1.01 (d, 3H, *J* = 5.0 Hz). ^{13}CNMR ($CDCl_3$): δ 171.73, 105.76, 94.21, 80.58, 50.87, 45.22, 43.59, 37.93, 36.30, 34.43, 33.74, 31.96, 25.84, 25.09, 20.52, 20.26. IR (KBr): 2930, 1740 cm^{-1}. FABMS: *m*/*z* 379.45 (M + H). Anal. ($C_{18}H_{25}O_5F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-chlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41c). Yield 62%; mp 116−117 °C. ^{1}H NMR ($CDCl_3$): δ 7.23 (d, 2H, *J* = 8.2 Hz), 7.09 (d, 2H, *J* = 8.3 Hz), 5.83 (s, 1H), 3.14−3.24 (m, 1H), 2.52−2.72 (m, 2H), 2.34−2.48 (m, 1H), 1.96−2.09 (m, 3H), 1.67−1.82 (m, 4H), 1.44 (s, 3H), 0.98 (d, 3H, *J* = 5.7 Hz). ^{13}CNMR ($CDCl_3$): δ 171.76, 146.47, 129.13, 127.09, 125.71, 122.45, 106.42, 93.92, 79.60, 50.81, 43.50, 38.09, 37.91, 36.30, 35.89, 33.75, 29.17, 26.87, 25.57, 25.44, 25.04. IR (KBr): 2951, 2925, 1740, 1491, 1112, 1031, 1000 cm^{-1}. FABMS: *m*/*z* 427(M + Li). Anal. ($C_{23}H_{29}O_5Cl$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-fluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41d). Yield 52%; mp 152−153 °C. ^{1}H NMR ($CDCl_3$): δ 7.15−6.93 (m, 4H), 5,83 (s, 1H), 3.22 (ddd, 1H, *J* = 5.5, 2.7, 7.0 Hz), 2.65 (ddd, 1H, *J* = 6.2, 8.0, 10.4 Hz), 2.58 (ddd, 1H, *J* = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, *J* = 4.4, 13.0, 15.1 Hz), 1.60−2.19 (m, 9H), 1.44 (s, 3H), 1.25−1.50 (m, 4H), 1.05 (m, 1H), 0.98 (d, 3H, *J* = 5.8 Hz). ^{13}CNMR ($CDCl_3$): δ 172.84, 143.47, 133.08, 128.6, 127.09, 125.71, 122.45, 106.42, 93.92, 79.60, 50.81, 43.50, 38.09, 37.91, 36.30, 35.89, 33.75, 29.17, 26.87, 25.57, 25.44, 23.84, 19.92. IR (KBr): 1736, 1382, 1261, 1184, 1116, 881, 850, 831, 798 cm^{-1}. FABMS *m*/*z*: 411 (M + Li). Anal. ($C_{23}H_{29}O_5F$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-trifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41e). Yield 62%; mp 112−114 °C. ^{1}H NMR ($CDCl_3$): δ 7.51 (d, 2H), 7.27 (d, 2H), 5.83 (s, 1H), 3.21 (ddd, 1H, *J* = 5.3, 2.5, 7.0 Hz), 2.65 (ddd, 1H, *J* = 6.1, 8.3, 10.2 Hz), 2.61 (ddd, 1H, *J* = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, *J* = 4.4, 13.0, 15.1 Hz), 1.60−2.21 (m, 9H), 1.44 (s, 3H), 1.25−1.40 (m, 4H), 1.05 (m, 1H), 0.96 (d, 3H, *J* = 5.6 Hz). ^{13}CNMR ($CDCl_3$): δ 171.79, 147.47, 131.56, 128.6, 127.09, 125.71, 122.45, 119.6, 104.42, 93.92, 79.60, 50.81, 43.50, 38.09, 37.91, 36.30, 35.93, 33.75, 29.17, 26.77, 25.57, 25.28, 23.81, 20.20. IR (KBr): 1734, 1383, 1261, 1187, 1116, 881, 856, 831, 796 cm^{-1}. FABMS *m*/*z*: 461 (M + Li). Anal. ($C_{24}H_{29}O_5F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-trifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41f). Yield 67%; mp 153−154 °C. ^{1}H NMR ($CDCl_3$): δ 7.52 (d, 2H), 7.29 (d, 2H), 5,83 (s, 1H), 3.22 (ddd, 1H, *J* = 5.5, 2.7, 7.0 Hz), 2.65 (ddd, 1H, *J* = 6.2, 8.0, 10.4 Hz), 2.58 (ddd, 1H, *J* = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, *J* = 4.4, 13.0, 15.1 Hz), 1.60−2.19 (m, 9H), 1.44 (s, 3H), 1.25−1.50 (m, 4H), 1.05 (m, 1H), 0.98 (d, 3H, *J* = 5.8 Hz). ^{13}CNMR ($CDCl_3$): δ 171.76, 146.47, 129.09, 125.69, 125.65, 105.81, 93.88, 79.59, 50.48, 43.50, 38.09, 37.91, 36.30, 35.93, 33.95, 29.07, 26.76, 25.55, 25.24, 23.71, 20.18. IR (KBr): 1736, 1382, 1261, 1184, 1120, 886, 850, 831, 798 cm^{-1}. FABMS *m*/*z*: 461 (M + Li). Anal. ($C_{24}H_{29}O_5F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,5-bistrifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41g). Yield 58%; mp 109−110 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (d, 1H), 7.31 (d, 2H), 5.83 (s, 1H), 3.22 (ddd, 1H, *J* = 5.5, 2.7, 7.0 Hz), 2.65 (ddd, 1H, *J* = 6.2, 8.0, 10.4 Hz), 2.58 (ddd, 1H, *J* = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, *J* = 4.4, 13.0, 15.1 Hz), 1.60−2.19 (m, 9H), 1.44 (s, 3H),

1.25−1.50 (m, 4H), 1.05 (m, 1H), 0.98 (d, 3H, J = 5.8 Hz). ^{13}CNMR ($CDCl_3$): δ 176.00, 140.07, 131.00, 112.45, 105.14, 92.46, 78.89, 50.93, 44.89, 39.62, 39.45, 35.86, 31.17, 29.24, 28.18, 27.97, 26.53, 23.97, 21.12. IR (KBr): 1740, 1382, 1261, 1184, 1116, 881, 850, 831, 798 cm^{-1}. FT-ICR MS *m/z*: 529.5 (M + H). Anal. ($C_{23}H_{28}O_5F_2$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,5-difluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin-10(3H)-one (41h). Yield 52%; mp 100−104 °C. ^{1}H NMR ($CDCl_3$): δ 7.15−6.93 (m, 3H), 5,83 (s, 1H), 3.22 (ddd, 1H, J = 5.5, 2.7, 7.0 Hz), 2.65 (ddd, 1H, J = 6.2, 8.0, 10.4 Hz), 2.58 (ddd, 1H, J = 6.2, 7.6, 8.4 Hz), 2.42 (ddd, 1H, J = 4.4, 13.0, 15.1 Hz), 1.60−2.19 (m, 9H), 1.44 (s, 3H), 1.25−1.50 (m, 4H), 1.05 (m, 1H), 0.98 (d, 3H, J = 5.8 Hz). ^{13}CNMR ($CDCl_3$): δ 173.90, 150.07, 142.00, 112.45, 105.14, 100.02, 90.46, 79.60, 50.93, 44.60, 39.62, 39.47, 35.86, 31.17, 29.24, 28.18, 27.91, 26.53, 23.97, 20.13. IR (KBr): 1736, 1382, 1261, 1184, 1116, 881, 850, 831, 798 cm^{-1}. FT-ICR MS *m/z*: 429 (M + H). Anal. ($C_{23}H_{28}O_5F_2$) C, H.

General Procedure for Reduction of the Lactone 41b−41h Derivative to Lactol 51b−h using DIBAL. Preparation of the 10-Dihydro 9β-Artemisinin Analogues. To a stirred solution of **41b−h** (1.0 equiv) in dry CH_2Cl_2 at −78 °C was added 1 M DIBAL in CH_2Cl_2 (1.1 equiv). After 1 h, the reaction was quenched with saturated $NaHCO_3$, diluted with CH_2Cl_2, and allowed to warm to room temperature. The mixture was diluted with CH_2Cl_2 and washed with 10% HCl/saturated NH_4Cl (1:15 v/v). The CH_2Cl_2 layer was then dried over $MgSO_4$, filtered, and concentrated in vacuo to give **51b−h** as a white solid in 89−96% yield.

General Procedure for Reduction of the Lactol 51b−h Derivative to the Pyran 13−19 using Et_3SiH/BF_3OEt_2. Preparation of 10-Deoxo 9β-Artemisinin Analogues. To a stirred solution of **51b−h** (1 equiv) in dry CH_2Cl_2 at −78 °C, Et_3SiH (4 equiv) was added. The reaction was stirred for 10 min, and $BF_3{\cdot}OEt_2$ (1.5 equiv) was added. The resultant solution was allowed to stir for 3 h at −78 °C. After 5 h, the reaction was quenched at −78 °C with pyridine (8 equiv) and was allowed to warm to room temperature. The reaction mixture was poured into aqueous saturated NH_4Cl and extracted with EtOAc. The combined organic layers were washed with NH_4Cl, dried ($MgSO_4$), and concentrated in vacuo to give a white solid. The crude products were purified by flash chromatography on silica gel (80:20 EtOAc/hexanes) to give **13−19** as a pure white crystalline compound in 55−85% yield.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(butyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (7). Yield 80%; mp 91−92 °C. ^{1}H NMR ($CDCl_3$): δ 5.85 (s, 1H), 3.80 (ddd, 1H, J = 1.2, 4.1, 11.5 Hz), 3.46 (dd, 1H, J = 11.8, 11.8 Hz), 2.40−2.50 (m, 1H), 2.40 (ddd, 1H, J = 4.0, 13.5, 14.6 Hz), 2.03 (ddd, 1H, J = 3.1, 4.9, 14.4 Hz), 1.85−19.4 (m, 1H), 1.71 (dq, 1H, J = 3.3 12.9 Hz), 1.50−1.62 (m, 4H), 1.45 (s, 3H), 1.21−1.38 (m, 5H), 0.98 (d, 3H, J = 6.2 Hz), 0.90 (dd, 3H, J = 7.0, 7.0 Hz). IR (CH_2Cl_2): 2945, 2925, 2858, 1461, 1373, 1098, 1064, 877 cm^{-1}. CIMS−NH_3: *m/z* 328 (M + NH_4), 311 (M + H), 293, 275, 265, 207. Anal. ($C_{18}H_{30}O_4$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(trifluoromethyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (9). Yield 85%; mp 125−128 °C. ^{1}H NMR ($CDCl_3$): δ 5.53 (s, 1H), 4.02 (s, 1H), 3.82 (d, 2H, J = 18 Hz), 2.31−2.25 (m, 2H), 2.12−2.03 (m, 5H), 1.93−189 (m, 2H), 1.71−1.69 (m, 2H), 1.62−1.49 (m, 8H), 1.41 (s, 3H), 1.29−125 (m, 2H), 0.95 (d, 3H, J = 5.0 Hz). FT-ICR MS: *m/z* 365 (M + H). Anal. ($C_{18}H_{27}O_4F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-phenylpropyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (10). Yield 92%; mp 80−81 °C. ^{1}H NMR ($CDCl_3$): δ 7.15−7.34 (m, 5H), 5.21 (s, 1H), 3.80 (ddd, 1H, J = 1.1, 4.2, 11.5 Hz), 3.45 (dd, 1H, J = 11.7, 11.7 Hz), 2.57−2.70 (m, 2H), 2.50 (dddd, 1H, J = 4.0, 7.6, 11.6, 11.6 Hz), 2.39 (ddd, 1H, J = 4.0, 13.4, 14.5 Hz), 2.03 (ddd, 1H, J = 3.0, 4.8, 14.7 Hz), 1.89 (dddd, 1H, J = 2.9, 3.8, 6.7, 13.6 Hz), 1.63 (dd, 2H, J = 7.7, 7.7 Hz), 1.44 (s, 3H), 1.27 (dd, 2H, J = 6.4, 11.3 Hz), 0.96 (d, 3H, J = 6.2 Hz). IR: 2927, 2867, 1452, 1097, 1067, 877 cm^{-1}. CIMS−NH_3: *m/z* 390 (M + NH_4), 373 (M + H), 355, 337, 327, 269. Anal. ($C_{23}H_{32}O_4$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-chlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (11). Yield 88%; mp 84−86 °C. ^{1}H NMR ($CDCl_3$): δ 7.12−7.30 (m, 4H), 5.19 (s, 1H), 3.78 (ddd, 1H, J = 1.3, 4.3, 7.2 Hz), 3.44 (dd, 1H, J = 11.7, 11.7 Hz), 2.60 (ddd, 2H, J = 5.1, 7.4, 7.4 Hz), 2.43−2.53 (m, 1H), 2.37 (ddd, 1H, J = 4.1, 13.4, 14.7 Hz), 2.01 (ddd, 1H, J = 3.0, 4.8, 14.7 Hz), 1.83−1.93 (m, 1H), 1.62 (dd, 2H, J = 7.8, 7.8 Hz), 1.42 (s, 3H), 1.25 (dd, 2H, J = 6.5, 11.1 Hz), 0.95 (d, 3H, J = 6.2 Hz). IR (KBr): 2926, 2913, 2866, 1494, 1454, 1091, 1067 cm^{-1}. DCIMS−NH_3: *m/z* 424 (M + NH_4), 407 (M + H). Anal. ($C_{23}H_{31}O_4Cl$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-fluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (12). Yield 85%; mp 76−77 °C. ^{1}H NMR ($CDCl_3$): δ 7.12−7.07 (m, 2H), 6.98−6.92 (m, 2H), 5.19 (s, 1H), 3.78 (dd, 1H, J = 3.2, 10.9 Hz), 3.43 (dd, 1H, J = 11.7, 11.7 Hz), 2.61−2.32 (m, 4H), 2.15−1.81 (m, 2H), 1.71−1.45 (m, 7H), 1.43 (s, 3H), 1.41−1.04 (m, 5H), 0.97 (d, 3H, J = 6.3 Hz). IR (KBr): 1633, 1511, 1454, 1216, 1193, 1159, 1126, 910, 877, 829, 761, 736 cm^{-1}. Anal. ($C_{23}H_{31}O_4F$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-methoxyphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (13). Yield 94%; mp 95−96 °C. ^{1}H NMR ($CDCl_3$): δ 7.07 (d, 2H, J = 8.7 Hz), 6.82 (d, 2H, J = 8.7 Hz), 5.19 (s, 1H), 3.79 (s, 3H), 3.43 (dd, 1H, J = 11.7, 11.7 Hz), 2.60 (ddd, 2H, J = 5.1, 7.4, 7.4 Hz), 2.43−2.53 (m, 1H), 2.37 (ddd, 1H, J = 4.1, 13.4, 14.7 Hz), 2.01 (ddd, 1H, J = 3.0, 4.8, 14.7 Hz), 1.83−1.93 (m, 1H), 1.62 (dd, 2H, J = 7.8, 7.8 Hz), 1.43 (s, 3H), 0.95 (d, 3H, J = 6.2 Hz). IR (KBr): 2958, 2920, 2859, 1612, 1514, 1462, 1377, 1242, 1099, 1090, 1063, 877, 810 cm^{-1}. FABMS: *m/z* 403 (M + H), 217, 201, 185, 121. Anal. ($C_{24}H_{34}O_5$) C, H. HRFAB calcd for $C_{24}H_{34}O_5$ + H, 403.2484; found, 403.2486.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,4-dichlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (14). Yield 93%; mp 128−129 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 1H, J = 8.2 Hz), 7.24 (d, 1H, J = 2.0 Hz), 6.98 (dd, 1H, J = 2.0, 8.2 Hz), 5.20 (s, 1H), 3.78 (dd, 1H, J = 5.1, 13.0 Hz), 3.44 (dd, 1H, J = 11.8, 11.8 Hz), 2.57 (ddd, 2H, J = 5.1, 7.4, 7.4 Hz), 2.41−2.50 (m, 1H), 2.36 (ddd, 1H, J = 4.1, 13.4, 14.7 Hz), 1.83−1.93 (m, 1H), 1.59 (dd, 2H, J = 7.7, 7.7 Hz), 1.41 (s, 3H), 1.25 (dd, 2H, J = 9.8, 9.8 Hz), 0.95 (d, 3H, J = 6.2 Hz). IR (KBr): 2952, 2927, 2864, 1473, 1377, 1192, 1126, 1099, 1061, 935, 875, 831 cm^{-1}. Anal. ($C_{23}H_{30}O_4Cl_2$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-chlorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (15). Yield 85%; mp 92−93 °C. ^{1}H NMR ($CDCl_3$): δ 7.00−7.7.15 (m, 4H), 5.19 (s, 1H), 3.56 (ddd, 1H, J = 1.3, 4.3, 7.2 Hz), 3.44 (dd, 1H, J = 11.7, 11.7 Hz), 2.60 (ddd, 2H, J = 5.1, 7.4, 7.4 Hz), 2.43−2.53 (m, 1H), 2.37 (ddd, 1H, J = 4.1, 13.4, 14.7 Hz), 2.01 (ddd, 1H, J = 3.0, 4.8, 14.7 Hz), 1.83−1.93 (m, 1H), 1.62 (dd, 2H, J = 7.8, 7.8 Hz), 1.42 (s, 3H), 1.25 (dd, 2H, J = 6.5, 11.1 Hz), 0.95 (d, 3H, J = 6.2 Hz). IR (KBr): 2920, 2915, 2860, 1490, 1452, 1090, 1067 cm^{-1}. FABMS: *m/z* 413 (M + Li). Anal. ($C_{23}H_{31}O_4Cl$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-fluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (16). Yield 82%; mp 76−77 °C. ^{1}H NMR ($CDCl_3$): δ 7.12−7−6.92 (m, 4H), 5.19 (s, 1H), 3.78 (dd, 1H, J = 3.2, 10.9 Hz), 3.43 (dd, 1H, J = 11.7, 11.7 Hz), 2.61−2.32 (m, 4H), 2.15−1.81 (m, 2H), 1.71−1.45 (m, 7H), 1.43 (s, 3H), 1.41−1.04 (m, 5H), 0.97 (d, 3H, J = 6.3 Hz). IR (KBr): 1633, 1511, 1454, 1216, 1193, 1159, 1126, 910, 877, 829, 761, 736 cm^{-1}. FABMS *m/z*: 397 (M + Li). Anal. ($C_{23}H_{31}O_4F$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*m*-trifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (17). Yield 80%; mp 93−94 °C. ^{1}H NMR ($CDCl_3$): δ 7.12−7−6.92 (m, 4H), 5.19 (s, 1H), 3.76 (dd, 1H, J = 3.2, 10.9 Hz), 3.47 (dd, 1H, J = 11.6, 11.5 Hz), 2.61−2.36 (m, 4H), 2.13−1.82 (m, 2H), 1.69−1.48 (m, 7H), 1.43 (s, 3H), 1.41−1.04 (m, 5H), 0.97 (d, 3H, J = 6.3 Hz). IR (KBr): 1636, 1516, 1457, 1212, 1197, 1158, 1126, 910, 877, 829, 767, 739 cm^{-1}. FABMS *m/z*: 447 (M + Li). Anal. ($C_{24}H_{31}O_4F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(*p*-trifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (18). Yield 80%; mp 79−80 °C. ^{1}H NMR ($CDCl_3$): δ 7.51 (d, 2H), 7.28 (d, 2H), 5.19 (s, 1H), 3.76 (dd, 1H, J = 3.2, 10.9 Hz), 3.47 (dd, 1H, J = 11.6, 11.5 Hz), 2.61−2.36 (m, 4H), 2.13−1.82 (m, 2H), 1.69−1.48 (m, 7H), 1.43 (s, 3H), 1.41−1.04 (m, 5H), 0.97 (d, 3H, J = 6.3 Hz). IR (KBr): 1636, 1516, 1457, 1212, 1197, 1158, 1126, 910, 877, 829, 767, 739 cm^{-1}. FABMS *m/z*: 447 (M + Li). Anal. ($C_{24}H_{31}O_4F_3$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,5-bistrifluoromethylphenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (19). Yield 76%; mp 109−110 °C. ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 1H), 7.59 (s, 2H), 5.19 (s, 1H), 3.76 (dd, 1H, J = 3.3, 11.52 Hz), 3.44 (dd, 1H, J = 11.67, 11.7 Hz), 2.72 (m, 2H), 2.5 (m, 1H), 2.35 (m, 1H), 2.1 (m, 1H), 1.8 (m, 1H), 1.46−1.7 (m, 6H), 1.42 (s, 3H), 1.0−1.28 (m, 6H), 0.96 (d, 3H, J = 6.09 Hz). FT-ICR MS *m/z*: 509.50 (M + H). Anal. ($C_{25}H_{30}O_4F_6$) C, H.

(+)-Octahydro-3,6α-dimethyl-3,12-epoxy-9β-(3′-(3,5-difluorophenyl)propyl)-12H-pyrano[4,3j]-1,2-benzodioxepin (20). Yield 70%; mp 119−121 °C. ^{1}H NMR ($CDCl_3$): δ 6.6 (m, 3H), 5.18 (s, 1H), 3.77 (dd, 1H, J = 3.66, 3.63 Hz), 3.46 (dd, 1H, J = 11.67, 11.67 Hz), 2.3−2.6 (m, 4H), 2.0 (m, 1H), 1.8 (m, 1H), 1.40−1.75 (m, 6H), 1.41 (s, 3H), 1.0−1.4 (m, 6H), 0.94 (d, 3H, J = 6.03 Hz). FT-ICR MS *m/z*: 409.50 (M + H). Anal. ($C_{23}H_{30}O_4F_2$) C, H.

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