C-16 Artemisinin Derivatives and Their Antimalarial and Cytotoxic Activities: Syntheses of Artemisinin Monomers, Dimers, Trimers, and Tetramers by Nucleophilic Additions to Artemisitene

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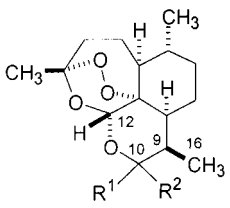
Nucleophilic additions of lithium keto and ester enolates and mono- and bifunctional Grignard reagents to artemisitene provided C-16-derived artemisinin monomers, dimers, trimers, and tetramers whose antimalarial and cytotoxic activities have been evaluated.

Introduction

Malaria, the disease caused by the protozoan parasite *Plasmodium falciparum*, is still one of the major endemic infectious diseases in many developing countries, especially in Asia, Africa, and South America, and kills over a million people each year. The prevalence of a multidrug resistant *P. falciparum* strain against antifolates and standard quinoline antimalarial drugs, together with the decline of support for antimalarial development from pharmaceutical companies, makes it imperative to search for new antimalarial drugs.[1]

The new clinically valuable antimalarial agent, artemisinin (qinghaosu), **1**, isolated from *Artemisia annua* L. (Asteraceae),[2,3] is a sesquiterpene lactone containing a unique endoperoxide bridge responsible for the antimalarial activity. It is generally believed that mechanism of action of artemisinin involves the formation of free radical intermediates resulting from interaction of the endoperoxide moiety with heme.[4−7] However, it is not entirely clear on how the free radicals cause the parasite death.[8,9] The effectiveness of artemisinin, however, is impaired by its low solubility and poor oral bioavailability. Chemical modifications of artemisinin have resulted in new derivatives that combine higher antimalarial efficacy and compounds' solubility. For example, derivatization of artemisinin at C-10 (see numbering in **1**) has yielded compounds **2**−**5**, of which **2**−**4** are now in use clinically. Compounds of this type have been shown to have ∼10-folds increase in their antimalarial activities as compared to the parent compound **1**.[10]

Lately, there were reports describing the synthesis of C-10-derived artemisinin and other trioxane dimers having linkers between the two core molecules. These compounds were much more potent against malaria parasites than artemisinin but also process antitumor activities.[11−13] Therefore, optimizing types and length, the linkage of the trioxane dimers may bring about compounds that are highly active against malaria parasites with low toxicity to the hosts.[14,15]



1 (artemisinin) $R^1 = R^2 = O$
2 (artemether) $R^1 = H$, $R^2 = OMe$
3 (arteether) $R^1 = H$, $R^2 = OEt$
4 (sodium artesunate) $R^1 = H$, $R^2 = OOCCH_2CH_2COONa$
5 (sodium artelinate) $R^1 = H$, $R^2 = OCH_2$-C$_6$H$_4$-COONa

An oxidized form of artemisinin, artemisitene (**6**), was found to be the minor product isolated together with the more abundant **1** from the American variant *A. annua* L.[16] Chemically, **6**, possessing an α-methylene lactone moiety, is most susceptible to nucleophilic attack in a Michael addition fashion to give the corresponding adduct (e.g., **8**). The overall process can be regarded as an easy access to C-16 derivatives of **1**. However, because of the fact that **6** coexists with **1** in the plant in low quantities and also that its isolation and purification from the plant had proved not to be a straightforward process, **6** was, therefore, not utilized as starting material in the preparation of artemisinin derivatives. Semisynthesis of **6** from the naturally abundant **1** would provide the solution to this drawback, and after unsuccessful attempts,[17] **6** was synthesized in 1990.[18] Thereafter, we reported the convenient one-pot synthesis of **6** via a selenoxide elimination route[19a] together with its reactions with nucleophiles in our study on correlation of antimalarial activity of artemisinin derivatives with their binding affinities with ferroprotoporphyrin IX.[19b] Similar methods have also been subsequently published.[20,21]

We now report on our continued effort to prepare C-16 derivatives of **1** from the reactions of **6** with various

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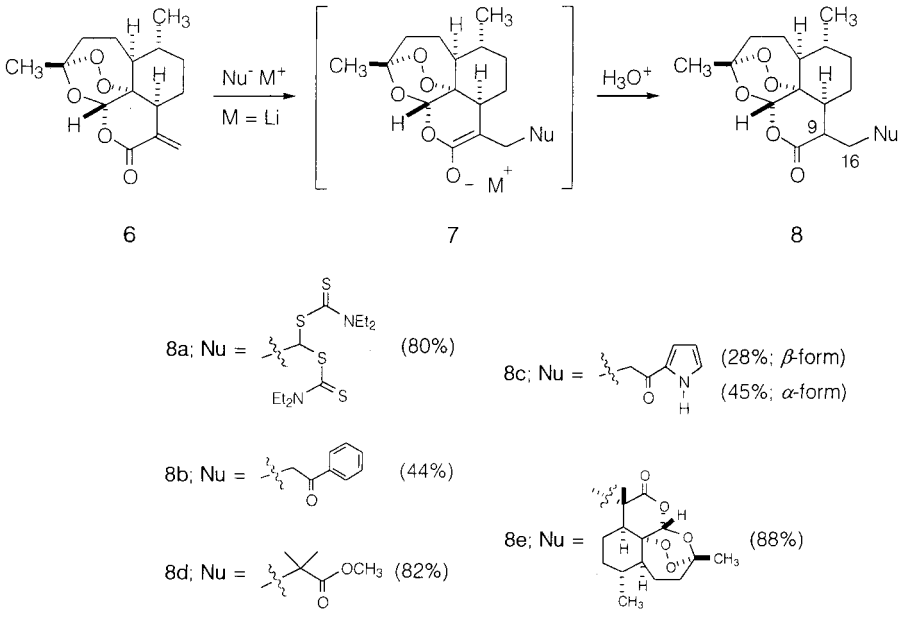
Scheme 1



types of nucleophiles and the in vitro antimalarial and cytotoxicity evaluations of products obtained against *P. falciparum*, human epidermoid carcinoma (KB), human breast cancer (BC), and African green monkey kidney fibroblast (vero cells), respectively.

Results and Discussion

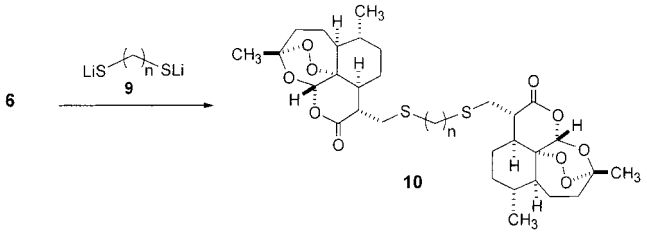
Chemical Syntheses. Three types of nucleophiles have been employed in the 1,4-nucleophilic additions to **6**.

(a) Organolithiums. Artemisitene (**6**), prepared from **1** by the reported selenoxide elimination approach,[19b] readily underwent 1,4-addition reactions with alkyllithium and lithium keto and ester enolates at −78 °C in tetrahydrofuran (THF) to give the corresponding enolate **7**, which, upon protonation during workup, provided the corresponding C-16 derivatives, **8a**−**e**, of artemisinin (Scheme 1).

The above reactions, except for **8c**, gave only thermodynamically less favorable C-9α stereochemical isomers of **8** due to the fact that protonation of the intermediate, lithium enolate (**7**), at low temperature took place on the more favorable β-face of the molecule. In the case of **8c**, the dianion generated from 2-acetylpyrrole with 2 equiv of lithium diisopropylamide (LDA) was insoluble in THF solution at −78 °C, and after the addition of **6**, the reaction turned into a clear yellow solution which, upon protonation with saturated aqueous NH_4Cl solution at low temperature followed by preparative liquid chromatography (PLC) separation (silica gel, EtOAc/hexane = 25/75 as the developing solvent), provided two products, the α- and β-stereoisomers of **8c** in 45 and 28%, respectively.

Because of the fact that nuclear magnetic resonance (NMR) data of many artemisinin derivatives have been well-documented, stereochemical identification of products described was straightforward, where upon the chemical shift of protons at C-9 situated on the α-face of the molecule resonates at about 1 ppm downfield from that of the β-counterpart. Also, a ^{13}C NMR chemical shift of the carbon attached to C-9 on the α-side appears at a much lower field than the opposite.[10] For example, the proton at C-9 and carbon at C-16 absorptions of **1** are observed at δ 3.39 and 12.50 ppm while those of *epi*-artemisinin appear at δ 2.28 and 20.48 ppm, respectively. These differences in chemical shifts have been used in the stereochemical identification of all compounds reported here. For compound **8c**, the α-H (β-isomer) at C-9 resonated at δ 3.31 ppm and that of the opposite isomer, the β-H, appeared at δ 2.30 ppm.

Scheme 2[a]

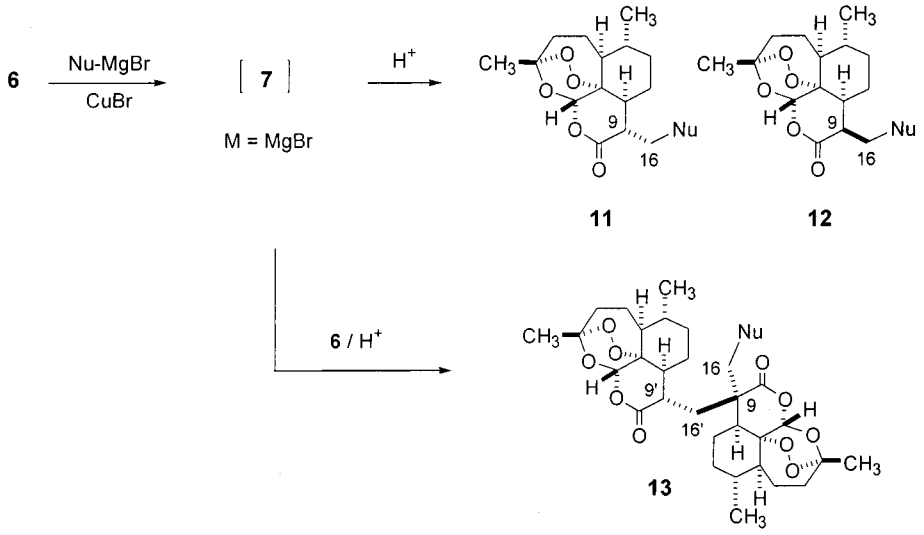


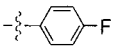
[a] Key: **10a**, *n* = 2 (86%); **10b**, *n* = 3 (91%); **10c**, *n* = 4 (97%); **10d**, *n* = 5 (93%).

Reaction of the anion derived from **1** to **6** was exceptionally clean and only one product, the dimer **8e**, was obtained in near quantitative yield (it was the only product identified by TLC of the crude reaction mixture, 88% after crystallization). The structure of **8e** (*m/e* 563.7 $(M + 1)^+$) has been deduced from its spectral data, and the complete stereostructure was confirmed by X-ray crystallographic analysis. Here, the sole 1,4-addition reaction to **6** surprisingly took place on the apparently more hindered α-side of **7** followed by protonation on the less hindered β-face of the molecule.

Related nucleophilic addition reactions between **6** and dithiolates, **9a**−**d**, derived from the corresponding dithiols, were also investigated. As shown in Scheme 2, dimeric artemisinin analogues **10a**−**d** with a linker, $S-(CH_2)_n-S$, of various length and flexibility were obtained in very good yields when the above reactants were allowed to react in THF at −78 °C. However, compounds **10a**−**d** were not very stable and spontane-

Scheme 3



	11	12	13
a; Nu = CH_2CH_3	61%	18%	9%
b; Nu = $CH_2CH_2CH_3$	41%	9%	13%
c; Nu = $CH_2CH_2CH_2CH_3$	55%	15%	21%
d; Nu = $CH_2CH_2CH_2CH_2CH_3$	47%	15%	9%
e; Nu = $CH_2CH_2CH_2Ph$	35%	8%	35%
f; Nu = 	75%	11%	4%
g; Nu = 	65%	8%	15%

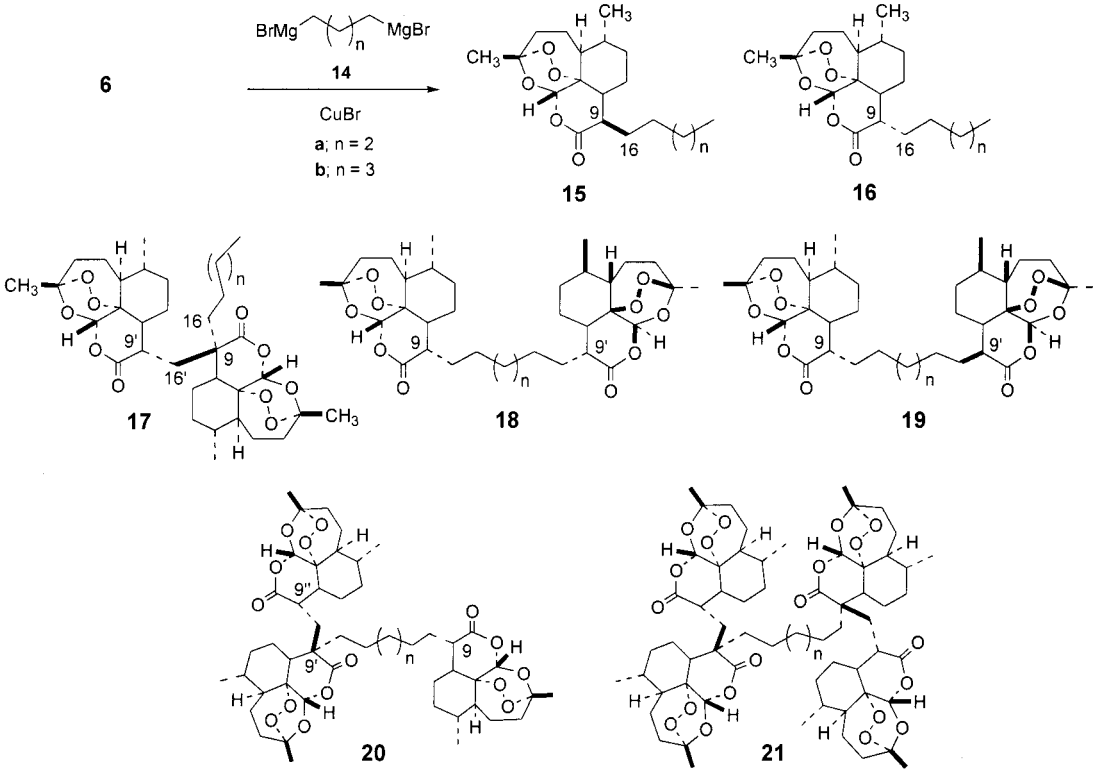
ously decomposed in solutions or upon storage at room temperature giving complex mixtures. Chemical yields of **10a**−**d** shown were calculated from the pure samples obtained directly from PLC purification without crystallization. NMR spectra of the products, dimer **10**, indicated that they are symmetrical molecules. For example, **10a** (*m/e* 677.5 $(M + Na)^+$ for $C_{32}H_{46}O_{10}S_2$) showed only one absorption at δ 2.31 ppm (dd, J = 11.5, 3.5 Hz) for the two identical protons at C-9 and C-9′ and also one singlet at δ 5.96 ppm assignable for equivalent protons at C-12 and C-12′. Chemical shifts of the protons described led to the conclusion for the α-stereochemistry at both C-9 and C-9′ of the dimers **10a**−**d**.

(b) Grignard Reagents. Grignard reagents have been employed as alternative Michael donors in the addition reactions to **6** (Scheme 3). The reactions were conducted in THF solution at −78 °C in the presence of a catalytic amount of Cu(I) salt to afford, upon workup at the same temperature, adduct **11** as a major product. However, in contrast to those of organolithiums, reactions of **6** with Grignard reagents also provided reasonable amounts of the C-9β-stereoisomers (**12**), and the dimer **13** resulted from further reaction of the emerging enolate **7** with another molecule of **6** as shown in Scheme 3. It should be noted that probably due to the difference in nature of the enolates, the stereochemistry at C-9 of dimer **13**, obtained from the attack of **7** to **6**, is opposite to that observed in **8e**.

(c) Bifunctional Grignard Reagents. Bifunctional Grignard reagents are of our interest in the further synthesis of novel artemisinin analogues. The reaction involved Michael addition of 1 equiv of the bis-Grignard reagent, **14**, to the exo double bond of **6** (2 equiv) at −78 °C to yield a mixture of artemisinin derivatives, which after PLC separation (silica gel, EtOAc/hexane = 30/70 as the developing solvent) afforded two isomeric artemisinin adducts (**15** (C-9, β-) and **16** (C-9, α-)), two unsymmetrical and one symmetrical bis adducts (**17**, **18** (C-9, α-; C-9′, β-), and **19** (C-9, α-; C-9′, α-)), respectively, and tris- (**20**) and tetrakis- (**21**) adducts as shown in Scheme 4.

Evidently, the bifunctional Grignard reagent reacted either on one end with the first molecule of **6** (giving **15** and **16** after protonation) followed by trapping the emerging enolate with another molecule of **6** (to give **17**) or at both ends with two molecules of **6** (yielding **18** and **19**) followed by further addition of the bis enolate to one or two molecules of **6** to provide the trimer, **20**, or tetramer, **21**, respectively. Mass, ^{1}H, and ^{13}C NMR spectral data analyses were employed for structural and stereochemical identification of all products obtained above. For example, two types of protons at C-9, the β-form at C-9 (δ 3.20 ppm) and α-form at C-9′ (δ 2.10 ppm) and two singlets at δ 5.86 and 5.92 ppm, assignable for two protons at C-12 and C-12′, were observed for the dimer **18a** (*m/e* 619.3569 $(M + 1)^+$). On the other hand, compound **19a** (*m/e* 619.3563 $(M + 1)^+$) showed identical protons at C-9 and C-9′ (δ 2.05 ppm) and at C-12 and C-12′ (δ 5.92 ppm). The trimer (*m/e* 900.6 $(M + 1)^+$) showed two sets of C-9 and C-9″ β-protons and

Scheme 4



cmpds	15	16	17	18	19	20	21
a; n = 2	3%	14%	7%	5%	4%	4%	7%
b; n = 3	3%	12%	7%	6%	16%	32%	11%

three singlets for three different protons at C-12, C-12′, and C-12″ in the ^{1}H NMR spectrum, while identification of the tetramer (*m/e* 1178.6 (M^+)) was surprisingly straightforward due to its symmetrical structural feature.

Biological Activities. All compounds prepared as described above were subjected to in vitro malaria screening system against *P. falciparum* (K1, multidrug resistant strain), and the assay was performed following the microculture radioisotope technique described by Desjardins[22] using both artemisinin and dihydroartemisinin as standards. Cytotoxicity against KB, BC, and vero cells was tested using the protocol described by Skehan.[23] IC_{50} values of the standard compound, ellipticine, were 2.44 ± 0.41, 2.03 ± 0.82, and 2.84 ± 0.41 μM, respectively, for KB, BC, and vero cells. Results are grouped into four categories, i.e., artemisinin derivatives substituted at C-16 by simple alkyls, aryls, and iron chelator (Table 1); artemisinin dimers linked by alkyl and alkyl disulfide chains (Table 2); other artemisinin dimers (Table 3); and artemisinin trimers and tetramers (Table 4).

It can be seen from Table 1 that activities against malaria parasites of most C-16 substituted artemisinin derivatives are comparable to artemisinin. In the cases where both α-isomers and β-isomers were obtained from 1,4-addition reactions of nucleophiles to **6**, the β-isomers are, in general, more potent against *P. falciparum* than their counterparts, suggesting stereochemistry dependent activity of these derivatives.[10]

Because artemisinin is believed to require iron for its antimalarial action, covalent linkage of artemisinin to iron chelator yielded more potent artemisinin derivatives.[24] The 1,4-addition of the anion derived from methylene-bis(diethyldithiocarbamate), an iron chelator, to **6** provided the artemisinin−iron chelator adduct, **8a**, showing much better antimalarial activity against *P. falciparum* than **1** as predicted with a good therapeutic index[25] of 3500 times of its cytotoxic effect to vero cells. This supports the belief that iron is essential in the mode of action of artemisinin.[4−7]

It has been reported that certain artemisinin dimers linked together at C-10 were much more potent against malaria parasites than artemisinin,[14,15] but as can be seen in Table 2, dimers **10a**−**d**, **18**, and **19** are not. It is important, however, to note that stereochemical orientations at C-9 and C-9′ of the two artemisinin molecules in these dimers play almost insignificant roles with regard to their biological activities, against both *P. falciparum* and KB, BC, and vero cells (Table 2).

Antimalarial activities of artemisinin dimers (Table 3), trimers, and tetramers (Table 4) are quite impressive because they are higher than artemisinin (with the exception of **21b**). Also, their differences in antimalarial activities and toxicities are quite large, except for compounds **13d**−**f**, which show comparatively high potency against vero cells.

Most interesting is compound **8e**, obtained from the addition of artemisinin anion to **6** (Scheme 1), whose activity against *P. falciparum* is 1 order of magnitude

Table 1. Antimalarial Activity and Cytotoxicity of Compounds **8a**−**e**, **11a**−**g**, and **12a**−**g**[a]

cmpds	antimalarial activity EC_{50} (nM) (therapeutic index)[b]	cytoxicity KB IC_{50} (μM)	BC IC_{50} (μM)	vero cells IC_{50} (μM)[c]
8a	1.9[d] (3.5×10^3)	2.5	2.4	6.6
8b	12.8[e] ($>1.0 \times 10^4$)	inactive	inactive	>130
8c (C-9α)	24.8[d] ($>5.2 \times 10^3$)	inactive	inactive	>130
8c (C-9β)	8.8[e] ($>1.5 \times 10^4$)	inactive	inactive	>130
8d	>26.1[d] ($>5.0 \times 10^3$)	inactive	inactive	>130
8e	1.0[d] (8.9×10^4)	23	13	89
11a	11 ($>1.2 \times 10^4$)	inactive	inactive	>130
12a	6.3 ($>2.1 \times 10^4$)	inactive	inactive	>130
11b	13.8 ($>9.4 \times 10^3$)	54	inactive	>130
12b	11.3 ($>1.2 \times 10^4$)	inactive	inactive	>130
11c	11.7 (1.6×10^3)	11	11	19
12c	8.9 ($>1.5 \times 10^4$)	inactive	inactive	>130
11d	13.8 (1.0×10^3)	6.2	6.2	14
12d	12.9 (1.2×10^3)	6.2	5.7	16
11e	10.3 (1.3×10^3)	5.2	5.0	13
12e	8.4 (1.9×10^3)	11	14	16
11f	12.9 (9.3×10^3)	35	41	120
12f	6.7 (1.9×10^4)	inactive	inactive	>130
11g	18.2 (7.1×10^3)	44	41	>130
12g	20.7 (6.3×10^3)	inactive	inactive	>130

[a] Artemisinin, $EC_{50} = 12.1$ nM; dihydroartemisinin, $EC_{50} = 3.0$ nM. [b] Therapeutic index = IC_{50} of vero cells/EC_{50} of artemisinin derivatives. [c] Maximum concentration = 130 μM with ellipticine as positive control. [d] Artemisinin, $EC_{50} = 11.7$ nM; dihydroartemisinin, $EC_{50} = 2.92$ nM. [e] Artemisinin, $EC_{50} = 7.81$ nM; dihydroartemisinin, $EC_{50} = 1.40$ nM.

better than that of the parent compound, **1**, and displays low cytotoxicity. X-ray crystal data of this compound indicated that in crystal form, **8e** arranged itself such that all oxygen atoms were engulfed by the hydrophobic part of its molecule as shown in Figure 1. A plausible explanation can be put forward that the antimalarial activity of dimer **8e** correlates with its lipophilicity due to the compound's better ability to penetrate the membrane. However, this explanation should be treated with caution because the conformation of **8e** in solution is still not known and also there is a report that the increase in lipophilicity may lead to higher cytotoxicity of artemisinin derivatives.[26]

X-ray Crystallographic Study of 8e. Crystal data of **8e**: $C_{30}H_{40}O_{10}$, MW = 560.62, monoclinic, $P2_1$, a = 9.214(1) Å, b = 11.282(1) Å, c = 14.181(1) Å, V = 1424.7(2) Å^3, $F(000) = 600$. With $Z = 2$, the calculated density was 1.307 g cm^{-3}. Data were collected from a 0.30 × 0.30 × 0.50 mm^3 crystal at room temperature on an Enraf-Nonius CAD4 diffractometer with Mo Kα radiation (λ = 0.710 73 Å) in θ−2θ scan mode. A total of 4761 independent reflections was measured (4292 observed, $|F_o| > 4\sigma|F_o|$) between 2 and 25°. There was

Table 2. Antimalarial Activity and Cytotoxicity of Compounds **10a**−**d**, **18a**,**b**, and **19a**,**b**[a]

cmpds	antimalarial activity EC_{50} (nM) (therapeutic index)[b]	cytoxicity KB IC_{50} (μM)	BC IC_{50} (μM)	vero cells IC_{50} (μM)[c]
10a	7.4	*d*	*d*	*d*
10b	13.0	*d*	*d*	*d*
10c	5.7	*d*	*d*	*d*
10d	12.0	*d*	*d*	*d*
18a	10.5 (9.5×10^2)	1.8	1.6	10
19a	1.4 (7.1×10^3)	2.3	1.6	10
18b	0.91[e] (3.1×10^3)	0.76	0.36	2.8
19b	1.1[e] (4.5×10^3)	1.1	1.1	4.9

[a] Artemisinin, $EC_{50} = 11.9$ nM; dihydroartemisinin, $EC_{50} = 2.96$ nM. [b] Therapeutic index = IC_{50} of vero cells/EC_{50} of artemisinin derivatives. [c] Maximum concentration = 130 μM with ellipticine as positive control. [d] Not performed due to compound's instability. [e] Dihydroartemisinin, $EC_{50} = 0.55$ nM.

Table 3. Antimalarial Activity and Cytotoxicity of Compounds **13a**−**g**[a]

cmpds	antimalarial activity EC_{50} (nM) (therapeutic index)[b]	cytoxicity KB IC_{50} (μM)	BC IC_{50} (μM)	vero cells IC_{50} (μM)[c]
13a	4.4 ($>3.0 \times 10^4$)	inactive	inactive	>130
13b	4.3 ($>3.0 \times 10^4$)	inactive	inactive	>130
13c	3.9 (1.6×10^4)	23	36	63
13d	5.0 (5.0×10^2)	4.0	1.3	2.5
13e	3.4[d] (7.1×10^2)	5.7	1.2	2.4
13f	2.3[e] (4.8×10^2)	7.7	7.4	1.1
13g	2.1[e] (2.6×10^3)	4.0	3.5	5.5

[a] Artemisinin, $EC_{50} = 12.1$ nM; dihydroartemisinin, $EC_{50} = 3.0$ nM. [b] Therapeutic index = IC_{50} of vero cells/EC_{50} of artemisinin derivatives. [c] Maximum concentration = 130 μM with ellipticine as positive control. [d] Artemisinin, $EC_{50} = 7.81$ nM; dihydroartemisinin, $EC_{50} = 1.40$ nM. [e] Dihydroartemisinin, $EC_{50} = 0.81$ nM.

Table 4. Antimalarial Activity and Cytotoxicity of Compounds **20a**,**b** and **21a**,**b**[a]

cmpds	antimalarial activity EC_{50} (nM) (therapeutic index)[b]	cytoxicity KB IC_{50} (μM)	BC IC_{50} (μM)	vero cells IC_{50} (μM)[c]
20a	2.4 (2.8×10^3)	1.7	1.2	6.6
20b	3.1 (3.2×10^3)	2.8	2.0	10
21a	5.8 (1.6×10^3)	2.0	2.8	9.2
21b	>20 ($>2.1 \times 10^3$)	5.4	9.6	>42

[a] Artemisinin, $EC_{50} = 12.1$ nM; dihydroartemisinin, $EC_{50} = 3.0$ nM. [b] Therapeutic index = IC_{50} of vero cells/EC_{50} of artemisinin derivatives. [c] Maximum concentration = 130 μM with ellipticine as positive control.

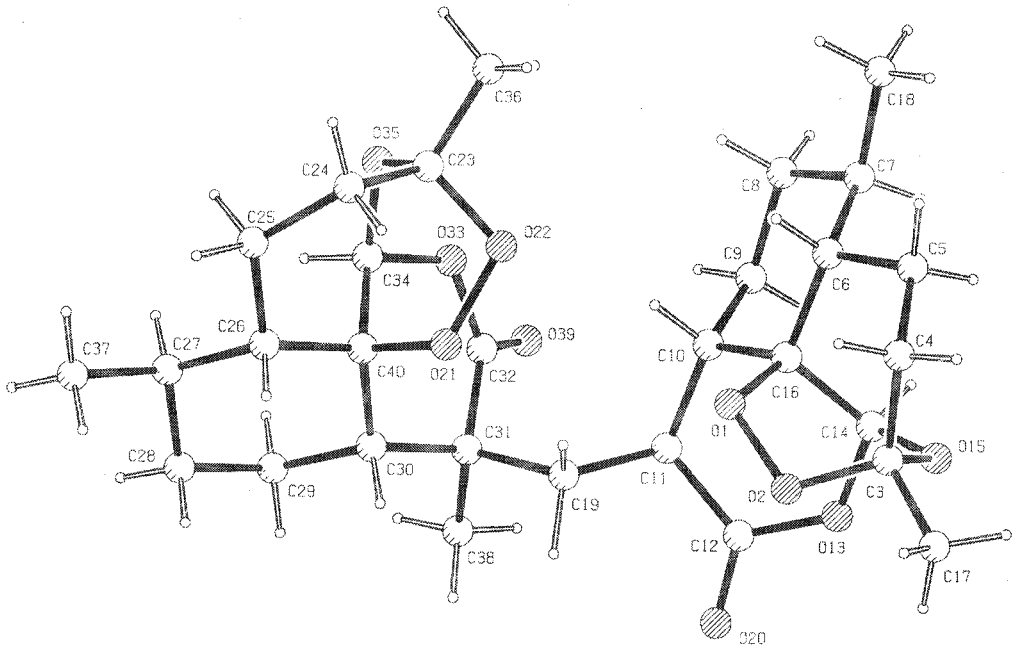


Figure 1. Computer-generated perspective drawing of **8e**.

no crystal decay during data collection, and no correction was made for absorption. The crystal structure was solved by direct methods using SHELXS-97, and then, all atoms except hydrogen atoms were refined anisotropically on F^2 using SHELXL-97 to give a final R-factor of 0.0449 (wR = 0.1253) with a goodness of fit = 0.909 and a data-to-parameter ratio of 13.3:1. Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, England (CCDC161500).

Conclusion

Nucleophilic additions of alkyllithium, lithium keto and ester enolates, Grignard, and bifunctional Grignard reagents to **6** have led to various C-16-derived artemisinin derivatives, which include artemisinin monomers, dimers, trimers, and tetramers. Most of these compounds showed antimalarial activity better than or comparable to that of artemisinin with no or low cytotoxicity against two tumor cell lines, KB, BC, and vero cells. Although some of these compounds are toxic to vero and also cancer cells at micromolar ranges, the cytotoxicities are generally over 500 times higher than their corresponding antimalarial activities (EC_{50} values). This indicates that these artemisinin derivatives are highly selective to malaria parasites with a large safety window over their cytotoxic effects.

Experimental Section

Chemistry. Melting points were determined by an Electrothermal melting point apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 and Bruker DRX 400. ESITOF mass spectra were obtained from a Micromass LCT mass spectrometer, and lock mass calibration was applied for the determination of the accurate mass. IR spectra were recorded on an FT-IR system 2000 (Perkin-Elmer) spectrometer. Elemental analyses were carried out by using a Perkin-Elmer elemental analyzer PE2400 series II and a Perkin-Elmer elemental analyzer 2400 CHN. Merck silica gel 60 PF_{254} was used for PLC. Solvents were distilled before used. Dried, oxygen free THF (freshly distilled from sodium/benzophenone) was used in all experiments. Reactions were carried out under nitrogen atmosphere.

Typical Procedure. Compound 8a. To a solution of LDA (0.70 mmol) in THF (1 mL) at −78 °C was slowly added a solution of methylene-bis(diethyldithiocarbamate) (183 mg, 0.59 mmol) in THF (2 mL), and the reaction mixture was left stirring at −78 °C for 45 min. A solution of **6** (150 mg, 0.54 mmol) in THF (2 mL) was added, and the reaction mixture was left stirring at −78 °C for 5 h. Saturated aqueous NH_4Cl solution workup at −78 °C followed by extraction with CH_2Cl_2, drying ($MgSO_4$), and evaporation gave the crude product, which was purified by PLC (silica gel, 25% EtOAc/hexane as eluent) to afford a single isomer (C-9β), **8a**, as yellow solid, 228.5 mg (80%), mp 133−135 °C. MS (LC/(+)ESI-MS) *m/e*: 591.9 $(M + 1)^+$. Anal. ($C_{26}H_{42}N_2O_5S_4$) C, H, N.

Compound 8b. Yield, 44%; white crystals (CH_2Cl_2/hexane); mp 119−120.5 °C. ESITOF exact mass *m/e*: 401.1945 $(M + 1)^+$. Anal. ($C_{23}H_{28}O_6$) C, H.

Compound 8c. The crude product was purified by PLC (silica gel, 25% EtOAc/hexane as eluent) to obtain two separable (β, α) C-9 stereoisomers in ratio of 1.0:1.6: β-isomer, 55.2 mg (28%), and α-isomer, 88.4 mg (45%), respectively.

Compound 8c (C-9β). White crystals (EtOAc/hexane); mp 65−67 °C (dec). ESITOF exact mass *m/e*: 309.1913 $(M + 1)^+$. Anal. ($C_{21}H_{27}NO_6$) C, H, N.

Compound 8c (C-9α). White crystals (EtOAc/hexane); mp 109.8 °C (dec). ESITOF exact mass *m/e*: 390.1614 $(M + 1)^+$. Anal. ($C_{21}H_{27}NO_6$) C, H, N.

Compound 8d. Yield, 82%; white crystals (CH_2Cl_2/hexane). mp 103−105 °C. MS (LC/(+)ESI-MS) *m/e*: 383.5 $(M + 1)^+$. Anal. ($C_{20}H_{30}O_7$) C, H.

Compound 8e. Yield, 88%; white crystals (CH_2Cl_2/hexane). mp 188−190 °C. MS (LC/(+)ESI-MS *m/e*: 563.7 $(M + 1)^+$. Anal. ($C_{30}H_{42}O_{10}$) C, H.

Compound 10a. Yield, 86%; white semisolid. MS (LC/(+)ESI-MS for $C_{32}H_{46}O_{10}S_2$ *m/e*: 677.5 $(M + Na)^+$.

Compound 10b. Yield, 91%; white semisolid. MS (LC/(+)ESI-MS) for $C_{33}H_{48}O_{10}S_2$ *m/e*: 691.8 $(M + Na)^+$.

Compound 10c. Yield, 97%; white semisolid. MS (LC/(+)ESI-MS) for $C_{34}H_{50}O_{10}S_2$ *m/e*: 706.0 $(M + Na)^+$.

Compound 10d. Yield, 93%; white semisolid. MS (LC/(+)ESI-MS) for $C_{35}H_{52}O_{10}S_2$ *m/e*: 720.4 $(M + Na)^+$.

General Procedure for Synthesis of Artemisinin Adducts from the Reaction of Grignard Regent with Artemisitene. Grignard reagent[27] was prepared in the usual manner from magnesium turnings (3 equiv) and bromo

compound (1.5 equiv) in THF (0.7 M) at such a rate as to maintain gentle reflux and stirred at room temperature for 2 h. The solution of Grignard reagent was added to a suspension of Cu(I)Br in THF (1 mL), and the reaction mixture was left stirring at −40 °C for 20 min. Then, a solution of **6** (1 equiv) in THF (1 mL/50 mg) was added and the reaction mixture was again left stirring at −78 °C for 2 h. Saturated aqueous NH_4Cl solution workup followed by extraction with CH_2Cl_2, drying ($MgSO_4$), and evaporation gave crude product. The crude product was purified by PLC (silica gel, 25% EtOAc/hexane as the eluent) to obtain two separable (α and *β*) C-9 stereoisomers (**11** and **12**) and a dimeric isomer (**13**), respectively.

Compound 11a. Yield, 61%; white crystals (CH_2Cl_2/hexane); mp 126−127 °C. ESITOF exact mass *m/e*: 311.1869 (M + 1)$^+$. Anal. ($C_{17}H_{26}O_5$) C, H.

Compound 12a. Yield, 18%; white crystals (CH_2Cl_2/hexane); mp 146−147 °C (lit[10] mp 149−150 °C). MS (LC/(+)ESI-MS) *m/e*: 311.4 (M + 1)$^+$. Anal. ($C_{17}H_{26}O_5$) C, H.

Compound 13a. Yield, 9%; white crystals (CH_2Cl_2/hexane); mp 178−180 °C. MS (LC/(+)ESI-MS) *m/e*: 591.7 (M + 1)$^+$. Anal. ($C_{32}H_{46}O_{10}$) C, H.

Compound 11b. Yield, 41%; white crystals (CH_2Cl_2/hexane); mp 114.7−116.5 °C. MS (LC/(+)ESI-MS) *m/e*: 325.4 (M + 1)$^+$. Anal. ($C_{18}H_{28}O_5$) C, H.

Compound 12b. Yield, 9%; white crystals (CH_2Cl_2/hexane); mp 130−132 °C (lit[10] mp 129−130 °C). MS (LC/(+)ESI-MS) *m/e*: 325.4 (M + 1)$^+$. Anal. ($C_{18}H_{28}O_5$) C, H.

Compound 13b. Yield, 13%; white crystals (CH_2Cl_2/hexane); mp 199−201 °C. MS (LC/(+)ESI-MS) *m/e*: 605.7 (M + 1)$^+$. Anal. ($C_{33}H_{48}O_{10}$) C, H.

Compound 11c. Yield, 55%; white crystals (CH_2Cl_2/hexane); mp 96.4−97 °C. MS (LC/(+)ESI-MS) *m/e*: 339.4 (M + 1)$^+$. Anal. ($C_{19}H_{30}O_5$) C, H.

Compound 12c. Yield, 15%; white crystals (CH_2Cl_2/hexane); mp 122−124 °C (lit[10] mp 122.5−123.5 °C). ESITOF exact mass *m/e*: 339.2170 (M + 1)$^+$. Anal. ($C_{19}H_{30}O_5$) C, H.

Compound 13c. Yield, 21%; white crystals (CH_2Cl_2/hexane); mp 190−192 °C. MS (LC/(+)ESI-MS) *m/e*: 619.7 (M + 1)$^+$. Anal. ($C_{34}H_{50}O_{10}$) C, H.

Compound 11d. Yield, 47%; colorless oil. ESITOF exact mass *m/e*: 353.2311 (M + 1)$^+$. Anal. ($C_{20}H_{32}O_5$) C, H.

Compound 12d. Yield, 15%; white crystals (CH_2Cl_2/hexane); mp 52−54 °C (lit[10] mp 80.5−82 °C). MS (LC/(+)ESI-MS *m/e*: 353.5 (M + 1)$^+$. Anal. ($C_{20}H_{32}O_5$) C, H.

Compound 13d. Yield, 9%; white crystals (CH_2Cl_2/hexane); mp 160−162 °C. MS (LC/(+)ESI-MS *m/e*: 633.8 (M + 1)$^+$. Anal. ($C_{35}H_{52}O_{10}$) C, H.

Compound 11e. Yield, 35%; colorless oil. ESITOF exact mass *m/e*: 401.2305 (M + 1)$^+$. Anal. ($C_{24}H_{32}O_5$) C, H.

Compound 12e. Yield, 8%; colorless oil.[10] ESITOF exact mass *m/e*: 401.2304 (M + 1)$^+$. Anal. ($C_{24}H_{32}O_5$) C, H.

Compound 13e. Yield, 35%; white crystals (CH_2Cl_2/hexane); mp 185.5 °C (dec). MS (LC/(+)ESI-MS *m/e*: 681.8 (M + 1)$^+$. Anal. ($C_{39}H_{52}O_{10}$) C, H.

Compound 11f. Yield, 75%; white crystals (CH_2Cl_2/hexane); mp 122−123 °C. ESITOF exact mass *m/e*: 377.1765 (M + 1)$^+$. Anal. ($C_{21}H_{25}FO_5$) C, H.

Compound 12f. Yield, 11%; white crystals (CH_2Cl_2/hexane); mp 121−123 °C. ESITOF exact mass *m/e*: 377.1716 (M + 1)$^+$. Anal. ($C_{21}H_{25}FO_5$) C, H.

Compound 13f. Yield, 4%; white crystals (CH_2Cl_2/hexane); mp 127−129 °C. ESITOF exact mass *m/e*: 657.2866 (M + 1)$^+$. Anal. ($C_{36}H_{45}FO_{10}$) C, H.

Compound 11g. Yield, 65%; white crystals (CH_2Cl_2/hexane); mp 131−133 °C. ESITOF exact mass *m/e*: 377.1756 (M + 1)$^+$. Anal. ($C_{21}H_{25}FO_5$) C, H.

Compound 12g. Yield, 8%; white crystals (CH_2Cl_2/hexane); mp 104−106 °C. ESITOF exact mass *m/e*: 377.1758 (M + 1)$^+$. Anal. ($C_{21}H_{25}FO_5$) C, H.

Compound 13 g. Yield, 15%; white crystals (CH_2Cl_2/hexane); mp 188−190 °C. ESITOF exact mass *m/e*: 657.2883 (M + 1)$^+$. Anal. ($C_{36}H_{45}FO_{10}$) C, H.

General Procedure for the Reaction of Artemisitene and 1,*n*-Dimagnesiumbromoalkane.[28] Magnesium turnings (5 equiv) are placed in a reaction flask. A mixture of 1,2-dibromoethane (0.1 mL) in THF (5 mL) was added, and the flask was warmed until gas evolution was apparent and then further for approximately 2 min. The THF was removed by a syringe and replaced by fresh THF (14 mL). 1,4-Dibromoalkane (1.1 equiv) was added, while the solution was stirred magnetically. Stirring was continued for 6 h. A solution of **6** (2 equiv) at −78 °C was added, and the reaction mixture was left stirring for 24 h. Saturated aqueous NH_4Cl solution workup followed by extraction with CH_2Cl_2, drying ($MgSO_4$), and evaporation gave the crude product. The crude product was purified by PLC (silica gel, 30% EtOAc/hexane as the eluent) to obtain monomeric, dimeric, trimeric, and tetrameric adducts.

Compound 15a. Yield, 3%; white crystals (CH_2Cl_2/hexane); mp 122−124 °C (lit[10] mp 122.5−123.5 °C). ESITOF exact mass *m/e*: 339.2170 (M + 1)$^+$. Anal. ($C_{19}H_{30}O_5$) C, H.

Compound 16a. Yield, 14%; white crystals (CH_2Cl_2/hexane); mp 96.4−97 °C. MS (LC/(+)ESI-MS) *m/e*: 339.4 (M + 1)$^+$. Anal. ($C_{19}H_{30}O_5$) C, H.

Compound 17a. Yield, 7%; white crystals (CH_2Cl_2/hexane); mp 190−192 °C. MS (LC/(+)ESI-MS) *m/e*: 619.7 (M + 1)$^+$. Anal. ($C_{34}H_{50}O_{10}$) C, H.

Compound 18a. Yield, 5%; white crystals (CH_2Cl_2/hexane); mp 176−178 °C. ESITOF exact mass *m/e*: 619.3569 (M + 1)$^+$. Anal. ($C_{34}H_{50}O_{10}$) C, H.

Compound 19a. Yield, 4%; white crystals (CH_2Cl_2/hexane); mp 187−189 °C. ESITOF exact mass *m/e*: 619.3563 (M + 1)$^+$. Anal. ($C_{34}H_{50}O_{10}$) C, H.

Compound 20a. Yield, 4%; white crystals (CH_2Cl_2/hexane); mp 149−151 °C. MS (LC/(+)ESI-MS) *m/e*: 900.6 (M + 1)$^+$. Anal. ($C_{49}H_{70}O_{15}$) C, H.

Compound 21a. Yield, 7%; white crystals (CH_2Cl_2/hexane); mp 179−180 °C. MS (LC/(+)ESI-MS) *m/e*: 1178.6 (M$^+$). Anal. ($C_{64}H_{90}O_{20}$) C, H.

Compound 15b. Yield, 3%; white crystals (CH_2Cl_2/hexane); mp 52−54 °C (lit[10] mp 80.5−82 °C). MS (LC/(+)ESI-MS) *m/e*: 353.5 (M + 1)$^+$. Anal. ($C_{20}H_{32}O_5$) C, H.

Compound 16b. Yield, 12%; colorless oil. ESITOF exact mass *m/e*: 353.2311 (M + 1)$^+$. Anal. ($C_{20}H_{32}O_5$) C, H.

Compound 17b. Yield, 7%; white crystals (CH_2Cl_2/hexane); mp 160−162 °C. MS (LC/(+)ESI-MS) *m/e*: 633.7 (M + 1)$^+$. Anal. ($C_{35}H_{52}O_{10}$) C, H.

Compound 18b. Yield, 6%; white crystals (CH_2Cl_2/hexane); mp 70−72 °C. MS (LC/(+)ESI-MS) *m/e*: 633.7 (M + 1)$^+$. Anal. ($C_{35}H_{52}O_{10}$) C, H.

Compound 19b. Yield, 16%; white crystals (CH_2Cl_2/hexane); mp 62−64 °C. MS (LC/(+)ESI-MS) *m/e*: 633.7 (M + 1)$^+$. Anal. ($C_{35}H_{52}O_{10}$) C, H.

Compound 20b. Yield, 32%; white crystals (CH_2Cl_2/hexane); mp 126.5−128 °C. MS (LC/(+)ESI-MS) *m/e*: 915.4 (M + 1)$^+$. Anal. ($C_{50}H_{72}O_{15}$) C, H.

Compound 21b. Yield, 11%; white crystals (CH_2Cl_2/hexane); mp 172−174 °C. MS (LC/(+)ESI-MS) *m/e*: 1193.6 (M + 1)$^+$. Anal. ($C_{65}H_{92}O_{20}$) C, H.

Antimalarial Activity. Continuous in vitro cultures of the asexual erythrocytic stage of *P. falciparum* (K1, multidrug resistant stain) were maintained. Quantitative assessment of antimalarial activity in vitro was determined using the microculture radioisotope technique based upon the method described by Desjardins.[22] Effective concentration (EC_{50}) represents the concentration that cause 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]hypoxanthine by *P. falciparum*.

Cytotoxicity Assay. The cytotoxicity of the artemisinin analogues against KB, BC, and vero cell lines was evaluated employing the colorimetric method as by Skehan and coworkers.[23] Ellipticine was used as the reference substance, exhibiting the activity toward KB and BC cell lines, with an EC_{50} of 2.44 ± 0.41 and 2.03 ± 0.41 μM, respectively.

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Supporting Information Available: NMR (^{1}H and ^{13}C) and IR spectroscopic data of all artemisinin derivatives. This material is available free of charge via the Internet at http://www/pubs.acs.org.

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