Mechanism-Based Design of Parasite-Targeted Artemisinin Derivatives: Synthesis and Antimalarial Activity of Benzylamino and Alkylamino Ether Analogues of Artemisinin

Paul M. O'Neill,* Laurence P. Bishop, Richard C. Storr,† Shaun R. Hawley, James L. Maggs, Stephen A. Ward, and B. Kevin Park

Departments of Chemistry and of Pharmacology and Therapeutics, The University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

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Several artemisinin derivatives linked to benzylamino and alkylamino groups were synthesized in order to enhance accumulation within the malaria parasite. The *in vitro* antimalarial activity was assessed against the chloroquine sensitive HB3 strain and the chloroquine resistant K1 strain of *Plasmodium falciparum*. In general the incorporation of amino functionality enhances the activity relative to artemisinin. The most potent analogue in the series was compound **6** which was severalfold more active than artemisinin against both strains of *P. falciparum* used in the study.

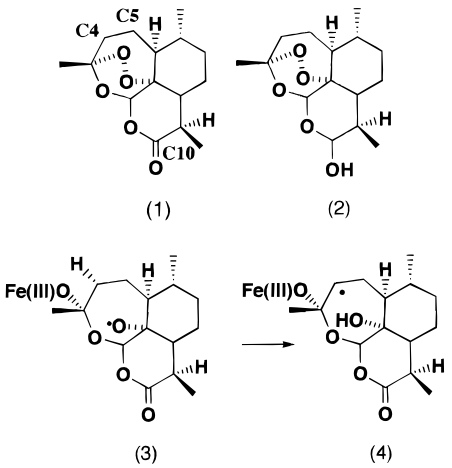
Introduction

Malaria is one of the world's most deadly diseases and is becoming an increasingly serious problem as malaria parasites develop resistance to drugs such as chloroquine and mefloquine. There is, therefore, considerable urgency to develop new classes of antimalarials. Artemisinin (**1**) (qinghaosu) is an unusual 1,2,4-trioxane which has been used clinically in China for the treatment of multidrug resistant *Plasmodium falciparum* malaria.[1] However the clinical application of artemisinin has been limited by the drug's pharmacokinetic properties. This has provided the impetus for the investigation of derivatives of this compound, some of which include esters and ethers of the the corresponding lactol, dihydroartemisinin (DHA) (**2**). Analogues of this type are currently being developed as potent and rapidly acting antimalarials.[2]

Studies on the mode of action of artemisinin and related 1,2,4-trioxanes have indicated that the peroxide bridge is cleaved homolytically by heme (ferriprotoporphyrin IX), a ubiquitous cellular component of *P. falciparum*, to give a reactive intermediate that alkylates vital parasite protein molecules.[3] There is evidence that this key intermediate is the radical at C-4 (**4**) formed by an intramolecular abstraction of the 4αH by the initially formed alkoxy radical **3**.[4]

Several "mechanism-based approaches" have been investigated for improving the antimalarial activity of artemisinin derivatives. In a recent study Posner has investigated the effect of radical-stabilizing groups at the C-4 position in the artemisinin framework.[5,6] It was proposed that incorporation of such groups would increase the formation of the carbon-centred radical and that this would be in turn reflected by increased antimalarial activity. In another approach Yuthavong synthesized a series of analogues covalently linked to iron chelators. The rationale behind this series of compounds was that the chelation of available "free iron" in the food vacuole of the parasite would locate available Fe(III)/Fe(II) species close to the peroxide bridge of the artemisinin derivative leading ultimately to enhanced cleavage and generation of the required "cytotoxic radical species".[7] However, neither of these approaches resulted in compounds with significantly greater antimalarial potency.

Chart 1. Structures of Artemisinin, Dihydroartemisinin, and Radical Intermediates **3** and **4**



Existing 4-aminoquinoline antimalarials such as chloroquine and amodiaquine incorporate a basic alkylamino side chain, the role of which is probably 2-fold. Firstly, protonation of the drug within the acidic vacuole of the parasite assists accumulation since the resultant cation cannot pass out of the parasite.[8] Secondly, the protonated alkylamino groups might also assist binding to the proposed cellular receptor, heme,[9] within the acidic food vacuole, i.e., the site of action of antimalarial peroxides.

We have linked artemisinin to benzylamino and alkylamino groups (Chart 2) with the aim of increasing the activity of artemisinin by enhancing the intracellular accumulation of drug within the parasite food vacuole by "ion trapping". This should increase the amount of drug available for interaction with heme and hence generation of the required alkylating species.

Chemistry

The preparation of the benzylamino ether derivatives of artemisinin is shown in Scheme 1. Artemisinin (**1**) was reduced with sodium borohydride to give DHA (**2**)

† Department of Chemistry.
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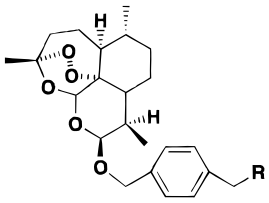
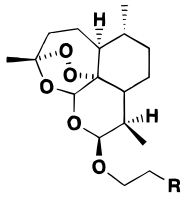
Chart 2. Structures of Amine-Substituted Artemisinin Derivatives

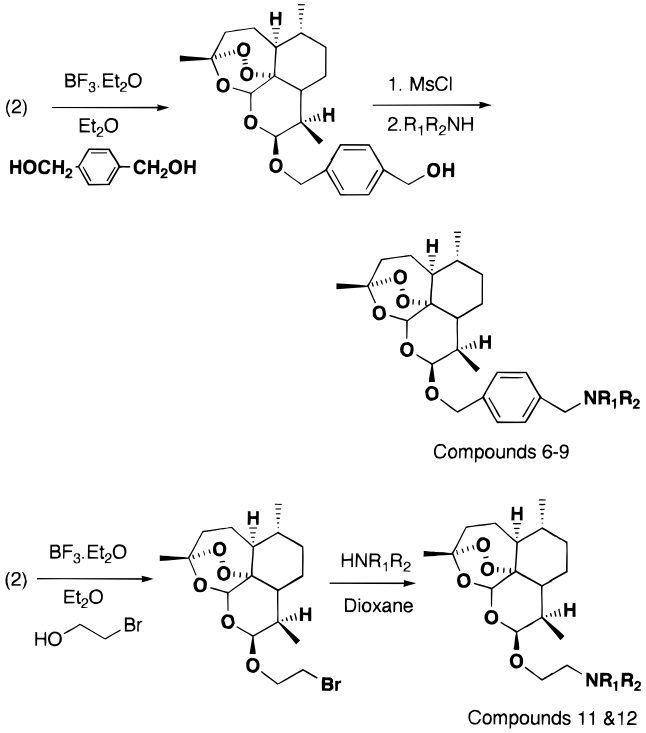


Compound No	R
(5)	OH
(6)	NEt_2
(7)	
(8)	
(9)	



Compound No	R
(10)	Br
(11)	NHMe
(12)	NHEt

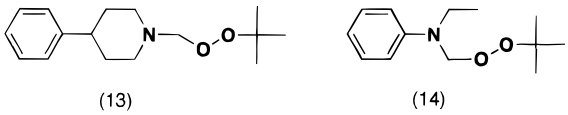
Scheme 1. Synthesis of Benzylamino and Alkylamino Ether Analogues of Artemisinin



according to the literature procedure.[10] Treatment of (**2**) with 4-(hydroxymethyl)benzyl alcohol, in the presence of BF_3 etherate catalyst, gave the required benzyl alcohol **5**, (Scheme 1) in 80% yield. NMR analysis of the product revealed a small coupling (J = 3.30 Hz, C9H-C10H) indicating that the stereochemistry of the product was β at the C-10 carbon atom. Treatment of the benzylic alcohol in dichloromethane with mesyl chloride in the presence of triethylamine at low temperature gave the mesylate which was used without further purification in the next step of the synthesis. The solvent was removed, dry dioxane was added, and the mesylate was allowed to react with 2 equiv of the appropriate amine for 1 h at 40 °C. The solvent was removed, and the required product was purified by preparative thin layer chromatography.

Two alkylamino ether derivatives were also synthesized for comparison according to Scheme 1. DHA was

Chart 3. Amine Peroxide Antimalarials



(13) (14)

coupled with 2-bromoethanol in anhydrous ether using BF_3 etherate catalysis to give a mixture of α and β stereoisomers.[11] The β isomer (**10**, R = Br) was separated by column chromatography to give the product as a yellow solid. The bromo compound was then allowed to react with the appropriate amine in dioxane at 50 °C for 1 h, and the product was finally purified by column chromatography.

Antimalarial Activity

The antimalarial activity of chloroquine and the artemisinin analogues was determined[12,13] against two strains of *P. falciparum*: the uncloned K1 strain (chloroquine resistant) and the HB3 strain (chloroquine sensitive) using standard techniques.

Results and Discussion

As formulated by De Duve,[14,15] the explanation of the weak base effect is based upon two main assumptions: (i) the neutral (uncharged) forms of the weak bases readily cross both plasma and vesicle membranes and (ii) these membranes are impermeable (or much less permeable) to the protonated forms of the bases. The logical consequences of these assumptions are consistent with the known effects of weak bases on both mammalian cells and plasmodia,[16] i.e., weak bases are concentrated by protonation in their nondiffusable form within acidic intracellular vesicles.

In addition to the wide variety of artemisinin analogues that have been examined to date,[17] simple peroxides such as *tert*-butyl hydroperoxide and hydrogen peroxide have been screened and found to have limited antimalarial potency.[18] Since both of these agents result in unwanted side effects, such as hemolysis of uninfected erythrocytes at parasiticidal concentrations, Vennerstrom synthesized a series of amine peroxides (e.g., **13** and **14**) with the idea that a selective drug delivery would circumvent toxicity to the host.[18] The rationale for the intended specificity of action was the selective concentration of these weak base derivatives in the acidic digestive vacuoles of the parasite (pH $\geq$ 5.0) where the oxidant sensitivity of the malaria parasite could be exploited to maximum effect. The study revealed that linking *tert*-butyl peroxide to various amine groups enhanced antimalarial activity by 1 order of magnitude.

The purpose of the present study was to explore the effect on antimalarial activity of linking artemisinin to different amine functionalities. It was anticipated that the incorporation of a moiety that can undergo protonation in the acidic food vacuole of the parasite would enhance cellular accumulation by ion trapping and thereby provide larger quantities of drug available for interaction with heme, the "cellular activator" of antimalarial peroxide drugs. It can be seen from Table 1 that all of the analogues tested have significant activity against both strains of *P. falciparum* used in the study. Of the benzylamino compounds, the derivatives **6** and **9** are the most active against the chloroquine sensitive

Table 1. Antimalarial Activity against the HB3 and K1 Strains of *P. falciparum*

drug	HB3 IC_{50} (nM)	±SE	K1 IC_{50} (nM)	±SE
chloroquine	20	1.1	250	7.1
artemisinin (**1**)	7.3	0.6	6.4	0.5
5	4.2	0.8	4.4	0.9
6	3.1	0.9	1.4	0.2
7	12.2	1.9	9.8	1.7
8	50	9	35	17
9	2.3	0.3	2.3	0.3
11	2.7	0.4	2.4	0.4
12	4.1	0.04	4.1	0.04

HB3 strain, having approximately 2-fold greater activity than artemisinin and being several times more active than chloroquine. Analogue **6** was also the most active compound tested against the chloroquine resistant K1 strain. However, although the derivatives **6**−**9** show good activity, it is clear that there is only slight improvement over compound **5** which was included in the study as a control. This analogue is also more potent than artemisinin but does not contain an amino group. Thus the observed increases in activity for analogues **6** and **9** may be on account of not only increased cellular accumulation but also increased lipophilicity brought about by the presence of the substituted benzene ring.

In general the rank order of drug activity within each amine series was similiar for both strains of plasmodia. Notably the morpholino compound **8** had reduced activity. The two alkylamino derivatives **11** and **12** had comparable activity in both strains with slightly improved activity relative to artemisinin. Interestingly none of the derivatives was subject to resistance; all of the analogues have equipotent activity against chloroquine resistant and sensitive isolates.

Summary and Conclusion

The aim of the present study was to develop simple chemical routes to a series of amine-functionalized artemisinin derivatives and to investigate the effect of amine substitution on antimalarial activity. The fact that all the compounds tested have good activity illustrates the potential of selectively targeting the malarial parasite, and further studies are in progress to examine whether linking artemisinin to a range of diprotic side chains can enhance antimalarial activity further.

Experimental Section

Antimalarial Testing. Two strains of *P. falciparum* from Thailand were used in this study: (a) the uncloned K1 strain, which is known to be chloroquine resistant, and (b) the HB3 strain, which is sensitive to all antimalarials.

Parasites were maintained in continuous culture using a method derived from that of Jensen and Trager.[12] Cultures were maintained in culture flasks containing human erythrocytes (2−5%) with parasitemia ranging from 0.1% to 10%. The parasites were suspended in RPMI 1640 medium supplemented with 25 mM HEPES buffer, 32 mM $NaHCO_3$, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O_2, 4% CO_2, and 93% N_2.

Antimalarial activity was assessed by an adaptation of the 48 h sensitivity assay of Desjardins et al.[13] which uses [^{3}H]-hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were dissolved in 100% ethanol and diluted to an appropriate concentration with complete medium (final concentrations contained less than 1% ethanol). Assays were performed in sterile 96-well microliter plates, each well containing 100 μL of medium which was seeded with 10 μL of a parasitized red blood cell mixture to give a resulting initial parasitemia of 1% with a 5% hematocrit. Control wells (which constituted 100% parasite growth) consisted of the above, with the omission of the drug.

After 24 h incubation at 37 °C, 0.5 μCi of [^{3}H]hypoxanthine was added to each well. After a further 24 h incubation, the cells were harvested onto filter mats, dried overnight, placed in scintillation vials with 4 mL of scintillation fluid, and counted on a liquid scintillation counter.

IC_{50} values were calculated by interpolation of the probit transformation of the log dose−response curve. Each compound was tested in triplicate against both strains to ensure reproducibility of the results.

Chemistry. Merck Kieselgel 60 F 254 precoated silica plates for TLC were obtained from BDH, Poole, Dorset, U.K. Column chromatography was carried out on Merck 938S silica gel. Infra red (IR) spectra were recorded in the range 4000−600 cm^{-1} using a Perkin Elmer 298 infrared spectrometer. Spectra of liquids were taken as films. Sodium chloride plates (Nujol mull) and solution cells (dichloromethane) were used as indicated.

Proton NMR spectra were recorded using Perkin Elmer R34 (220 MHz) and Bruker (400 and 200 MHz) NMR spectrometers. Solvents are indicated in the text, and tetramethylsilane was used as an internal reference. Mass spectra were recorded at 70 eV using a VG7070E mass spectrometer. The samples were introduced using a direct insertion probe. In the text the parent ion (M^+) is given followed by peaks corresponding to major fragment losses with intensities in parentheses.

10β-[[*p*-(Hydroxymethyl)benzyl]oxy]dihydroartemisinin (5). Dihydroartemisinin (0.5 g, 1.77 mmol) was dissolved in anhydrous ether (70 mL) under nitrogen. BF_3·-Et_2O (0.25 mL) was then added by syringe followed by 4-(hydroxymethyl)benzyl alcohol (0.37 g, 2.65 mmol). The reaction mixture was left to stir for 20 h, after which water was added. The ether layer was washed with sodium sulfate solution (30% w/v) and dried with magnesium sulfate and the solvent removed under reduced pressure to give the crude product as an oil. Column chromatography (silica, 40% ethyl acetate/60% petroleum ether) gave the product as a colorless viscous syrup (0.56 g, 78%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.08−7.20 (m, 4H, Ar-H), 5.30 (s, 1H, C-H at C12), 4.75 (d, 1H, J = 3.30 Hz, C-H at C10), 4.73 (d, 1H, 4.52, J = 12.10 Hz, $-OCH_2Ar$), 4.52 (s, 2H, CH_2OH), 4.35 (d, 1H, J = 12.10 Hz, $-OCH_2Ar$), 2.50 (m, 1H), 2.25 (dt, 1H), 1.10−1.95 (m, 10H), 1.30 (s, 3H, CH_3 at C3), 0.90 (d, 3H, CH_3 at C6), 0.85 (d, 3H, CH_3 at C9); ^{13}CNMR ($CDCl_3$, 400 MHz) 140, 127, 126, 104, 101, 87, 81, 69, 65, 52, 44, 37, 36, 34, 30, 26, 24.5, 20, 12; LCMS (75 V, MeOH−AcOH) 437 (M + MeOH + 1, 100), 422 (M^+ + NH_4, 65), 405 (M + 1, 1), 359 (M + 1 − CO_2H_2, 9), 267 (M + 1 − $CHPhCH_2OH$ − H_2O, 75), 221 (14); CIMS 422 (M^+ + NH_4, 8), 376 (M^+ − HCO_2H, 12), 359 (M^+ − HCO_2H − NH_3, 100), 317 (8), 284 (39), 267 (36), 251 (16), 221 (96), 138 (33); IR (CH_2Cl_2) 3510 (OH), 1420, 1260, 1200, 890 (O-O), 815 (O-O). Anal. C,H.

10β-[[*p*-[(Diethylamino)methyl]benzyl]oxy]dihydroartemisinin (6). The benzyl alcohol derivative **5** (0.10 g, 0.246 mmol) was dissolved in dry dichloromethane (10 mL) under nitrogen, and the solution was cooled to 0 °C. Mesyl chloride (0.42 g, 0.36 mmol) was added, and the solution was allowed to stir for 45 min (TLC). The solvent was removed under reduced pressure, and the residue was dissolved in dioxane (5 mL). The reaction vessel was flushed with nitrogen, and diethylamine (0.035 g, 0.50 mmol, 0.05 mL) was added via syringe. The reaction mixture was heated at 50 °C for 1 h, and the solvent was removed under reduced pressure. The residue was applied to a preparative TLC plate (1% MeOH/99% DCM), and the product was obtained as a colorless oil, 0.52 g (49%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.11−7.31 (m, 4H, Ar-H), 5.40 (s, 1H, C-H at C12), 4.90 (d, 1H, J = 3.10 Hz, CH at C10), 4.84 (d, 1H, J = 12.10 Hz, $-OCH_2Ar$), 4.45 (d, 1H, J = 12.10 Hz, $-OCH_2Ar$), 3.50 (s, 2H, CH_2N), 2.60 (m, 1H), 2.45 (q, 4H, NCH_2CH_3), 2.32 (dt, 1H), 1.10−2.12 (10H, m), 1.40 (s,

3H, CH_3 at C3), 0.96 (t, 6H, NCH_2CH_3), 0.89 (d, 3H, CH_3 at C6), 0.85 (d, 3H, CH_3 at C9);^{13}CNMR ($CDCl_3$, 400 MHz) 136, 128, 127, 104, 101, 87, 81, 69, 57, 52, 46, 44, 37, 36, 34, 30, 26, 24, 20, 12, 11; LCMS (75 V, 50−75% MeOH−AcOH) 460 (M^+ + MeOH + 1, 100), 401(M^+ − HCO_2H), 372 (23), 195 (14), 176 (M + 1 − DHA, 15); IR (CH_2Cl_2) 3056, 2987, 1421, 1258, 1193, 1175, 1158, 1139, 1099, 895 (O-O), 820 (O-O). Anal. C,H,N.

10*β*-[[*p*-(Piperidinomethyl)benzyl]oxy]dihydroartemisinin (7). This compound was prepared as described for **6**. The yield was 40%.: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.10−7.30 (m, 4H, Ar-H), 5.45 (s, 1H, C-H at C12), 4.90 (d, 1H, CH at C10), 4.86 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 4.50 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 3.53 (s, 2H, CH_2N), 2.65 (m, 1H), 2.40 (m, 5H), 1.62 (m, 4H, NCH_2CH_2-), 1.44 (s, 3H, CH_3 at C3), 1.15−2.10 (m, 11H), 0.96 (d, 3H, CH_3 at C6), 0.92 (d, 3H, CH_3 at C9); ^{13}C NMR ($CDCl_3$, 400 MHz) 137, 136, 129, 104, 101, 87, 81, 69, 67, 63, 54, 52, 44, 37, 34, 30, 26, 25, 24.64, 24.48, 24.03, 20, 13, 9; LCMS (85 V, 75% MeOH−AcOH) 494 (M + Na, 100), 472 (M^+ + 1, 100); IR (CH_2Cl_2) 3050, 2900, 1430, 1260, 1175, 895 (O-O), 820 (O-O). Anal. C,H,N.

10*β*-[[*p*-(Morpholinomethyl)benzyl]oxy]dihydroartemisinin (8). This compound was prepared as described for **6**. **8**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.18−7.30 (m, 4H, Ar-H), 5.45 (s, 1H, C-H at C12), 4.91 (d, 1H, J = 4.10 Hz, CH at C10), 4.89 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 4.50 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 3.70 (m, 4H, NCH_2CH_2O), 3.50 (s, 2H, CH_2N), 2.67 (m, 1H), 2.45 (m, 4H, NCH_2CH_2O-), 1.45 (s, 3H, CH_3 at C3), 1.25−2.10 (m, 11H), 0.97 (d, 3H, CH_3 at C6), 0.93 (d, 3H, CH_3 at C9); LCMS (85 V, 75% MeOH−AcOH) 474 (M^+ + 1, 100), 190 (10); IR (CH_2Cl_2) 3050, 895 (O-O), 820 (O-O). Anal. C,H,N.

10*β*-[[*p*-(Pyrrolidinomethyl)benzyl]oxy]dihydroartemisinin (9). This compound was prepared as described for **6**. The yield after preparative TLC was 40%: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.43 (m, 4H, Ar-H), 5.31 (s, 1H, C-H at C12), 4.95 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 4.91 (d, 1H, J = 3.50 Hz, C-H at C10), 4.55 (d, 1H, J = 12.65 Hz, $-OCH_2Ar$), 4.06 (s, 2H, CH_2N), 3.06 (m, 4H,NCH_2CH_2), 2.67 (m, 1H), 2.40 (dt, 1H), 2.05 (m, 4H, NCH_2CH_2), 1.34 (s, 3H, CH_3 at C3), 1.22−1.87 (m, 10H), 0.96 (d, 3H, CH_3 at C6), 0.94 (d, 3H, CH_3 at C9); LCMS (75 V, 50−75% MeOH−AcOH) 458 (M^+ + 1, 100), 444 (7), 430 (23), 412 (7), 174 (28); IR (CH_2Cl_2) 3060, 2951, 1421, 1272, 895 (O-O), 820 (O-O). Anal. C,H,N.

10*β*-(2-Bromoethoxy)dihydroartemisinin (10). Dihydroartemisinin (0.25 g, 0.88 mmol) was dissolved in dry ether, and the reaction vessel was flushed with nitrogen. 2-Bromoethanol (2 equiv) was added via syringe, and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed under reduced pressure, and the mixture of α and β isomers was separated by column chromatography (hexane/ethyl acetate, 9:1): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.38 (s, 1H, C-H at C12), 4.71 (d, 1H, J = 3.60 Hz, C-H at C10), 3.78−4.11 (m, 2H), 3.48 (m, 2H, CH_2Br), 2.65 (m, 1H), 2.40 (dt, 1H), 1.10−1.95 (m, 10H), 1.30 (s, 3H, CH_3 at C3), 0.90 (s, 3H, CH_3 at C6), 0.89 (s, 3H, CH_3 at C9); MS 374, 376 (M^+, 5), 314, 316; IR (CH_2Cl_2) 895 (O-O), 818 (O-O). Anal. C,H.

10*β*-[2-(*N*-Methylamino)ethoxy]dihydroartemisinin (11). The bromo compound **11** (0.20 g, 5.33 mmol) was dissolved in dioxane; 2 equiv of aqueous methylamine was added, and the solution was heated at 50 °C for 1.2 h. The product was purified by preparative TLC to give **11** as an oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.36 (s, 1H, C-H at C12), 4.76 (d, 1H, J = 3.30 Hz, C-H at C10), 3.50−3.93 (m, 2H), 2.73 (m, 2H, CH_2N), 2.42 (s, 3H, N-CH_3), 2.40 (dt, 1H), 2.11 (m, 1H, NH), 1.10−1.95 (m, 11H), 1.30 (s, 3H, CH_3 at C3), 0.90 (s, 3H, CH_3 at C6), 0.89 (s, 3H, CH_3 at C9); MS 374, 376 (M^+, 5), 314, 316; IR (CH_2Cl_2) 895 (O-O), 818 (O-O). Anal. C,H,N.

10*β*-[2-(Ethylamino)ethoxy]dihydroartemisinin (12). The bromo compound **11** (0.20 g, 5.33 mmol) was dissolved in dioxane; 2 equiv of aqueous ethylamine (70%) was added, and the solution was heated at 50 °C for 1.2 h as for **11**. The product was purified by preparative TLC to give **12** as an oil which was converted to the corresponding maleate salt: ^{1}H NMR ($CDCl_3$, 200 MHz) 5.94 (s, 2H, CH:CH), δ 5.36 (s, 1H, C-H at C12), 4.62 (d, 1H, J = 3.30 Hz, C-H at C10), 3.32−4.01 (2H, m, $-OCH_2$), 2.80−3.20 (4H, m, CH_2NCH_2), 2.40 (dt, 1H), 1.10−1.95 (m, 10H), 1.30 (s, 3H, CH_3 at C3), 1.12 (t, 3H, NCH_2CH_3), 0.90 (s, 3H, CH_3 at C6), 0.89 (s, 3H, CH_3 at C9); IR (KBr) 1700, 1630, 1580, 1470, 1360, 825 (O-O). Anal. C,H,N.

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