DIHYDROARTEMISININ AS CHIRAL
TEMPLATE FOR THE PREPARATION OF
OPTICALLY PURE 1, 2, 4-TRIOXANES
4.1 Introduction

The discovery of artemisinin 1, as the active principle of the Chinese traditional drug *Artemisia Annua*, has stimulated a great deal of interest in malaria chemotherapy.\(^{1,2}\) The peroxide group present in the form of 1,2,4-trioxane is essential for the antimalarial activity of artemisinin and its derivatives. Several synthetic 1, 2, 4-trioxane based on this lead have shown promising antimalarial activity.\(^3\)

As part of this endeavor, our laboratory had earlier developed a new photooxygenation route for the preparation of 1, 2, 4-trioxanes.\(^4\) The key step in this method are

(i) Preparation of properly substituted allylic alcohols.

(ii) Photooxygenation of allylic alcohols to give \(\beta\)-hydroxyhydroperoxides, and

(iii) Condensation of \(\beta\)-hydroxyhydroperoxides with an aldehydes / ketones to furnish 1, 2, 4-trioxanes.

Using this method our group has synthesized a large number of trioxanes. Several of these trioxanes have shown promising antimalarial activity.\(^5\) This methodology,
however, produces a racemic mixture of $\beta$-hydroxyhydroperoxides which in turn furnish racemic trioxanes. In this chapter, using dihydroartemisinin as a chiral template and geraniol derived alcohol, we have explored the possibility of preparation of enantiomerically pure $\beta$-hydroxyhydroperoxides and their subsequent use in the preparation of optically pure 1,2,4-trioxanes.

4.2 Chemistry

For the preparation of optically active 1, 2, 4-trioxanes, we adopted the following retro-synthetic strategy. To implement this strategy, dihydroartemisinin (DHQ) was prepared from artemisinin using the known procedure. Acetate alcohol was prepared in 4-step from geraniol as given in (Scheme 1). Geraniol was acetylated to geranyl acetate in 92% yield which on epoxidation furnished epoxides in 96% yield. Periodic acid mediated
cleavage of 5 furnished geranyl aldehyde 6 in 59% yield which on NaBH₄ reduction furnished acetate alcohol 7 in 99% yield.

BF₃·OEt₂ catalysed condensation of DHQ 2 and acetate alcohol 7 in dry DCM at subzero temperature (-5°C to 0°C) furnished diastereomeric mixture of β- and α-isomers 8a and 8b respectively in the ratio 7:1 in 97% yield which upon purification by column chromatography furnished pure β-isomers 8a and mixture of 8a and 8b (Scheme 2). Deacetylation of 8a with K₂CO₃/MeOH furnished artemisinin-linked allyl alcohol 9a in 76% yield. Singlet oxygen mediated photooxygenation of 9a in CH₃CN in the presence of methylene blue as photo-sensitizer at -10°C to 0°C furnished an inseparable diastereomeric mixture of β-hydroxyhydroperoxide 10 in 21% yield. NaBH₄ reduction of 10 furnished inseparable diastereomeric mixture of diol 11 in 65% yield, which on acetylation furnished again inseparable mixture of diacetate 12 in 75% yield. Our strategy was based on the assumption that the diastereomeric mixture of β-hydroxyhydroperoxides would be separable into pure isomers. Having failed to achieve this separation, we condensed the mixture of hydroperoxides with cyclohexanone hoping that the trioxanes would be separable. Thus, hydroperoxide 10 on acid-catalysed condensation with cyclohexanone furnished artemisinin-linked trioxane 13 in 85% yield as a mixture of diastereomers, which could not be separated by column chromatography.

Scheme 1: Reagents and Conditions: (a) Ac₂O, Et₃N, DMAP, DCM, r.t. 10 min. (b) PBA, DCM, NaHCO₃, 0°C, 2.5 h. (c) HIO₄, THF, 0°C, 40 min. (d) NaBH₄, MeOH, 0°C, 0.5 h.

¹H NMR, ¹³C NMR and TLC of 13 showed 50:50 mixtures of two diastereomers thereby indicating that there is no chiral induction in this reaction. This fact was further
confirmed from $^1$H NMR, $^{13}$C NMR and TLC of 12 which also showed 50:50 inseparable mixtures of two diastereomers.

Scheme 2: Reagents and Conditions: (a) BF$_3$.Et$_2$O, DCM, 0°C, 3h. (b) K$_2$CO$_3$, MeOH, 0°C, 1h. (c) hv, CH$_3$CN, methylene blue, -10°C to -5°C, 6h. (d) Cyclohexanone, Conc. HCl, r.t., 1h. (e) NaBH$_4$, MeOH, 0°C, 15 min.

4.3 Antimalarial Activity

Having failed in our objective to prepare optically active 1, 2, 4-trioxanes, we assessed the key intermediate and the final trioxane (8a, 9a, 11, 12 and 13) against multidrug-resistant *P. yoelii nigeriensis* in Swiss mice at 48 mg/kg x 4 days by oral route using Peter's procedure using $\beta$-arteether as positive control, which given orally at 48 mg/kg x 4 days provides 100% protection to the mice infected with multidrug-resistant *P.*
yoelii nigeriensis. At 24 mg/kg × 4 days, it provides only 20% protection. All the newly prepared derivatives except 8a and 9a provided 100% protection at 48 mg/kg × 4 days and these compounds were further screened at 24 mg/kg × 4 days. None of these compounds provided 100% protection at this dose. The results are summarized in Table 1.

4.4 Conclusion

Using DHQ as chiral auxiliary, we have made an effort to extend our photooxygenation methodology for the preparation of optically pure 1, 2, 4-trioxanes. However, these efforts were not successful. However in this process we have prepared an artemisinin-1, 2, 4-trioxane hybrid which is equipped with two 1, 2, 4-trioxane pharmacophore. This hybrid compound has shown antimalarial activity which is comparable with that of β-arteether.

Table 1: Blood schizontocidal activity of artemisinin analogs against multi-drug-resistant (MDR) strain P.yoelii in Swiss mice via oral route.8,9

<table>
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<tr>
<th>Compd.</th>
<th>Log P</th>
<th>Dose mg/kg × 4 days</th>
<th>% Suppression of Parasitaemia on day 4a,b</th>
<th>Cured / Treated</th>
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<td>8a</td>
<td>4.61</td>
<td>48</td>
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<td></td>
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<td>24</td>
<td>79.55</td>
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<td>9a</td>
<td>4.38</td>
<td>48</td>
<td>59.15</td>
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<td>11</td>
<td>3.47</td>
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<td>12</td>
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<tr>
<td>β-Arteether</td>
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<td>48</td>
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Percent suppression = [(C-T)/C] × 100, where C= parasitaemia in control group, and T= parasitaemia in treated group. A 100% suppression of parasitaemia means no parasites were detected in 50 oil immersion fields during microscopic observation.

4.5 Experimental

4.5.1 General. All glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on complab melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FT-IR RXI spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were recorded using Bruker Supercon Magnet DPX-200 or DRX-300 spectrometers (operating at 200 and 300 MHz respectively for $^1$H; 50 and 75 MHz respectively for $^{13}$C ) using CDCl$_3$ as solvent. Tetramethylsilane (δ 0.00 ppm) served as an internal standard in $^1$H NMR and CDCl$_3$ (δ 77.0 ppm) in $^{13}$C NMR. Chemical shifts are reported in parts per million (ppm). Splitting patterns are described as singlet (s), doublet (d), triplet (t) and multiplet (m). In NMR, numbering of atoms is presented according to the usual numbering in artemisinin as indicated in the text. Fast atom bombardment mass spectra (FABMS) were obtained on JEOL SX-102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Glycerol or m-nitrobenzyl alcohol was used as matrix. Electrospray mass spectrometry (ESMS) were recorded on a Micromass Quattro II triple quadruple mass spectrometer. Elemental
analyses were performed on Vario EL-III C H N S analyzer (Germany) and values were within ±0.4 % of the calculated values except where noted. Column chromatography was performed over Merck silica gel (particle size: 60-120 Mesh) procured from Qualigens (India), flash silica gel (particle size: 230-400 Mesh). All chemicals and reagents were obtained from Aldrich (Milwaukee, WI), Lancaster (England) or Spectrochem (India) and were used without further purification. Log P values of the compounds were calculated using Chem Draw Ultra 7.0 software.

4.5.2 Acetic acid 3,7-dimethyl-octa-2,6-dienyl ester (4): To a solution of 3 (30.0 g, 0.195 mol) and acetic anhydride (50.0 mL, 0.531 mol) dissolved in dichloromethane (200 mL) was added triethylamine (50.0 mL, 0.360 mol) dropwise and dimethyl aminopyridine (DMAP, 100 mg) at r.t. The mixture was stirred at the same temperature for 10 min. The reaction mixture was then quenched with saturated sodium bicarbonate solution (50 mL) and extracted with dichloromethane (3 × 100 mL). The organic layer was washed with 10% aqueous HCl solution (2 × 50 mL), then with water, dried over anhyd. Na₂SO₄ and concentrated under reduced pressure. The crude product on column chromatography over silica gel using EtOAc-Hexane (1:9) as eluant gave pure geranyl acetate 4 (35.18 g, 92% yield) as an oil; FT-IR (neat, cm⁻¹) 2965.1, 2923.6, 1741.8, 1669.5, 1447.1, 1373.9, 1236.0, 1025.5; ¹H NMR (200 MHz, CDCl₃) δ 1.60 (s, 3H, CH₃), 1.68 (s, 3H, CH₃), 1.69 (s, 3H, CH₃), 2.05 (s, 7H), 4.58 (d, 2H, J = 7.0 Hz, CH₂OAc), 5.08 (t, 1H, Olefinic H), 5.34 (t, 1H, Olefinic H); FABMS (m/z):197 [M + H]⁺.

4.5.3 Acetic acid 5-(3, 3-dimethyl-oxiranyl)-3-methyl-pent-2-enyl ester (5): To a magnetically stirred solution of geranyl acetate 4 (25.0 g, 0.127 mol) in DCM (50 mL) at 0 °C containing NaOAc (17.41 g, 0.128 mol) was added slowly and dropwise a freshly prepared perbenzoic acid (PBA, 50 mL) dissolved in DCM over 0.5 h. The mixture was
stirred for another 2 h. Then poured the reaction mixture into sat. NaHCO₃ solution (500 mL) and the organic layer was separated. The aqueous layer was extracted with DCM (2 x 50 mL). The extracts were combined and washed with ice-cold 1N NaOH aqueous solution (100 mL). The organic layer was then washed with water, dried over anhyd. Na₂SO₄ and concentrated under reduced pressure to furnish the crude geranyl epoxide 5 (26.0 g, 96% yield) as an oil; FT-IR (neat, cm⁻¹) 2962.9, 2930.0, 1740.9, 1457.6, 1378.2, 1236.3, 1032.3, 757.2; ¹H NMR (200 MHz, CDCl₃) δ 1.26 (s, 3H, CH₃), 1.31 (s, 6H, 2 x CH₃), 1.72 (s, 2H, CH₃), 2.05 (s, 3H, COCH₃), 2.67-2.73 (m, 2H), 4.07-4.10 (m, 1H), 4.59 (d, 2H, J = 7.0 Hz, CH₂0Ac), and 5.38 (t, 1H); FABMS (m/z): 213 [M + H]⁺.

4.5.4 Acetic acid 3-methyl-6-oxo-hex-2-ynyl ester (6): To a magnetically stirred solution of epoxide 5 (25.5 g, 0.12 mol) in Et₂O (375 mL) at 0 °C was added dropwise HIO₄·2H₂O (25 g, 0.108 mmol) dissolved in THF (150 mL) over 40 min. The slurry was stirred for an additional 0.5 h and poured into H₂O (250 mL). The layers were partitioned and the aqueous layer was extracted with Et₂O (3 x 50 mL). The combined extracts were washed with sat. NaHCO₃ (2 x 50 mL), then washed with water, dried over anhyd. Na₂SO₄ and concentrated under reduced pressure to furnish the crude aldehyde 6. The crude product on column chromatography over silica gel using EtOAc-Hexane (15:85) as eluant gave pure 6 (12.0 g, 59% yield) as an oil; FT-IR (neat, cm⁻¹) 3021.9, 2933.2, 2726.5, 1732.7, 1443.9, 1371.8, 1239.0, 1027.7, 757.9; ¹H NMR (200 MHz, CDCl₃) δ 1.72 (s, 3H, CH₃), 2.05 (s, 3H, COCH₃), 2.34-2.41 (m, 2H), 2.54-2.62 (m, 2H), 4.58 (d, 2H, J = 6.9 Hz, CH₂0Ac), 5.36 (t, 1H), 9.77 (d, 1H, J= 1.33 Hz); FABMS (m/z): 171 [M + H]⁺.

4.5.5 Acetic acid 6-hydroxy-3-methyl-hex-2-enyl ester (7): To the solution of aldehyde 6 (10.0 g, 0.058 mol) in MeOH (50 mL) was added NaBH₄ (2.23 g, 0.058 mol) slowly
portionwise and stirred the reaction mixture for 0.5 h. Neutralised the reaction mixture with glacial AcOH till it becomes neutral, solvent was evaporated from reaction mixture, and then diluted with distilled water (50 mL) and extracted with ether (3 × 50 mL). The organic layer was washed with brine, dried over anhyd Na₂SO₄ and evaporated under vacuum to furnish the crude product, which on column chromatography over silica gel using EtOAc-Hexane (15:85) as eluant furnished acetate alcohol 7 (10.07 g, 99% yield) as an oil; FT-IR (neat, cm⁻¹) 3459.9, 2937.9, 1738.0, 1444.3, 1373.4, 1238.9, 1034.4, 759.5; ¹H NMR (200 MHz, CDCl₃) δ 1.72 (s, 3H, CH₃), 2.04 (s, 3H, COCH₃), 3.63 (t, 2H, J = 6.4 Hz, CH₂OH), 4.57 (d, 2H, J = 7.0 Hz, CH₂OAc), 5.34-5.41 (m, 1H, olefinic H); FABMS (m/z): 173 [M + H]+.

4.5.6 Etherification of Dihydroartemisinin: To a solution of dihydroartemisinin 2 (7.69 g, 0.027 mol) and acetate alcohol 7 (10.0 g, 0.058 mol) in dry dichloromethane (40 mL) was added BF₃.OEt₂ (1 ml) at -10°C to -5°C. The reaction mixture was stirred at the same temperature for 3h, neutralized with saturated sodium bicarbonate solution (50 mL) and extracted with dichloromethane (3 × 50 mL). The organic layer was washed with water (25 mL), dried over anhyd. Na₂SO₄ and concentrated under reduced pressure. The resultant crude product, on column chromatography over silica gel using EtOAc-Hexane (1:49) as eluant gave pure β-isomer 8a (1.13 g, 9.5% yield) and a mixture of α- and β-isomer 8a & 8b (10.68 g, 90% yield); the pure α-isomer 8b could not be separate out. The combined yield of 8a & 8b being 98%.

8a (β-isomer): Oily; FT-IR (neat, cm⁻¹) 2939.6, 2875.4, 1739.3, 1451.2, 1373.6, 1236.6, 1102.4, 1024.4, 758.2; ¹H NMR (200 MHz, CDCl₃) δ 0.90 (d, 3H, J = 7.4 Hz, CH₃), 0.95 (d, 3H, J = 5.8 Hz, CH₃), 1.21-2.13 (m, 14H), 1.44 (s, 3H, CH₃), 1.70 (s, 3H, CH₃), 2.06 (s, 3H, COCH₃), 2.29-2.43 (m, 1H), 2.58-2.64 (m, 1H ), 3.35 (td, 1H, J = 9.6 Hz, 6.1 Hz, OCH₂), 3.83 (td, 1H, J = 9.5 Hz, 6.2 Hz, OCH₃), 4.58 (d, 2H, J = 7.0 Hz, CH₂OAc), 4.77
DHQ as chiral template

(d, 1H, J = 2.9 Hz, C_{10}-H), 5.34 (t, 1H, J = 7.2 Hz, Olefinic H), 5.38 (s, 1H, C_{12}-H); $^{13}$C NMR (50 MHz, CDCl$_3$) δ 13.40 (CH$_3$), 16.68 (CH$_3$), 20.71 (CH$_3$), 21.35 (CH$_3$), 24.81 (CH$_2$), 25.03 (CH$_2$), 26.52 (CH$_3$), 28.08 (CH$_2$), 31.26 (CH), 35.00 (CH$_2$), 36.49 (CH$_2$), 36.78 (CH$_2$), 37.81 (CH), 44.81 (CH), 52.93 (CH), 61.61 (CH$_2$), 68.08 (CH$_2$), 81.40 (C), 88.21 (CH), 102.32 (CH), 104.35 (C), 119.07 (C), 141.98 (C), 171.33 (C); FABMS (m/z): 439 [M + H]$^+$; Anal. For (C$_{24}$H$_{36}$O$_7$): Calcd C 65.73 H 8.73; Found C 65.63 H 8.71.

4.5.7 Preparation of Allyl alcohol 9a (Deacetylation of 8a): To a solution of 8a (0.55 g, 1.25 mmol) in MeOH (20 mL), was added K$_2$CO$_3$ (excess) at $^0$C and stirred the reaction mixture for 1h. The reaction mixture was evaporated, then added distilled water (20 mL) and extracted with dichloromethane (3 × 20 mL). The organic layer was washed with water (10 mL), dried over anhyd. Na$_2$SO$_4$ and concentrated under reduced pressure. The resultant crude product, on column chromatography over silica gel using EtOAc-Hexane (1:9) as eluant gave pure allyl alcohol 9a (0.38 g, 76% yield) as an oil; FT-IR (neat, cm$^{-1}$) 3456.4, 3019.8, 2933.2, 2875.4, 1595.9, 1441.5, 1379.6, 1216.6, 1102.0, 1015.3, 762.0; $^1$H NMR (200 MHz, CDCl$_3$) δ 0.91 (d, 3H, J = 7.4 Hz, CH$_3$), 0.96 (d, 3H, J = 6.0 Hz, CH$_3$), 1.21-2.13 (m, 14H), 1.43 (s, 3H, CH$_3$), 1.67 (s, 3H, CH$_3$), 2.29-2.38 (m, 1H), 2.58-2.65 (m, 1H), 3.36 (td, 1H, J = 9.5 Hz, 6.1 Hz, OCH$_2$), 3.83 (td, 1H, J = 9.4 Hz, 6.3 Hz, OCH$_2$), 4.15 (d, 2H, J = 6.8 Hz, CH$_2$OH), 4.77 (d, 1H, J = 3.0 Hz, C$_{10}$-H), 5.40 (s, 1H, C$_{12}$-H), 5.45 (t, 1H, Olefinic H); $^{13}$C NMR (50 MHz, CDCl$_3$) δ 12.29 (CH$_3$), 13.43 (CH$_3$), 19.93 (CH$_3$), 20.76 (CH$_3$), 24.88 (CH$_2$), 25.07 (CH$_2$), 26.56 (CH$_2$), 28.43 (CH), 30.08 (CH$_2$), 31.19 (CH$_2$), 32.31 (CH), 33.88 (CH$_3$), 35.04 (CH$_2$), 36.82 (CH$_2$), 37.88 (CH$_2$), 38.27 (CH$_2$), 39.16 (CH), 42.64 (CH), 44.81 (CH), 45.15 (CH), 51.56 (CH), 52.97 (CH), 68.04 (CH$_2$), 74.05 (CH$_2$), 81.60 (C), 88.32 (CH), 102.36 (CH), 104.54 (C), 116.54 (C), 138.18 (C); FABMS (m/z): 397 [M + H]$^+$; ESMS (m/z): 414 [M + NH$_4$]$^+$, 419 [M + Na]$^+$; Anal. For (C$_{22}$H$_{36}$O$_6$): Calcd C 66.64 H 9.15; Found C 66.62 H 9.07.
4.5.8 Photooxygenation of Allyl alcohol: A solution of allylic alcohol 9a (0.350 g, 0.88 mmol) and methylene blue (5 mg) in acetonitrile (30 mL), maintained at 0 °C, was irradiated with a 500 W tungsten-halogen lamp, while oxygen was bubbled slowly into the reaction mixture for 6h. Solvent was evaporated under vacuum at r.t. to furnish crude product which on column chromatography over deactivated silica gel (12% v/w of water) using EtOAc-Hexane (2: 8) as eluant furnished pure 10 (0.080 g, 21% yield) as an oil; Oily; FT-IR (neat, cm$^{-1}$) 3442.5, 2930.7, 2832.4, 2717.7, 1591.6, 1462.2, 1435.8, 1363.1, 1023.2, 775.1; $^1$H NMR (200 MHz, CDCl$_3$) δ 0.91 (d, 3H, $J$ = 7.4 Hz, CH$_3$), 0.95 (d, 3H, $J$ = 5.9 Hz, CH$_3$), 1.21-2.14 (m, 12H), 1.43 (s, 3H, CH$_3$), 1.62 (s, 2H), 2.29-2.43 (m, 1H), 2.59-2.64 (m, 1H ), 3.40-3.53 (m, 1H), 3.73-3.90 (m, 3H), 4.50-4.56 (m, 1H), 4.79 (d, 1H, $J$ = 3.2 Hz, C$_{10}$-H), 5.07 & 5.15 (2 x s, 2H), 5.39-5.42 (m, 1H), 8.68 (d, 1H, $J$ = 12.1 Hz, OOH); FABMS (m/z): 429 [M + H]$^+$.

4.5.9 Preparation of Diol (11): To the solution of β-hydroxyhydroperoxide 10 (0.096 g, 0.22 mmol) in MeOH (20 mL) was added NaBH$_4$ (0.025 g, 0.65 mmol) slowly portionwise and stirred the reaction mixture for 15 min. Neutralised the reaction mixture with glacial AcOH till it becomes neutral, solvent was evaporated from reaction mixture, and then diluted with distilled water (10 mL) and extracted with ether (3 x 25 mL). The organic layer was washed with brine, dried over anhyd Na$_2$SO$_4$ and evaporated under vacuum to furnish the crude product, which on column chromatography over silica gel using EtOAc-Hexane (3:7) as eluant furnished diol 11 (0.06 g, 65% yield) as an oil; FT-IR (neat, cm$^{-1}$) 3448.0, 3019.7, 2951.6, 1661.0, 1447.6, 1380.6, 1216.6, 1099.6, 1021.3, 761.5; $^1$H NMR (200 MHz, CDCl$_3$) δ 0.90 (d, 3H, $J$ = 7.4 Hz, CH$_3$), 0.95 (d, 3H, $J$ = 6.1 Hz, CH$_3$), 1.22-2.13 (m, 14H), 1.44 (s, 3H, CH$_3$), 2.29-2.38 (m, 1H), 2.61-2.64 (m, 1H ), 3.36-3.58 (m, 2H), 3.71 (dd, 1H, $J$ = 11.1 Hz, 2.7 Hz, CH$_3$OH), 3.80-3.88 (m, 1H), 4.21 (m, 1H), 4.78 (d, 1H, $J$ = 2.8 Hz, C$_{10}$-H), 4.98 & 5.16 (2 x s, 2H, Olefinic H), 5.39 (s, 1H,
\textbf{4.5.10 Procedure for Synthesis of Diacetate (12):} To a solution of diol 11 (0.100 g, 0.24 mmol) and acetic anhydride (0.32 mL, 3.40 mmol) dissolved in dry dichloromethane (10 mL) was added triethylamine (0.32 mL, 2.30 mmol) dropwise and dimethyl aminopyridine (DMAP, 1 mg) at 0°C. The mixture was stirred at the same temperature for 15 min. The reaction mixture was then quenched with saturated sodium bicarbonate solution (5 mL) and extracted with dichloromethane (3 \times 10 mL). The organic layer was washed with 10% aqueous HCl solution (2 \times 10 mL), then with water, dried over anhyd. Na$_2$SO$_4$ and concentrated under reduced pressure. The crude product on column chromatography over silica gel using EtOAc-Hexane (1:9) as eluant gave pure diacetate 12 (0.09 g, 75% yield) as an oil; FT-IR (neat, cm$^{-1}$) 3016.4, 2946.1, 1741.8, 1448.4, 1374.1, 1227.8, 1098.9, 1025.7, 758.7; $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 0.91 (d, 3H, $J = 7.4$ Hz, CH$_3$), 0.95 (d, 3H, $J = 5.9$ Hz, CH$_3$), 1.26-2.18 (m, 14H), 1.43 (s, 3H, CH$_3$), 2.05 (s, 3H, CHCOCH$_3$), 2.09 (s, 3H, CH$_2$COCH$_3$), 2.36-2.38 (m, 1H), 2.60-2.62 (m, 1H), 3.37-3.42 (m, 1H), 3.86 (td, 1H, $J = 9.7$ Hz, 6.2 Hz, OCH$_2$), 4.10 (dd, 1H, $J = 11.9$ Hz, 7.7 Hz, OCH$_2$), 4.25 (dd, 1H, $J = 11.9$ Hz, 3.4 Hz, OCH$_2$), 4.77 (d, 1H, $J = 3.2$ Hz, C$_{10}$-H), 4.99 & 5.12 (2 \times s, 2H, Olefinic H), 5.36-5.41 (m, 1H), 5.38 (s, 1H, C$_{12}$-H); $^{13}$C NMR (50 MHz, CDCl$_3$) $\delta$ 13.43 (CH$_3$), 20.74 (CH$_3$), 21.16 (CH$_3$), 21.42 (CH$_3$), 24.89 (CH$_2$), 25.07 (CH$_2$), 26.58 (CH$_3$), 28.33 (CH$_2$), 29.91 (CH$_2$), 31.31 (CH), 35.03 (CH$_2$), 36.84 (CH$_2$), 37.86 (CH), 44.84 (CH), 52.98 (CH), 64.80 (CH$_2$), 68.05 (CH$_2$), 74.11 (CH$_2$), 81.51 (C),
88.30 (CH), 102.37 (CH), 104.47 (C), 113.43 (CH₂), 144.36 (C), 170.40 (C), 171.04 (C);
FABMS (m/z): 497 [M + H]<sup>+</sup>; Anal. For (C₂₆H₄₀O₉): Calcd C 62.88 H 8.12; Found C 62.97 H 8.12.

4.5.11 Synthetic Protocol for the Preparation of Hybrid Trioxane (13): To a solution of β-hydroxyhydroperoxide 10 (0.08 g, 0.187 mmol) in CH₂Cl₂ (15 mL) was added cyclohexanone (0.4 mL, 4.08 mmol) in CH₂Cl₂ (15 mL) and conc. HCl (0.02 ml). Reaction mixture was stirred at r.t. for 1h and poured into saturated aq NaHCO₃ solution (10 mL). Organic layer was separated; aqueous layer was extracted with dichloromethane (3 × 10 mL). Combined organic layer was dried over anhyd. Na₂SO₄ and evaporated under vacuum at r.t. to furnish the crude product, which on column chromatography over silica gel using EtOAc-Hexane (1: 99) as eluant furnished 13 (0.08 g, 85% yield) as an oil; FT-IR (neat, cm⁻¹) 2937.0, 2869.8, 1653.9, 1582.5, 1449.0, 1371.2, 1194.1, 1102.4, 1026.1, 990.0, 760.5; <sup>1</sup>H NMR (200 MHz, CDCl₃) δ 0.90 (d, 3H, J = 7.4 Hz, CH₃), 0.95 (d, 3H, J = 6.0 Hz, CH₃), 1.21-2.13 (m, 24H), 1.43 (s, 3H, CH₃), 2.29-2.38 (m, 1H), 2.58-2.66 (m, 1H), 3.38 (td, 1H, J = 9.5 Hz, 6.2 Hz, OCH₂), 3.71 (dd, 1H, J = 11.7 Hz, 2.8 Hz), 3.80-3.87 (m, 1H), 3.93 (dd, 1H, J = 11.3 Hz, 21.66 Hz), 4.70 (dd, 1H, J = 9.3 Hz, 1.7 Hz), 4.77 (d, 1H, J = 2.9 Hz, C₁₀-H), 5.03 & 5.04 (2 × s, 2H, Olefinic H), 5.38 (s, 1H, C₁₂-H); <sup>1</sup>C NMR (50 MHz, CDCl₃) δ 13.44 (CH₃), 20.75 (CH₃), 22.67 (CH₃), 24.90 (CH₂), 25.07 (CH₂), 25.93 (CH₂), 26.59 (CH₃), 28.49 (CH₂), 30.97 (CH₂), 31.32 (CH), 35.03 (CH₂), 36.85 (CH₂), 37.87 (CH₂), 44.85 (CH), 52.99 (CH), 62.63 (CH₂), 68.00 (CH₂), 81.47 (C), 88.32 (CH), 102.41 (CH), 104.49 (C), 114.50 (CH₂), 144.01 (C);
FABMS (m/z): 509 [M + H]<sup>+</sup>; Anal. For (C₂₆H₄₀O₉): Calcd C 66.12 H 8.72; Found C 66.42 H 8.63.
4.6 References and Notes


8. (a) Peters, W. Techniques for the study of drug response in experimental malaria. In Chemotherapy and drug resistance in malaria; Academic Press: London, 1970; pp 64-136. (b) In vivo antimalarial efficacy test: The blood schizontocidal activity of the test compounds was evaluated in rodent model using multi-drug resistant strain of *Plasmodium yoelii nigeriensis*. The colony bred Swiss mice of either sex (20 ± 2 g) were inoculated intraperitoneally with 1x10^5* P. yoelii* (MDR) parasites on day zero and treatment was administered to group of five mice at each dose, from day 0 to 3, once daily. The drug dilutions of all compounds were prepared in groundnut oil so as to contain the required amount of the drug (0.6 mg/kg for a dose of 48 mg/kg, 0.3 mg for a dose of 24 mg/kg and 0.15 mg for a dose of 12 mg/kg) in 0.1 mL and administered orally for each dose. Parasitaemia level were recorded from thin blood smears on day 4 and subsequently twice a week till day 28. The animals which did not develop patent infection till day 28 were recorded as cured. Mice treated with β-arteether served as positive control.
9. (a) 100% suppression of parasitemia means no parasites were detected in 50 oil immersion microscopic fields (parasites if at all present, are below the detection limit). The parasites present below the detection limit can multiply and eventually can be detected during observation on subsequent days. In such cases though the drug is providing near 100% suppression of the parasitaemia on day 4 but will not provide full protection to the treated mice in the 28 day survival assay. Multidrug-resistant Plasmodium yoelii nigeriensis used in this study is resistant to chloroquine, mefloquine and halofantrine. (b) 100% protection means none of the treated mice developed patent infection during the 28 days observation period and hence were recorded as cured. Similarly, 20% protection means only 1 out of 5 mice was cured.

\( ^1H \) NMR Spectra of 8a

\( ^{13}C \) NMR Spectra of 8a
$^{1}H$ NMR Spectra of 9a

$^{13}C$ NMR Spectra of 9a
\[ ^1\text{H NMR Spectra of 11} \]

\[ ^13\text{C NMR Spectra of 11} \]
$^1$H NMR Spectra of 12

$^{13}$C NMR Spectra of 12