Studies in Photooxygenation of Unsaturated Alcohols: Synthesis of Novel Antimalarial Agents

A Summary of the Thesis Submitted

To

University of Lucknow

For Award of The Degree of

Doctor of Philosophy (Ph.D.)

In Chemistry

By

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M.Sc. (Organic Chemistry)

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Lucknow, India

2004

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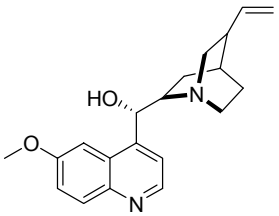
Chapter 1 Singlet Oxygen Mediated Synthesis of Antimalarials

Malarial infection

Malaria is a parasitic disease which is caused by various species of *Plasmodium* protozoa. Together with AIDS and TB, malaria is responsible for largest number of deaths annually. The malarial threat, though highest in Sub-Saharan Africa, South-East Asia and South America, is not limited to these regions, mainly due to travelers/migrants from malarial to non-malarial regions. The high rate of mortality associated with malaria can be attributed to the increasing cases of **drug-resistance** of *Plasmodium falciparum*, the most deadly of the four human infecting malarial parasites. The other three species that infect humans are *P. vivax*, *P. ovale*, and *P. malariae*.

Malaria chemotherapy

Chloroquine **1** is one of the most inexpensive, readily available, and probably most prescribed drugs for the chemotherapy of malaria. However, unfortunately it has been rendered ineffective in many parts of the world, due to the emergence of multidrug-resistant *P. falciparum*. Quinine **2** is one of the oldest known drugs against malaria. It is an alkaloid isolated from the bark of *Cinchona*. Despite its use for over 350 years, quinine is effective against all forms of malaria including the severe cases of *P. falciparum* malaria. However due to some serious side effects quinine finds a limited use in this area. Combination therapy, developed to combat drug resistance, is also under threat due to emergence of multidrug-resistant parasites. Although several individual and combination drug therapies are available against malaria each has its limitations due to one or more of the liabilities associated with toxicity, resistance and/or cost.



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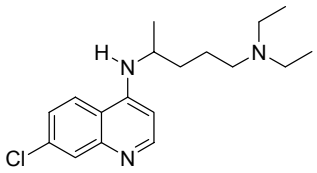

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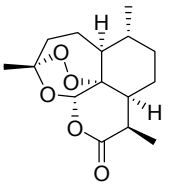
Artemisinin and related peroxides

Artemisinin (qinghaosu) **3**, isolated in early 1970's by Chinese scientists from *Artemisia annua*, is a tetracyclic sesquiterpene 1,2,4-trioxane. With its unusual and unique structure, high antimalarial efficacy, and negligible toxicity artemisinin has fascinated both chemists and biologists for past three decades. Due to its poor solubility in both oil and water, several oil and water soluble derivative of artemisinin with even better activity profile have been synthesized. These include dihydroartemisinin **4**, artemether **5**, arteether **6**, artesunate **7** and artelinic acid **8**. Artemisinin and its derivatives are the only class of antimalarial against which no clinically relevant resistance has been reported.



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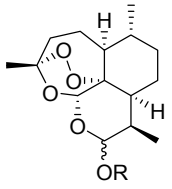
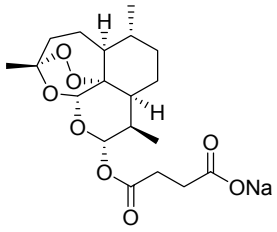


4, R=OH
5, R=Me
6, R=Et



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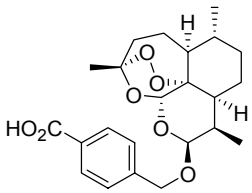

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Singlet oxygen mediated synthesis of antimalarials

Singlet oxygen is an extremely useful synthetic tool for regio- and stereo-specific introduction/insertion of oxygen into organic substrates. Synthesis of several complex natural products and organic compounds of biological interest are amongst the most important applications of the singlet oxygen chemistry. In the recent past synthesis of *antimalarials*, such as artemisinin, structurally simple 1,2,4-trioxanes and other peroxides is probably one of the well explored potential of the singlet oxygen chemistry. This chapter reviews some important reactions of singlet oxygen with organic substrates and their application towards the synthesis of novel antimalarial compounds.

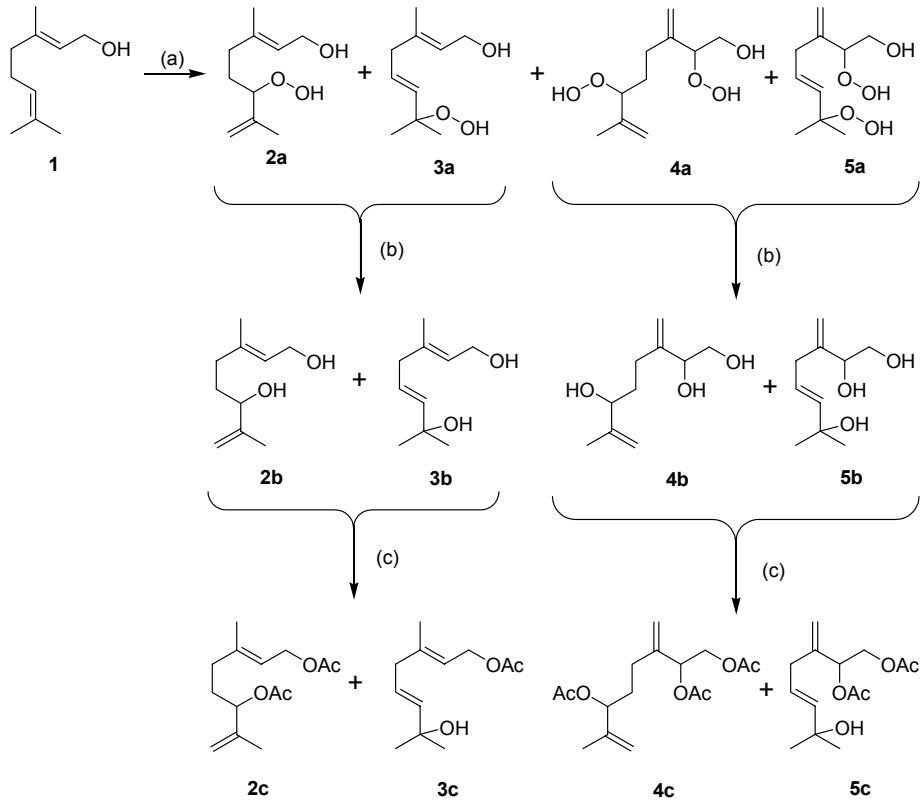
Chapter 2 Synthesis and Antimalarial Activity of New 6-Arylalkylvinyl and Alkylvinyl Substituted 1,2,4-Trioxanes

Introduction

Our laboratory has earlier reported a singlet oxygen-mediated synthesis of 1,2,4-trioxanes. Photooxygenation of appropriately substituted allylic alcohols is the key step of this synthesis. In continuation of these studies we have explored the potential of geraniol **1**, an acyclic monoterpene allylic alcohol, as a starting material for the preparation of several novel hydroxy-functionalized 1,2,4-trioxanes. This chapter describes details of this study.

Photooxygenation of geraniol

Methylene blue sensitized photooxygenation of geraniol **1** in MeCN at –5^{o}C for 6h, furnished an inseparable mixture of monohydroperoxides **2a** & **3a** in 22.6% yield and of dihydroperoxides **4a** & **5a** in 52.8% yield (Scheme 2.1). The $NaBH_4$ reduction of the mixture of **2a** and **3a**, in MeOH at 0^{o}C gave an inseparable 1:1 mixture (^{1}H NMR) of respective alcohols (diols) **2b** and **3b** in 78.3% yield. A similar reduction of the mixture of **4a** and **5a** also gave an inseparable 1:1 mixture (^{1}H NMR) of respective alcohols (triols) **4b** and **5b** in 83.8% yield. Independent acetylation of the mixture of diols **2b** and **3b**, and that of triols **4b** and **5b** with Ac_2O, Et_3N and DMAP (cat.) in CH_2Cl_2 gave acetyl derivatives **2c** & **3c**, and **4c** & **5c** (in nearly quantitative yields), which were easily separated and fully characterized from their spectral data (IR, NMR & MS). Triacetate **4c** was obtained as an inseparable 1:1 mixture of diastereomers (^{13}C NMR), implying that dihydroperoxide **4a** is also a 1:1 mixture of diastereomers. The ^{1}H NMR of diacetate **5c** showed it to be the *trans* isomer (*J*=15.6 Hz), implying that dihydroperoxide **5a** also has the trans stereochemistry. This is, in fact, one of the inherent characteristic of the $^{1}O_2$–ene reaction.

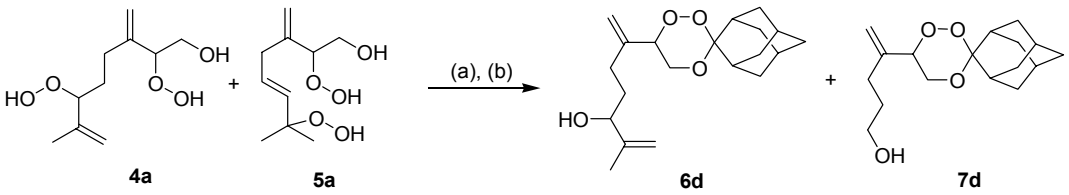


Reagents and conditions (a) *h*ν, O_2, methylene blue, MeCN, –5°C, 6h. (b) $NaBH_4$, MeOH, 0°C, 1h. (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t., 10 min.

Scheme 2.1 Photooxygenation of geraniol **1**

Preparation of 1,2,4-trioxanes

Acid catalyzed condensation (ketalization) of the mixture of **4a** and **5a** with 2-adamantanone in CH_2Cl_2 followed by reduction with $NaBH_4$ in MeOH at 0 °C furnished a mixture of trioxanes **6d** and **7d** in 9.5 and 9.4 % yields respectively (Scheme 2.2). Trioxanes **6a-c** and **7a-c** were prepared by similar reaction of these hydroperoxides with acetone, cyclopentanone and cyclohexanone (Figure 2.1). Trioxanes **6a-d** were obtained as inseparable 1:1 mixture of stereoisomers. Trioxanes **7a-d** are derived from **5a** by acid catalyzed cleavage of the tertiary hydroperoxy group ('Hock cleavage', Scheme 2.3).



Reagents and conditions (a) 2-Adamantanone, TsOH, CH_2Cl_2, r.t., 1h then 0°C overnight. (b) $NaBH_4$, MeOH, 0°C, 1h.

Scheme 2.2 Preparation of 1,2,4-trioxanes

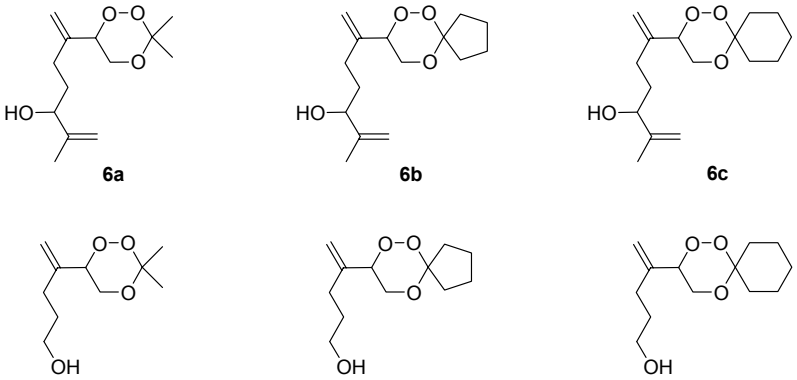
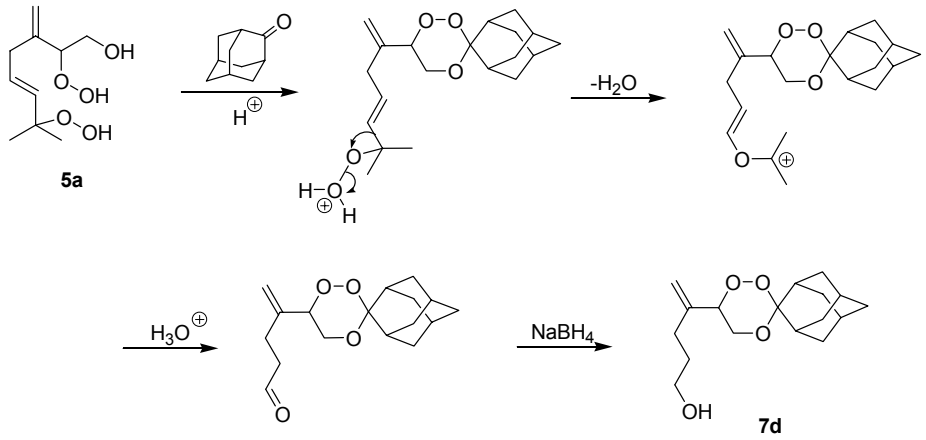


Figure 2.1



Scheme 2.3 Mechanism of formation of trioxanes **7**

Antimalarial activity of trioxanes

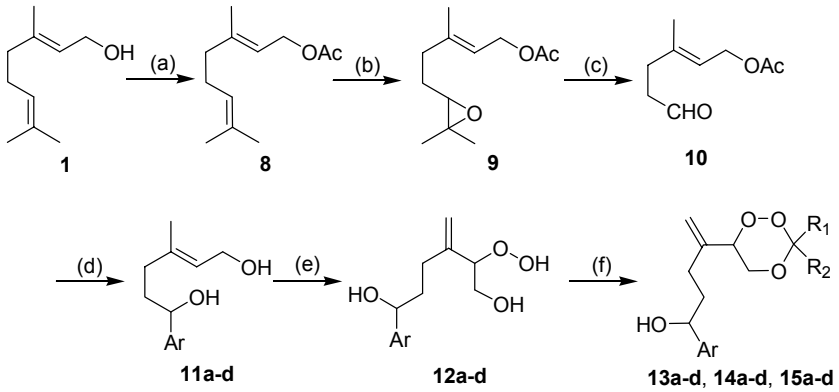
Trioxanes **6a-d** and **7a-d** were tested for their antimalarial activity *in vitro* against chloroquine susceptible strain (**NF 54**) of *Plasmodium falciparum* at 1000, 500, 250, 125, 62, 31, 15.5 ng/mL (2-fold serial dilution). Compound **6d** showed the most promising activity in this assay.

Synthesis of a new series of geraniol derived 1,2,4-trioxanes with improved results

As a sequel to the earlier study, in the present study we have used aldehyde acetate **10**, easily accessible from geraniol in three steps, to prepare a series of new hydroxy-functionalized 1,2,4-trioxanes that have a structural similarity with trioxanes **6a-d**.

Geraniol was converted to aldehyde acetate **10** using a literature procedure (Scheme 2.4).[5] Reactions of **10** with 6-equivalents of Grignard reagents prepared from bromobenzene, 4-bromochlorobenzene, 1-bromonaphthalene and 4-bromobiphenyl furnished allylic alcohols **11a-d** in 60-75% yields (Scheme 2.4). Surprisingly, reaction of aldehyde **10** with 6-equiv of n-hexylmagnesiumbromide furnished tetrahydrofuran derivative **16** instead of the desired allylic alcohol **17** (Scheme 2.5). Methylene blue sensitized photooxygenation of allylic alcohols **11a-d** in MeCN furnished β-hydroxyhydroperoxides **12a-d** in 30-45% yield, as inseparable mixture of diastereomers (analyzed from ^{1}H NMR). Acid catalyzed condensation of β-hydroxyhydroperoxides **12a-d** with cyclopentanone, cyclohexanone, and 2-adamantanone furnished hydroxy-functionalized 1,2,4-trioxanes **13a-d**, **14a-d**, **15a-d** in 50-74% yields (Scheme 2.4, Figure 2.2), again as inseparable mixture of diastereomers (analyzed from ^{1}H and ^{13}C NMR). Oxidation of trioxanes **14a** and **15a** with chromiumtrioxide-pyridine complex in CH_2Cl_2 furnished keto-functionalized trioxanes **18** and **19** in 92 and 89% yields respectively (Figure 2.2).

The most interesting feature of this study is the regioselective photooxygenation of allylic alcohols **11a-d**. This exclusive formation of a single regioisomeric hydroperoxide can be ascribed to the increased stability of the peroxyepoxide transition-state, through the 'hydrogen-bonding' effects, for this type of H-abstraction. In other words this is a consequence of the well known 'cis-effect' in the singlet-oxygen chemistry.



Reagents and conditions (a) Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t., 10 min. (b) MCPBA, NaOAc, CH_2Cl_2, 0 ^oC, 5h. (c) H_5IO_6, THF-Et_2O, 0 ^oC, 1h. (d)) (i) ArMgBr, dry Et_2O, 0^oC to r.t., 3h (ii) H_2O, 0^oC. (e) *h*ν, O_2, methylene blue, MeCN, <0^oC, 4-6h. (f) ketone, TsOH, CH_2Cl_2, r.t., 1h. (**a**, Ar = Ph; **b**, Ar = 4-ClC_6H_4; **c**, Ar = 1-naphthyl; **d**, Ar = 4-PhC_6H_4)

Scheme 2.4 Preparation of a second series of geraniol derived 1,2,4-trioxanes

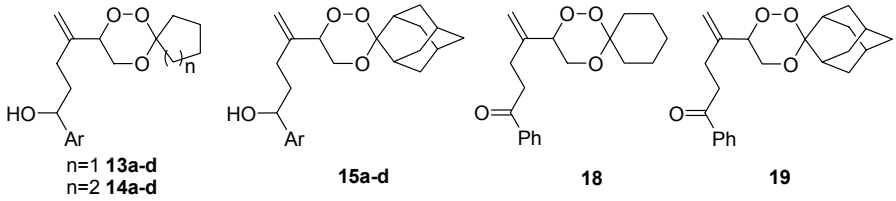
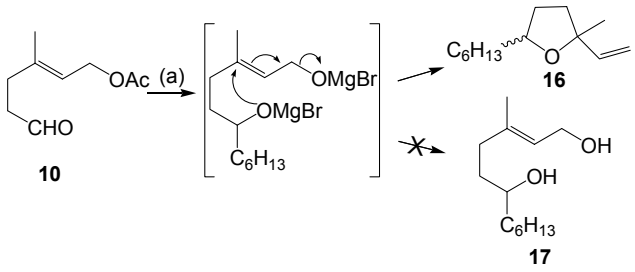


Figure 2.2 (**a**, Ar = Ph; **b**, Ar = 4-ClC6H4; **c**, Ar = 1-naphthyl; **d**, Ar = 4-PhC6H4)



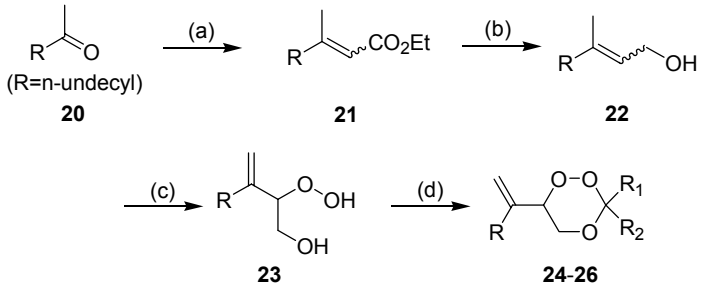
Reagents and conditions (a) (i) n-hexylMgBr, dry Et_2O, 0^oC to r.t., 4h (ii) H_2O, 0^oC.

Scheme 2.5.

Synthesis of 6-undecylvinyl substituted 1,2,4-trioxanes

Since synthesis of allylic alcohol **17** followed by its conversion to corresponding 1,2,4-trioxanes could not be achieved (Scheme 2.5) we switched over to another strategy to obtain structurally related trioxanes. Thus n-undecyl substituted allylic alcohol **22** was prepared as an inseparable 3:1 mixture of *E* and *Z* isomers in good yield from commercially available 2-tridecanone **20**, by means

of Wadsworth-Emmons olefination followed by reduction of **21** with $LiAlH_4$ (Scheme 2.6). Methylene blue sensitized photooxygenation of allylic alcohol **22** in MeCN furnished β-hydroxyhydroperoxide **23** in 43% yield. Acid catalyzed condensation of 2**3** with cyclopentanone, cyclohexanone, and 2-adamantanone, furnished trioxanes **24**, **25**, and **26** in 65, 76, and 72% yields respectively (Scheme 2.6, Figure 2.3).



Reagents and reaction conditions (a) $(OEt)_2P(O)CH_2CO_2Et$, NaH, DME, r.t. 24h. (b) $LiAlH_4$, Et_2O, 0°C, 6h. (c) *hν*, O_2, methylene blue, MeCN, <0°C, 7h. (d) Ketone, TsOH, CH_2Cl_2, r.t., 1h.

Scheme 2.6

24 n=1, R=n-undecyl
25 n=2, R=n-undecyl

26 R=n-undecyl

Figure 2.3

Antimalarial activity of trioxanes

All the trioxanes were screened for antimalarial activity against multidrug-resistant *P. yoelii* in mice at a dose of 96 mg/kg by both intramuscular (i.m.) and oral routes. The trioxanes showing activity at 96 mg/kg were further evaluated at 48 mg/kg and 24 mg/kg. Trioxanes **15a** and **15b** showed most promising activity in this assay.

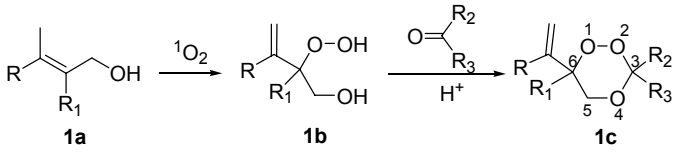
Conclusion

In conclusion, we have prepared several novel 1,2,4-trioxanes using geraniol, an abundantly available natural product, which show promising antimalarial activity.

Chapter 3 Carbohydrates as Chiral Templates: Attempted Enantio- and Diastereo-Selective Photooxygenation of Allylic and Homoallylic alcohols

Introduction

Photooxygenation of allylic alcohols, followed by acid catalyzed condensation of the β-hydroxyhydroperoxides with aldehydes and ketones, constitutes an important method for the synthesis of 1,2,4-trioxanes (Scheme 3.1).

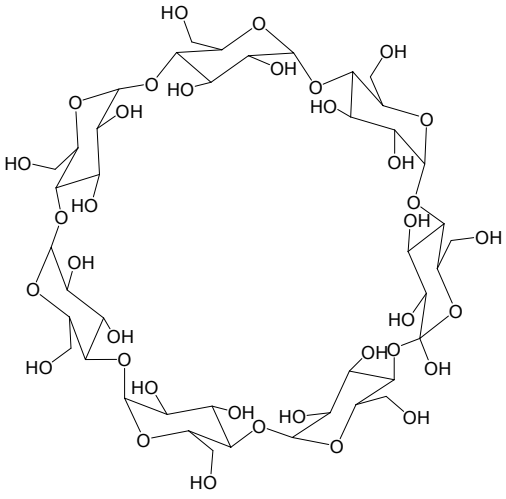


Scheme 3.1

Since photooxygenation furnishes a racemic mixture of β-hydroxyhydroperoxides **1b**, the resultant trioxanes **1c** are racemic mixtures. Several trioxanes synthesized using this method in our laboratory have shown excellent antimalarial activity both in rodent and simian models. Since the enantiomers can have different order of activity/toxicity, it is desirable to prepare enantiomerically pure 1,2,4-trioxanes. In this chapter we record our attempts towards this goal.

β–cyclodextrin

β–Cyclodextrin (**2**) is a cyclic heptasaccharide of glucose and is prepared by enzymatic degradation of starch. Both primary and secondary hydroxyl groups in **2** are projected away from the molecule and thus its inner cavity has hydrophobic character. The inner cavity of **2** has a diameter of 0.62 nm. This unique property of **2** makes it a suitable host for host-guest interactions with small and relatively non polar organic molecules. Organic compounds (the



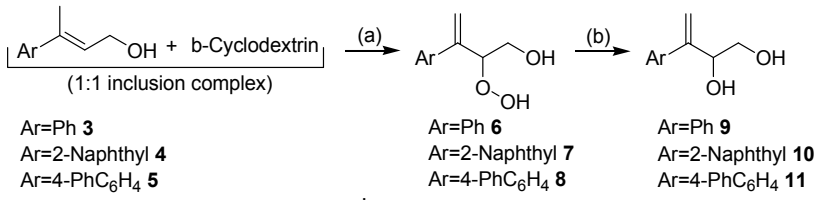
β-Cyclodextrin
2

guests) of suitable sizes form 1:1 cage or inclusion complexes with **2** (the host), in aqueous solutions, by displacing water from the inner cavity. There are several reports where inclusion complexes of small organic compounds with **2** have been shown to undergo enantio-, diastereo-, and/or regioselective reactions.

Photooxygenation of inclusion complexes of allylic alcohols 3-5 with β–cyclodextrin

Due to the above mentioned characteristics of β–cyclodextrin we selected it as our first chiral template for achieving enantioselective photooxygenation of allylic alcohols **3-5**. Alcohols **3-5** were easily prepared by a literature procedure. The inclusion complexes of **3-5** with **2** were prepared, in quantitative yield, by their drop-wise addition (as neat or as a saturated solution in acetone) to a saturated aqueous solution of **2**. Methylene blue sensitized photooxygenation of a suspension of the inclusion complex of **3** in water furnished hydroperoxide **6** that was reduced *in situ* with $NaBH_4$ to furnish diol **9** in 14.6 % yield. A similar sequence of reactions of the inclusion complexes of **4** and **5** with β–cyclodextrin gave diols **10** (7.4%) and **11** (7.0%) respectively (Scheme 3.4).

Optical rotations of **9-11** were determined and specific rotation $[\alpha]_D$ was found to be –1.45 (c=3.25, EtOH), –10.30 (c=2.0, EtOH) and –6.16 (c=0.35, EtOH) for **9**, **10** and **11** respectively. Thus diol **10** obtained from 2-naphthyl substituted allylic alcohol gave the highest specific rotation. Diol **10** was further examined by chiral HPLC (LiChroCART® 250-4 Chiradex® column, E. Merck) for its optical purity, which showed it to have an enantiomeric excess of 6.5%.



Reagents and conditions (a) hν, O_2, methylene blue, H_2O, 5-10 ^oC, 25-30h. (b) $NaBH_4$, MeOH, 0 ^oC, 1h.

Scheme 3.4 Photooxygenation of inclusion complexes of **3-5**.

As can be seen from the above data this work suffered from two limitations (i) poor yields in the photooxygenation step and (ii) poor enantioselectivity of the reaction. Thus no additional efforts were made on these lines and we switched over to an other chiral template 3,4,6-tri-*O*-benzyl- β-D-glucose.

Tri-*O*-benzyl-D-glucal

Commercially available D-(+)-glucose-derived 3,4,6-tri-*O*-benzyl-D-glucal (**12**) (Figure 3.1) is one of most extensively used precursor of 3,4,6-tri-*O*-benzyl-β-D-glucopyranosides. These glucosides can easily be prepared by reaction of alcohols with the α-epoxide (**13**) of **12**. The epoxide **13** (Figure 3.1) is an unstable compound and can only be prepared in two ways, (i) treatment of **12** with dimethyl dioxirane (DMD) or (ii) treatment of **12** with MCPBA in presence of excess KF, under strictly anhydrous conditions. The DMD mediated preparation suffers from a serious limitation that it is not suitable for large scale preparations. On the other hand the combination of MCPBA and KF is devoid of this limitation and thus we have used this method for our study.

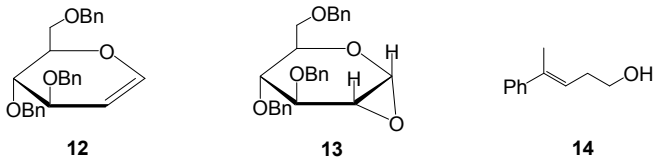
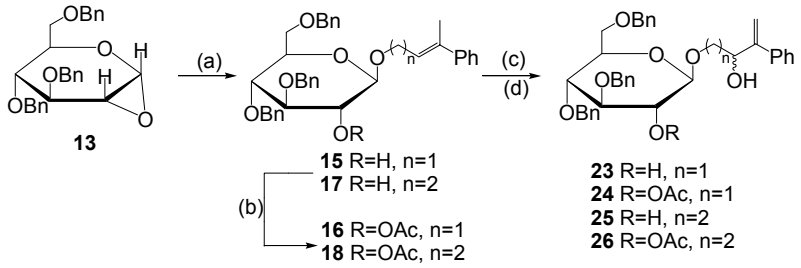


Figure 3.1

Preparation and photooxygenation of 3,4,6-tri-*O*-benzyl-β-D-glucopyranosides of allylic alcohol 3 and homoallylic alcohol 14

Condensation of allylic alcohol **3** with **13** in benzene in presence of silica gel and molecular sieves 3Å (MS 3A) at r.t. gave β-glucopyranoside **15** (41.6%). A similar reaction of homoallylic alcohol **14** (Figure 3.1) with **13** gave β-glucopyranoside **17** (29.9 %). Acetylation of both **15** and **17** using Ac_2O, Et_3N, DMAP (cat.) in CH_2Cl_2 gave corresponding acetates **16** and **18** in quantitative yields. Methylene blue sensitized photooxygenation of **15**, **16**, **17** and **18** in MeCN gave hydroperoxides **19**,

20, **21** and **22** respectively which on *in situ* reduction with Ph_3P gave corresponding alcohols **23** (78.0%), **24** (88.5%), **25** (73.2%) and **26** (56.4%) respectively (Scheme 3.5). The NMR data of **23-26** showed them to be a 1:1 mixture of diastereomers implying that the reaction was not at all diastereoselective.



Reagents and conditions (a) **3** or **14**, silica gel, MS 3A, C_6H_6, r.t., stir, 24h. (b) Ac_2O, Et_3N, DMAP (cat.), CH_2Cl_2, r.t., 10 min. (c) hν, O_2, methylene blue, MeCN, -5^oC, 3-4h. (d) Ph_3P, MeCN, r.t., 1h.

Scheme 3.5

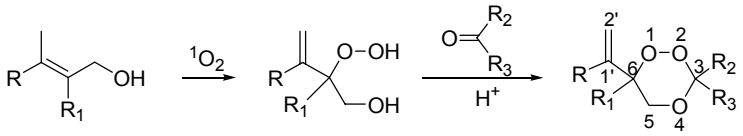
Conclusion

In our attempts to prepare optically pure β-hydroxhydroperoxides we have studied photooxygenation of inclusion complexes of allylic alcohols **3-5** with β–cyclodextrin and β-glucosides **15-18** derived from allylic and homoallylic alcohols **3** and **14**, and α-epoxide of 3,4,6-tri-*O*-benzyl-D-glucal. In both the attempts we have not been able to get the desired results.

Chapter 4 Photooxygenation of 3-Aryl-2-Cyclohexenols: Synthesis and Antimalarial Activity of Novel *trans*-Fused Bicyclic 1,2,4-Trioxanes

Introduction

Our laboratory has earlier developed a new, convenient and high yielding method for the preparation of 1,2,4-trioxanes. Preparation of β–hydroxyhydroperoxides by reaction of allylic alcohols with singlet oxygen and acid catalyzed condensation of the hydroperoxides with aldehydes or ketones are the key steps of this method (Scheme 4.1).



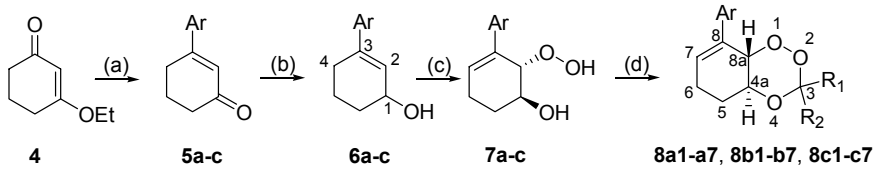
Scheme 4.1

In this chapter we report the extension of this method for the preparation of bicyclic 1,2,4-trioxanes. Highlight of this methodology is the regio- and stereo-selective photo-oxygenation of 3-aryl-2-cyclohexenols.

Preparation of *trans*-fused bicyclic 1,2,4-trioxanes

3-Aryl-2-cyclohexenones **5a-c** were prepared by a literature procedure. Thus, reaction of 3-ethyl-2-cyclohexenone **4** with Grignard reagents prepared from bromobenzene, 1-bromo-4-chlorobenzene and 4-bromobiphenyl followed by treatment with 10% H_2SO_4 gave **5a-c** in 70-84% yields. Reduction of **5a-c** with $NaBH_4$ in MeOH or CH_2Cl_2-MeOH (2:1) furnished 3-aryl-2-cyclohexenols **6a-c** (95-98% yield) as the sole products. No double bond reduction was observed under these conditions. Methylene blue sensitized photo-oxygenation of **6a-c** in MeCN gave 2-hydroperoxy-3-aryl-3-cyclohexenols **7a-c** in 22–35% yields (41–54% based on recovered starting). These β–hydroxyhydroperoxides **7a-c** on acid catalyzed condensation with benzaldehyde, 4-

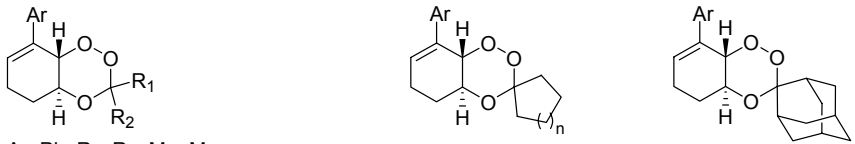
biphenylcarbaldehyde, acetone, cyclopentanone, cyclohexanone, cycloheptanone and 2-adamantanone, using catalytic concd HCl, at 0°C gave bicyclic trioxanes **8a1-a7**, **8b1-b7**, **8c1-c7** in 12–37% yields (Scheme 4.2, Figure 4.1).



(**a**, Ar=Ph; **b**, Ar=4-ClC_6H_4; **c**, Ar=4-PhC_6H_4)

Reagents and reaction conditions (a) (i) ArMgBr, Et_2O, 0°C to r.t., 3h. (ii) 10% aq H_2SO_4, 0°C. (b) $NaBH_4$, MeOH, 0°C, 1h. (c) O_2, hν, methylene blue, MeCN, 0°C, 18h. (d) aldehyde/ketone, concd HCl, CH_2Cl_2, 0°C, 3-6h. (**a**, Ar=Ph; **b**, Ar=4-ClC_6H_4; **c**, Ar=4-PhC_6H_4)

Scheme 4.2 Synthesis of bicyclic 1,2,4-trioxanes.



8a1 Ar=Ph; R_1, R_2=Me, Me
8a2 Ar=Ph; R_1, R_2=Ph, H
8a3 Ar=Ph; R_1, R_2=4-PhC_6H_4, H
8b1 Ar=4-ClC_6H_4; R_1, R_2=Me, Me
8b2 Ar=4-ClC_6H_4; R_1, R_2=Ph, H
8b3 Ar=4-ClC_6H_4; R_1, R_2=4-PhC_6H_4, H
8c1 Ar=4-PhC_6H_4; R_1, R_2=Me, Me
8c2 Ar=4-PhC_6H_4; R_1, R_2=Ph, H
8c3 Ar=4-PhC_6H_4; R_1, R_2=4-PhC_6H_4, H

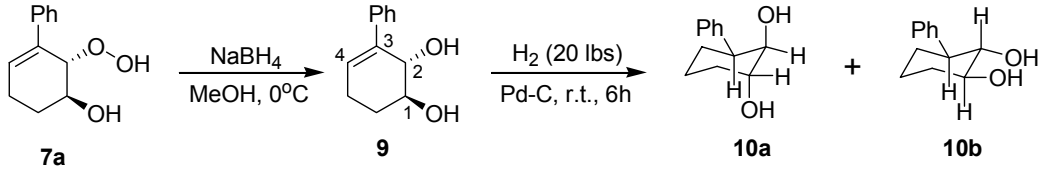
8a4 n=1, Ar=Ph
8b4 n=1, Ar=4-ClC_6H_4
8c4 n=1, Ar=4-PhC_6H_4
8a5 n=2, Ar=Ph
8b5 n=2, Ar=4-ClC_6H_4
8c5 n=2, Ar=4-PhC_6H_4
8a6 n=3, Ar=Ph
8b6 n=3, Ar=4-ClC_6H_4
8c6 n=3, Ar=4-PhC_6H_4

8a7 Ar=Ph
8b7 Ar=4-ClC_6H_4
8c7 Ar=4-PhC_6H_4

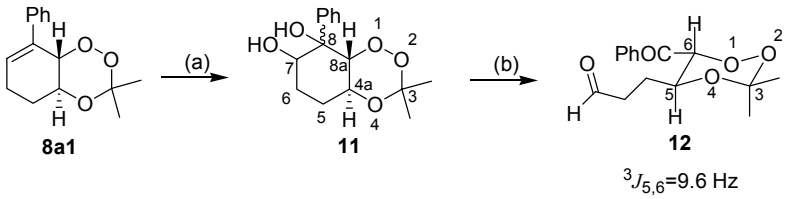
Figure 4.1.

Stereochemical assignments

Stereochemical assignments of hydroperoxides and trioxanes are based on the chemical transformations presented in Schemes 4.3 and 4.4.



Scheme 4.3



Reagents and reaction conditions (a) OsO_4 (cat.), 70% TBHP, $Et_3BnNOAc$, Me_2CO, r.t., 2d, 63.6%. (b) $Pb(OAc)_4$, PhH, r.t., stir, 1h, 83.9%.

Scheme 4.4

Antimalarial activity

All the trioxanes were subjected to in vivo antimalarial activity against multidrug-resistant *Plasmodium yoelii* in mice at a dose of 96 mg/kg by intramuscular (i.m.) and oral routes. Trioxane **8c6** showed the most promising activity in this assay.

Conclusion

In conclusion we have developed a photooxygenation route for the preparation of *trans*-fused bicyclic 1,2,4-trioxanes. Stereoselective photooxygenation of 3-aryl-2-cyclohexenols and acid catalyzed condensation of *trans*-2-hydroperoxy-3-aryl-3-cyclohexenols with aldehydes and ketones are the key steps of this method. Several new trioxanes prepared by this method have been tested for their antimalarial activity against multidrug resistant *P. yoelii* in mice.

Chapter 5 Fe(II)-Mediated Chemistry of 1,2,4-Trioxanes: Relevance to Their Mechanism of Action

Introduction

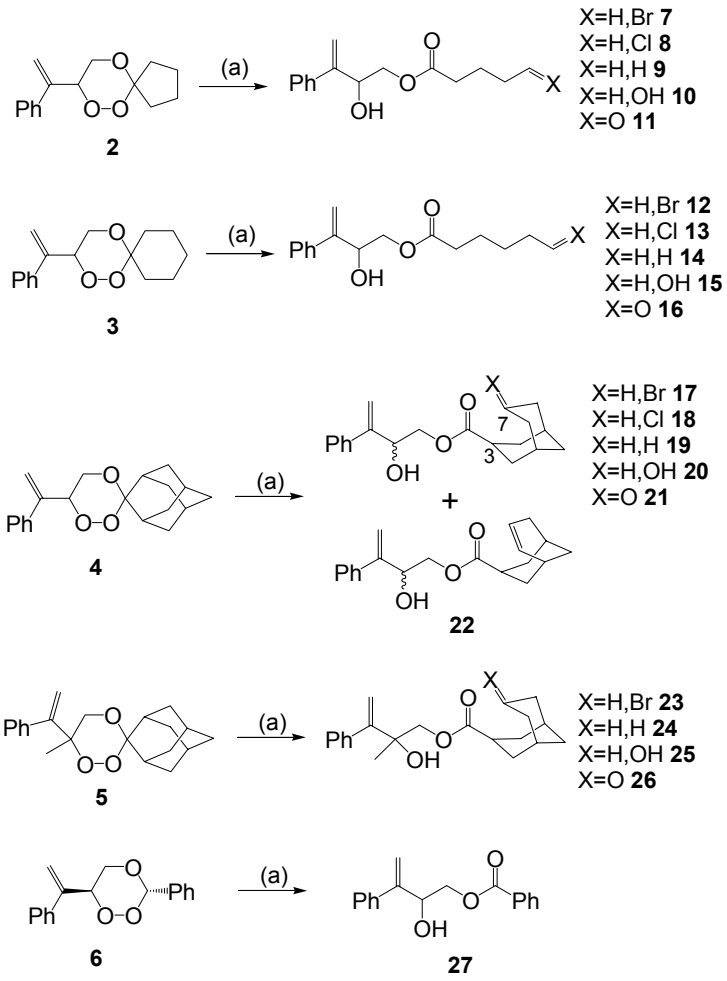
Several studies conducted on artemisinin have clearly proved essentiality of peroxide group (as 1,2,4-trioxane) for antimalarial activity. Biological studies have concluded that artemisinin is active at the blood stage of the malarial infection. Since parasite invaded erythrocytes are rich in heme iron(II), formed by host cell hemoglobin digestion, it is believed that a possible mode of action of artemisinin involves its interaction with heme iron(II). Based upon this hypothesis several studies involving Fe(II)-mediated chemistry of artemisinin and synthetic 1,2,4-trioxanes have been reported.

In order to study whether the antimalarial activity of 6-arylvinyl-1,2,4-trioxanes, prepared earlier in our laboratory, can be correlated with their reactivity towards ferrous ions, we have studied Fe(II)-mediated chemistry of five trioxanes **2-6**. This chapter describes details of this study.

Fe(II)-mediated reactions of 1,2,4-trioxanes

Trioxanes **2-6** were prepared by published procedure. Reaction of trioxane **2** with $FeBr_2$ (0.5 equiv) in anhydrous THF in absence and presence of additives such as water or L-(+)-ascorbic acid gave bromo ester **7** as the major product (Scheme 5.1). Similar reaction of **2** with $FeCl_2{\cdot}4H_2O$ (1.0 equiv) in MeCN gave chloro ester **8** as the major product. Treatment of trioxane **2** with catalytic $FeBr_2$ (0.1 equiv) and N-acetyl-(L)-cysteine (2.0 equiv) gave esters **7** and **9** as the major products together with unreacted **2**. Reaction of trioxane **2** with catalytic hemin (0.1 equiv) and 1.5 equiv of reduced glutathione (GSH) in aq MeCN for 24h under aerobic condition gave a mixture of three products **9** (3.0%), **10** (1.5%) and **11** (14.1%), together with unreacted **2** (32.0%). The same reaction under anaerobic condition (argon atmosphere) gave **9** (9.0%) as the only isolable product. On similar lines, reaction of trioxane **3** with $FeBr_2$ and $FeCl_2{\cdot}4H_2O$ gave corresponding halo derivatives **12** and **13** as

the major products (Scheme 5.1). Reaction of trioxane **3** with hemin/GSH under aerobic condition also gave three products **14** (4.9%), **15** (3.7%) and **16** (14.1%) together with unreacted **3** (20.0%), and only **14** (9.9%) under anaerobic condition. Treatment of trioxane **4** with $FeBr_2$ (0.5 equiv) in anhydrous THF gave bromo ester **17** in 33.3% yield (as an inseparable 1:1 mixture of diastereomers) along with ene ester **22** in 18% yield (also as an inseparable 1:1 mixture of diastereomers). Use of water as an additive led to an increased production of **17** (47.6%) partly compensated by a decreased yield of **22** (10.0%). Treatment of trioxane **4** with $FeCl_2 \cdot 4H_2O$ (1.0 equiv) gave a complex mixture of products from which only two compounds **18** and **22** could be characterized. Treatment of trioxane **4** with hemin/GSH under aerobic condition gave two products **20** (24.6%) and **21** (1.9%) together with unreacted **4** (40%). Similar treatment of **4** under anaerobic condition gave compound **19** (7.9%) as the only isolable product together with unreacted **4** (16%). Thus, trioxane **2** and **3** on treatment with hemin/GSH/air gave aldehydes **11** and **16** as major products whereas trioxane **4** under similar conditions gave diol **20** as the major product. Reaction of trioxane **5** with $FeBr_2$ (0.5 equiv) resulted in a slow and incomplete reaction to furnish bromide **23** (20.0%) as the major product, together with **25** (9.4%), **26** (2.1%) and unreacted **5** (30%). Reaction of trioxane **5** with hemin/GSH was also very slow and gave **25** (9.5%) and **26** (2.3%) together with unreacted **5** (60.0%) under aerobic and **24** (7.9%) and unreacted **5** (34.0%) under anaerobic conditions. Reaction of trioxane **6** with both $FeBr_2$ (0.5 equiv) and $FeCl_2 \cdot 4H_2O$ (1.0 equiv) in anhydrous THF and MeCN respectively gave the same product **27** in 32.0% yield (Scheme 5.1). In compounds **17**, **18**, **20**, **23** and **25** the stereochemistry at C-7 is unassigned.



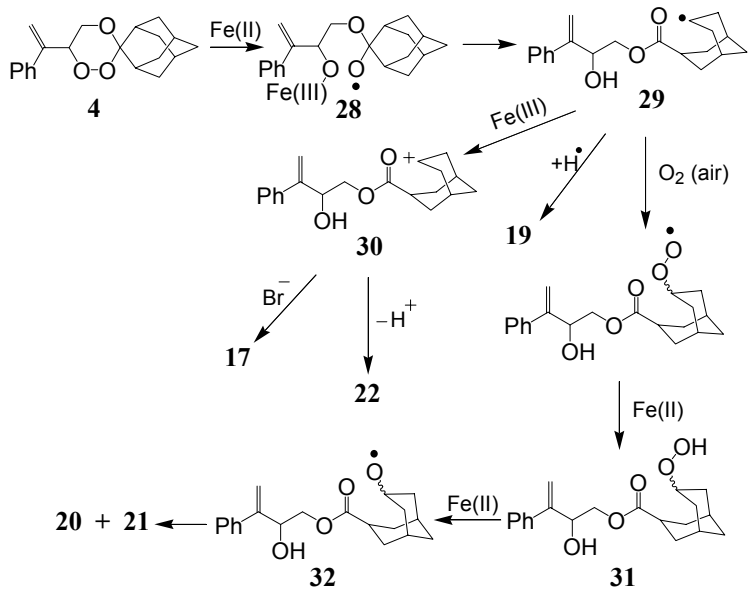
Reaction conditions (a) Fe(II) reagent, additive, solvent, air/argon, r.t., stir (see Tables 5.1 & 5.2 for details).

Scheme 5.1.

Mechanism of Fe(II)-mediated reactions of trioxanes

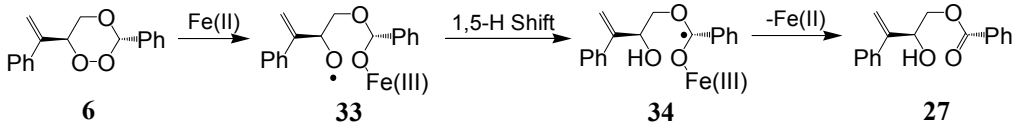
Formation of several products in the Fe(II) mediated reactions of trioxanes **2-5** under different conditions as detailed in Scheme 5.1, can be rationalized by a mechanism incorporating free radical and carbocation intermediates as exemplified in Scheme 5.2 for trioxane **4**. The reaction of trioxane **4** with Fe(II) ions causes reductive cleavage of the peroxide linkage to produce oxy radical **28** which rearranges to a C-centered radical **29**. The radical **29** on oxidation with Fe(III) is converted to the carbocation **30** that can react with bromide ion to give **17** or lose a proton to give alkene **22**. Alternaively the radical **29** can be trapped as **19** or as hydroperoxide **31** by reaction respectively with

H (present as RH) or O_2 (from air). The hydroperoxide **31** in turn can react with Fe(II) to give both **20** and **21** *via* **32** (Scheme 5.2).



Scheme 5.2. Mechanism of Fe(II) mediated reactions.

The formation of monobenzoate **27** from trioxane **6** can be rationalized by a 1,5-H transfer (Scheme 5.3) as suggested by Bloodworth and Shah in their work on $FeSO_4{\cdot}7H_2O$ mediated reaction of monocyclic 1,2,4-trioxanes.[12]



Scheme 5.3. Mechanism of formation of **27**

Results and discussion

It appears from this study that the active antimalarial trioxanes **2-4** rapidly undergo O–O cleavage followed by a C–C cleavage to produce C-centered free radicals that could alkylate parasite's biomolecules leading to a breakdown of its living mechanism. In addition primary free radicals

formed from trioxanes like **2** and **3**, can also react with molecular oxygen (a diradical) to ultimately produce aldehydes like **11** and **16** that could also fulfill the role of an alkylating agent. On the other hand trioxane **4** can produce oxy-radical **32** that can oxidize parasite proteins containing thiol residues, which is also evident from the fact that diol **20** is the major product of reaction of **4** with hemin/GSH/air. The relative poor antimalarial activity of trioxane **5** could be due to its poor reactivity towards Fe(II). Inability of trioxane **6** to form C-centered free radical could explain its poor antimalarial activity.

5.5 Conclusion

In conclusion we have studied the Fe(II)-mediated chemistry of five trioxanes and tried to correlate their antimalarial activity with their reactivity towards Fe(II).